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ABSTRACT BOOK

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In the Name of God

12th International Congress of Immunology & Allergy of Iran

Milad Tower-Tehran-Iran

"Immunology: A Dynamic & Innovative Science"

29th April-2nd May, 2014

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Welcome Message
In the Name of God

It is our pleasure to welcome you to the 12th International Congress of Immunology and Allergy of Iran, which is being held in Tehran from 29 April-2 May 2014. Founded in 1999, Iranian Society for Immunology and Allergy (ISIA) has become the largest association of immunologists in Iran. ICIA has been dedicated to the advancing of research and education in immunology in Iran, and in addition to its meetings, workshops and immunology courses, has been publishing the "Iranian Journal of Immunology", one of the first immunology journals in the middle-east region. Having organized and completed 11 International Congresses over the last two decades, ICIA is now honored to hold its 12th biannual International Congress in April-May 2014. The mission of the congress is to contribute to the standards of basic and clinical research in immunology and also to provide a platform for further communication among basic researchers and clinical scientists.

One of the strengths of this scientific meeting is the presence of internationally recognized and distinguished immunologists including Dr Rolf Zinkernagel, 1996 Nobel Prize winner in Physiology and Medicine. We would like to use this opportunity to thank all of our distinguished speakers, including Iranian expatriate scientists, for joining this congress. We strongly believe that the presence of world-renowned scientists will not only enhance the scientific aspect of this meeting, but also inspire a new generation of scientists and students to pursue their dreams, something that will shape the future of immunology and biomedical sciences in 21st century.

This scientific event would not be possible without the efforts of numerous colleagues, from department heads and principal investigators in different universities, to our young, energetic postgraduate students. Herein, we would like to thank all of them for their invaluable efforts and conviction. We hope that you enjoy this congress, and that your interactions with your colleagues from different institutions will be both professionally and personally rewarding.

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Plenary Abstracts

Cell Migration and Cell-Cell Communication in the Adaptive Immune System

Mempel TR
Massachusetts General Hospital and Harvard Medical School, Boston, USA

The development of T cells, their survival and clonal expansion, as well as their differentiation into diverse effector and regulatory cell populations is continually guided by their interactions with various types of antigen-presenting cells that collect and interpret information on the 'antigenic landscapes' of our tissues. My laboratory uses advanced intravital microscopy approaches to explore how these interactions are orchestrated through the highly regulated trafficking of T cells to different lymphoid and non-lymphoid tissues as well as their positioning into specific tissue microenvironments, and how the intracellular signals that regulate gene expression in T cells are activated in the dynamic context of these transient interactions. I will present results from our ongoing studies on T cell responses against solid tumors, through which we aim to understand where and how T regulatory cells impinge on the anti-tumor activity of cytotoxic effector T cells.

Cellular dynamics during HIV infection in humanized mice

Mempel TR
Massachusetts General Hospital and Harvard Medical School, Boston, USA

A variety of cell contact-mediated mechanisms have been identified that greatly enhance the otherwise low efficiency of HIV spread among T cells *in vitro*. These mechanisms share the common principles that concentrating infectious virus at the molecularly structured interfaces between cells increases the viral 'payload' per target cell, and that physiological mechanisms of intercellular communication, such as adhesion, cell polarization, and secretion, can be exploited to facilitate virus transfer. Such interfaces have been described between dendritic cells or macrophages and T cells, and between T cells, and have been named infectious and virological synapses, respectively.

We have recently begun to use multiphoton intravital microscopy to study the migratory behavior of human T cells in the lymphoid tissues of BLT humanized mice, and their interactions with their environment *in vivo*. Our initial results suggest that HIV-infected T cells migrate at reduced speeds, but nevertheless continue to roam through lymphoid tissues, which supports the spread of infection. During their migratory activity, they undergo viral envelop-dependent tethering interactions with $CD4^+$ cells in their vicinity, which facilitate cell fusion and syncytia formation, and may also serve the formation of virological synapses.

We hypothesize that infection of migratory immune cells, such as T cells, may be a pathogen strategy that allows cell-associated virus to overcome anatomical barriers and to shield itself against humoral immune factors in the extracellular environment during its dissemination

within and between different tissues through contact-dependent spread. Results form our current studies that are geared towards specifying the role of cell migration in HIV-1 infectious spread will be presented.

Immune Suppressor Mechanisms in Cancer

Greten TF
National Cancer Institute, Bethesda, USA

Tumors have developed multiple pathways to escape from anti-tumor immunity. Myeloid derived suppressor cells (MDSC) accumulate in cancer bearing individuals and are characterized by their profound immune suppressor function. Two different subsets of MDSC can be identified, monocytic Gr-1$^{dull/int.}$ (CD11b^{+}Ly6C^{high}Ly6G^{-}) and granulocytic Gr-1^{high} (CD11b^{+}Ly6C^{low}Ly6G^{high}) cells. Increased numbers of MDSC can be found in tumors, spleen, blood and liver of tumor bearing mice. We have studied hepatic MDSC in mice with primary liver tumors (HCC) as well as mice with subcutaneous tumors. In contrast to mice injected with tumor cells, which all demonstrated a rapid accumulation of MDSC in spleen and livers, mice with genetically or chemically induced HCC demonstrated an accumulation of MDSC only at late stages. Enhanced expression of genes associated with MDSC generation (GM-CSF, VEGF, IL6, IL1b) and migration (MCP-1, KC, S100A8, S100A9) was observed in mice with subcutaneous tumors. Only KC expression was in creased in mice with DEN-induced HCC. We next studied the role of hepatic MDSC in the context of inflammation. Unexpectedly MDSC not only failed to suppress inflammatory responses in a model of immune mediated hepatitis, but instead exacerbated liver damage. This damage was mediated by reactive oxygen species and accompanied by a significant increase in the expression of pro-inflammatory cytokines, costimulatory molecules and IFN-gamma-dependent CD40 upregulation on CD11b^{+}Gr-1^{+} cells. CD40 ligation impaired arginase dependent suppressor function of hepatic MDSC in vitro and in vivo and caused ROS mediated liver damage in tumor bearing mice. In summary our results demonstrate how hepatic MDSC accumulate in tumor bearing mice in the liver and how their phenotype and effector function can change in a setting of acute inflammation.

Personalized medicine and standardized immunophenotyping

Kariminia A
Oncology Lab, UBC, Vancouver , Canada

It has been known that healthy immune system is highly heterogeneous and the immunological changes due to various diseases have not been deeply investigated. In order to accurately measure differences in the human immune system it is necessary to have precise and standardized assays to differentiate true biological changes from technical flaws. Flow cytometry technique has hugely leveraged medical diagnosis/monitoring in the past few decades however the necessity of standardization is being more and more appreciated due to the presence of diversity of reagents, applications and instrumentation. Recent improvements towards standardization of human immuno-phenotyping and cell responses will be addressed and discussed.

The Leucocyte-Cancer Cell Hybrid Theory of Metastasis

Pawelek JM
Yale School of Medicine

This presentation focuses on the leukocyte-cancer cell hybrid theory as a mechanism for cancer metastasis. In the theory, fusion of a leucocyte, such as a macrophage, with a cancer cell results in a hybrid with the migratory capabilities of the leucocyte and the "immortality" of the cancer. Beginning from the first proposal of the theory more than a century ago and continuing today with the first proof in a human cancer, the hybrid theory offers a unifying explanation for metastasis. In this scenario, leukocyte fusion with a cancer cell is a secondary disease superimposed upon the early tumor, giving birth to a new, malignant cell with a leukocyte-cancer cell hybrid epigenome.

Hymenoptera venom allergy in patients with and without mastocytosis

Oude Elberink JN
University Medical Center Groningen, Dept of Allergology

Hymenoptera venom allergy (HVA) is a typical IgE-mediated reaction due to sensitization to one or more allergens of the venom, with a prevalence of 0.1-3% in the general population. Severity of systemic reactions varies from less severe (dermal reactions) to anaphylactic shock. Diagnosis is based on history and demonstration of specific IgE. Recommended treatment is Venom Immunotherapy which in general is very efficacious.
Elevated baseline serum tryptase levels coincide with an increased risk for both severe systemic reaction to Hymenoptera venom and systemic side effects during immunotherapy. Increased tryptase levels may also indicate the presence of mastocytosis, a disease characterized by a clonal proliferation of abnormal mast cells. . The prevalence of HVA is high (20 - 30%) in patient populations with any form of mastocytosis; systemic reactions are more severe compared to the general population, specific IgE levels are often very low or even absent and VIT is less efficacious in patients with mastocytosis Surprisingly, HVA prevalence does not increase constantly with increasing levels of mast cell load parameters: after a gradual increase to a maximum of near 50%, it declines with further rising levels.
This talk aims to answer the most important clinical questions concerning diagnosis and treatment of insect venom allergy comparing patients with and without mastocytosis.

Mast cell activation syndrome

Oude Elberink JN
University Medical Center Groningen, Dept of Allergology

Signs and symptoms of mast cell disorders are caused by the episodic release of vasoactive and inflammatory mediators from mast cells. There is no single symptom that is specific to mast cell activation. Patients with mast cell activation disorders present with recurrent symptoms of mast cell activation, in combination with objective evidence of mast cell mediator release. Two mast cell activation disorders have been recently proposed and defined: Monoclonal mast cell activation syndrome (MMAS) and Mast cell activation syndrome (MCAS)

Some patients present with recurrent episodes of anaphylaxis, while others have experienced less severe symptoms resembling allergic reactions, for which no consistent trigger or cause has been identified. The release of mast cell mediators in patients with mast cell disorders may be precipitated by a variety of stimuli, although not all triggers cause symptoms in every

patient. Potential provoking factors include a variety of medication, Hymenoptera venoms, alcohol, emotional stress, infections, physical stimuli and surgical interventions. By the time a mast cell disorder is considered, patients have typically undergone evaluation for a wide array of disorders, both allergic and nonallergic.

Modeling human B cell malignancies and Epstein-Barr-Virus infection in mice

Rajewsky K
Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

Given that human cancers are genetic diseases in that they are driven by oncogenic mutational events, the modern methods of targeted mutagenesis in mouse embryonic stem cells and, more recently, mouse zygotes, provide an ideal approach to study tumor pathogenesis in genetically tailored mouse models. I will exemplify this for human B cell lymphomas, arising either spontaneously in the course of the germinal center reaction or as a consequence of infection by Epstein-Barr-Virus, a γ-herpes virus specifically infecting and transforming human B cells. Mice can be genetically programmed to develop specific classes of lymphomas by targeting oncogenic events known from the analysis of the human tumors into specific stages of B cell differentiation in the mouse. The resulting tumors in the genetically engineered animals often mimic their human counterparts closely, also at the level of additional somatic mutations accumulating during tumor progression. The functional impact of such mutations can then be studied and evaluated with respect to therapeutic targeting. As pre-clinical models, such mice also allow one to study mechanisms of tumor dissemination and immune surveillance.
This presentation will describe the clinical manifestations, evaluation, diagnosis, and treatment of MCAS.

The complement system in health and disease

Meri S
Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Finland

The complement system plays a central role in body homeostasis and clean-up. Complement can destroy microbes and sense alterations in cells and tissues that have lost their viability. Viable cells carry complement inhibitors (CD35, CD46, CD55 and CD59) and surface polyanions that promote binding of the soluble inhibitor factor H. Factor H has an ability to discriminate between host cells and foreign cells on the basis of active recognition of structures of self (glycosaminoglycans, sialic acid, phospholipids). Errors in discrimination may lead to inadvertent complement activation against self-tissues. Complement attack against endothelial cells and blood cells causes vascular injury, leukocyte and platelet activation and hemolysis, i.e. thrombotic microangiopathy (TMA). The main form of TMA is atypical hemolytic uremic syndrome (aHUS). aHUS can be caused by mutations in factor H, CD46, factor I, thrombomodulin, C3 or factor B. Also autoantibodies against factor H or MCP can cause aHUS. aHUS may lead to kidney failure and thrombotic complications in brain, lungs and other tissues. Insufficient complement activity can predispose to infections (e.g. by pneumococci or meningococci). Pathogenic microbes can mimic host surface structures or hijack host complement inhibitors to escape complement attack. Such mechanisms have been found e.g. for pneumococci, group A and B streptococci, meningococci, borrelia spirochetes

and for many enteric gram-negative pathogens. Furthermore, recent studies suggest that complement is involved in tolerance to intestinal pathogens. An appropriate balance between activating and suppressing functions of complement is thus central to prevent microbial and autoreactive inflammatory diseases.

Obesity and immune system

Shidfar F
School of Health, Iran University of Medical Sciences

The World Health Organization (WHO) has reported that obesity has been growing at an alarming rate, accounting for approximately 35% of the population. The rapid rise in the rate of obesity is a critically important health issue for the developing countries. Obesity is associated with alterations in immunity, a chronic low-grade inflammation in which there are elevated circulating pro-inflammatory cytokines. Several hypotheses have been proposed: first, the overload of nutrients in adipocytes induces intracellular stress, resulting in the activation of inflammatory cascades .Second; overloading of adipocytes with fat overwhelmingly increases the infiltration of macrophages. These processes may cause the subsequent differentiation and activation of cytotoxic T cells, which initiate and propagate inflammatory cascades .Third; as adipose tissues enlarge, tissues become relatively hypoxic. Hypoxia within adipose tissue may activate inflammatory pathways .Fourth; overloaded adipocytes can themselves directly activate immune pathogen-sensors that cause chronic inflammation. Diet is an important regulatory factor on immune response in obese persons .Over-nutrition leads to immunoactivation due to a susceptibility to an inflammatory condition .Low glycemic index or low glycemic load carbohydrates,Omega 3 fatty acids,Vitamins A and C, Magnesium ,flavonoids , phytostrogens , probiotics and prebiotics can decrease inflammatory markers and can cause benefical effects in obese persons but saturated fatty acids and also trans fatty acids have shown deleterious effects on immune systems .

Transfusion Medicine in the Future

Pourfathollah AA
Professol of Immunology in Tarbiat Modares University and Managing Directing of Iranian Blood Transfusion Organization

Blood transfusion experienced up and downs in its long time history. Blood as therapeutic tool has been used since 300 BC, when bloodletting was common to reduce human's pains.
Today blood transfusion is advancing with an unexpected pace and we will witness great developments at following fields in near future:

- Increasing the number of voluntary non-remunerated blood donors will provide sufficient and safe blood sources.
- Development of autologous blood transfusion and patient blood management will reduce the need for allogenic blood transfusion.
- Use of blood components and blood alternatives which imitate the functions of blood will increase; for instance, successful use of recombinant erythropoietin will pave the way for efficient cytokine as new blood alternate.
- Modern technologies will be applied in different fields including information technology, automation, virus inactivation, producing blood components and targeted therapies.

Myeloid-Derived Suppressor Cells and tumor cell cross talk

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MDSCs are a heterogeneous group of cells, but in mice, all of them appear to share expression on their surfaces of the granulocytic myeloid (Gr-1) markers Ly6C and/or Ly6G, which are typically characteristic of macrophages and macrophage marker CD11b (Gr-1+CD11b+). MDSCs are known to inhibit the proliferation of CD4+and CD8+T cells, to block the activation of NK cells, and to polarize T helper maturation to a Th2 (tumor promoting) phenotype. Recent reports show that COX2, PGE2, and IL-6 may have a role in the differentiation and proliferation of MDSCs, but beyond that, little is known of the cytokine or other secreted factors that may lead to the differentiation of MDSCs from less differentiated monocyte progenitors.MDSCs and DCs do share a common progenitor: MDSCs can be converted to DCs with all-trans retinoic acid. Proliferation of MDSCs also seem to be inversely correlated with levels of mature DCs in cancer, as was noted by Mattei, et al., in a mouse strain deficient in the transcription factor IRF-8. In IRF-8-deficient (Irf8−/−) mice, melanoma cells grew more rapidly, leading to higher number of lung metastases. MDSCs also have a key role inM1/M2 balance. M1macrophages are activated by IFN-γ and express low levels of IL-10 and high levels of IL-12, which reinforces Th1 populations and drives maturation of DCs. In the presence of IL-10, direct contact between MDSCs and M1 macrophages causes down-regulation of IL-12 transcription and MHC class II (MHC-II) expression in macrophages, which effectively shuts down APC activity in DCs and macrophages, profoundly limiting immunosurveillance by the adaptive immune system. MDSCs also inhibit the cytotoxic functions of NK, B and T cells by the secretion of arginase-1 (ARG1) and NOS2 in thetumor microenvironment (TME).

Some groups have reported thatMDSCs promote tumor development by enhancing angiogenesis and by inhibiting T lymphocyte-mediated antitumor immunity a xenograft model of lung metastases induced by 4T1 breast cancer cells in BALB/c mice confirmed the important role that MDSCs play in tumor immune suppression .ELISPOT analysis demonstrated that MDSCs significantly lowered IFN-γ expression in tumor-bearing tissues. IFN-γ is a potent antitumor cytokine, suggesting that MDSCs play a major role in tumor-induced immunosuppression. MDSCs also help the proliferation of immunosuppressive T cells commonly called TREGs; the clonal expansion of TREGs is dependent on TGF-β and IL-10 secreted by Gr-1+CD11b+ MDSCs .High levels of MDSCs in the TME and peripheral blood have been correlated with poor prognosis and shorter median survival times for several distinct cancer types.

MDSC promote tumor escape by inhibiting T cell responses in vivo. MDSC accumulation has been demonstrated to participate in the suppression of immune responses to tumor antigens In tumor-bearing mice, MDSCs accumulate in the bone marrow, peripheral blood(PB) and secondary immune tissues (such as spleen and lymph nodes), and increased numbers of MDSCs were detected in the blood of most cancer patients. The STAT family of transcription factors plays major roles in MDSC function and altered immune response to cancer. Recently, some novel signaling molecules were found to induce MDSC expansion via the STAT3 pathway. Myeloid cell specific overexpression of apoptosis inhibitor 6 (Api6) induced constitutive activity of STAT3 in myeloid cells and systemic expansion of MDSCs. In the lung, this led to chronic inflammation and lung adenocarcinoma.

Tumor derived MDSCs promote tumor growth, whereas an infusion of MDSCs from the spleen of tumor-bearing mice does not distinctly affect tumor growth as compared with non-infused

mice. These data indicate that tumor and spleen-derived MDSCs have different functions, even though they have similar morphology and phenotypes. Tumor MDSCs have no alteration of ROS level, compared with those from naive CD11bGr-1 IMC, but have a high level of NO and Arg1. Tumor MDSCs suppressed both antigen-specific and nonspecific T cells. In contrast, splenic MDSCs contain high levels of ROS and modest levels of NO and Arg1 activity, and only suppress nonspecific T cells.

These functions are thought to be related to the fact that MDSCs express MHC class I, but not MHC class II, and are mediated by cell-cell contact. Tumor MDSCs promote more metastasis and invasion of tumor cells, compared with splenic MDSCs. In the tumor-bearing host, MDSCs are distinctly increased in not only spleen and other lymph organs,but also in other organs, including lungs and livers, where MDSCs could facilitate tumor metastasis to these organ sites.

In different tumor models, MDSCs induce increased numbers of Tregs through different mechanisms. In colon tumor models, $CD11b^{+}Gr\text{-}1^{+}CD115^{+}$ MDSCs induce Treg expansion via secretion of IL-10 and TGF-β. Interestingly, the production of NO was not required for MDSC induction of Tregs, whereas NO produced by iNOS has been shown to be involved in the T cell dysfunction induced by MDSCs (Huang et al., 2006). In contrast, in an ovarian cancer model, the induction of Tregs by MDSCs was associated with the expression of cytotoxic lymphocyte 4 antigen (CTLA4).

In addition to affecting immune function, MDSCs play an important role in tumor angiogenesis. MDSCs promote increased vascularization in tumors, leading to decreased apoptosis of tumor cells, and decreased hypoxic and necrotic regions within tumors, correlating with increased tumor burden.

Immunology of Aging

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Aging is a complex process that involves every cell and organ in the body and that leads to the deterioration of many biological functions over the lifespan of an individual. The reduced capacity to regrow injured tissues or organs and an increased propensity to infections and cancers are probably the most prominent hallmarks of senescence. In both developed and developing nations, the number and proportion of older people are increasing. By 2030, 30% of the population will be over 65 years old. The immune system is vital for the well-being and general health of all individuals, especially elderly, and profoundly affected by aging. Since the immune system interacts with every organ and tissue in the body, focused research on the immunology of aging is needed to further our understanding of the aging process. It is clear that the immune system (innate and adaptive) undergoes age-associated alterations, collectively termed immunosenescence. The sum of these changes is a dysregulation of many processes that normally ensure optimal immune function. Aging influence not only the renewal potential of this system but also the elements of the cytokine network essential for communication between its different parts. Aging is accompanied by numerous functional and phenotypic changes in T cells, B cells and monocytes/macrophages. In addition, autoimmunity, infections and occurrence of cancer increased in aged people.

Keywords: Aging, Immunology, biological functions

One Key for thousands locks: Antioxidant effects of Silimarin

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Reactive oxygen/nitrogen species (ROS, RNS) including Free radicals such as superoxide ($O2^{-}$), hydrogen peroxide (H2O2), and possibly hydroxyl radical (HO) are highly reactive and short-lived molecules. Due to unpaired electrons in their orbit, free radicals are unstable molecules that attack other substance within their reach and grab electron from them to stabilize themselves. ROS are the product of cellular aerobic metabolisms within the mitochondria. So, any chemical or infectious agent or genetic defect that can interfere with the function of mitochondria can lead to production of large amounts of ROS. In addition to mitochondria (a principal source of endogenous free radicals), peroxisomal fatty acid metabolism, cytochrome P-450 reaction and phagocytic cells are other sites of oxidant generation.

Flavonoids are phenolic compounds widely distributed in plants and Silymarin is a flavonoid complex which obtained from fruit and seed of Silybum Marinum (milk thistle). It is mainly composed of mixture of silybin, silychristin and silydianin mixture. Besides its strong hepatoprotective effect, silymarin exhibits anti-carcinogenic, antioxidant, iron chelating, anti-inflammatory, and immunomodulatory activities.

Various studies indicated that silymarin and its main active component silibinin exhibit immunomodulatory effects with both immunostimulatory and immunosuppressive activities in dose dependent manner. It increases lymphocyte proliferation, interferon gamma (IFNγ), interleukin (IL)-4, and IL-10 secretions by stimulated lymphocytes in a dose-dependent manner. It has been shown that in vitro treatment of peripheral blood mononuclear cells (PBMC) with silymarin causes restoration of the thiol status and increases in T cell proliferation and activation.

Silymarin inhibit T cell proliferation and pro-inflammatory cytokine secretion after incubation with 100 µM silymarin , and reduce mitogen activated protein kinase (MAPK) activity. Evidence indicated that silibinin significantly down regulated the pro-inflammatoryTH1 cytokines in vitro and exvivo, and is a potent anti-inflammatory agent through its ability to suppress inflammatory cytokines such as TNF-α and IL-1β. Molecular studies have demonstrated that silibinin inhibits the production of inflammatory cytokines through inhibition of NF-Kb signaling pathway. Besides it is demonstrated that silymarin suppress TNF-induced activation of NF-Kb and suppress the TNF-induced production of oxygen intermediated and lipid peroxidation.

We found that treatment of peripheral blood mononuclear cells with20 mug/ml silymarin causes intracellular glutathione restoration and peripheral blood mononuclear cell proliferation in thalassemia patients. The stimulatory effect of silymarin on the response of human peripheral blood lymphocytes to mitogens has been reported in patients with alcoholic cirrhosis following daily oral administration of 280 mg Legalon® .

It has been recently reported that combination therapy of desferrioxamine and silymarin was more effective than desferrioxamine monotherapy in reducing iron burden. Because of its free radical scavenging, immunostimulating, and iron chelating properties, silymarin has been suggested as an alternative therapy for β- thalassemia major.

Veterinary Immunogenetics; selection for disease resistance as an approach to the control of animal disease

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Resistance or susceptibility to numerous diseases such as viral, bacterial, parasitic and autoimmune diseases is associated with immune response genes. Moreover, many other non immunological traits such as production in livestock are controlled by these genes. Farmers and breeders can exploit genetic variation to identify and use animals that are relatively resistant to disease. There are a number of advantages in using resistant stock including increased production, improved animal welfare, reduced environmental contamination by drugs, delayed development of drug-resistant strains of pathogens or parasites and improved return on investment of time and money. These are considerable advantages and breeding for disease resistance is widely practiced in the livestock industries. Although it is desirable and feasible, the practical approaches should be varied for different area and populations upon the prevalence of infection and disease as well as the effectiveness of treatment. The key concepts in veterinary Immunogenetics will be introduced and we discuss its role in process of breeding for disease resistance.

Transfusion Related Immunomodulation (TRIM)

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Nearly 1 g of foreign antigens are entered in recipients' circulation after blood transfusion. Allogenic blood transfusion (ABT) has two opposite effects: Alloimmunization and Suppression.
These effects are affected by many factors like: storage time of blood products, the presence of white blood cells, underlying disease, presence of specific HLA in recipients and donors.
TRIM is a transient immunosuppression effect related to transfusion of blood products which increases risk of infections after blood transfusion, cancer recurrent and prolongs kidney graft survival.
It seems accumulation of soluble factors in blood units during storage time prepares condition such that induces cellular down regulation of immune system in recipients.
Keywords: Immunomodulation, Allogenic Blood Transfusion, TRIM

Features of a successful biopharmaceutical manufacturing in Iran

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Biopharmaceutical industry in Iran has emerged in late 90s. The first technology transfer was supported by Pasteur Institute of Iran for the recombinant HBS vaccine. Since then many private companies have been entered in this field resulted in introducing of more than 16 different biosimilar products in national market.
Aryogen Biopharma Company established in 2009 and released 4 recombinant medicinal

products after obtaining the quality, preclinical and clinical approval from Iran Food and drug organization. Recombinant activated human blood coagulation Factor VII (AryoSeven™), Ethanercept (Altebrel™), Rituximab (Zytux™), and Trastuzumab (Hercease™) are produced under the highest biopharmaceutical production standards. These high quality and affordable biosimilars are available for more people already have not access to the life-saving therapeutics. More than 170 qualified personnel including biotechnologists, pharmacists, physicians, and engineers are involved in different steps of production and marketing.
Aryogen is a role model of successful national planning for promoting knowledge based companies in Iran.

A case series of patients with Hyper IgM syndromes (13 cases) from Mofid hospital during 2005- 2013

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Background: Hyper IgM syndrome is a primary immunodeficiency with different phenotypes and genotypes. Until now, it is classified to6 types due to CD 40L, CD40, AID, UNG deficiency, associated with ectodermal dysplasia and type 4 as unknown causes. Patients have different manifestations that include: infections, autoimmunity and susceptibility to cancer especially hepatic cancer. We reported 13 cases of this syndrome with some special presentation and mutation analysis results.
Methods: 90 patients with signs and symptoms of immunodeficiency that was referred to Mofid Hospital during 2005-2013 was reviewed.13 patients with Hyper IgM diagnosis was selected and clinical ,laboratory and mutation analysis results of them was studied.
Results: Patients were 9 male and 4 female and had between 1 -12 years age.2 patients were brothers and one of them was presented with progressive glomerulonephritis with no responses to routine treatment since 7 years age. 2 male patients were siblings of twin mothers.2 patients got rituximab, one of them for huge splenomegaly and other for progressive lymphoproliferation of waldayer rings in mouth. One 2 yrs old boy showed oral candidiasis without any sign and symptoms of another infections .One 7 yrs old boy had hyper IgM with growth hormone deficiency.4 male patients had CD40 mutation and 2 of them had new mutations.3 female patients had ataxia telangiectasia and A.T like syndromes.
Conclusion: Report these numbers of cases from one center was guided us to importance of early diagnosis and treatmentof these patients and need to know about variable presentation of this syndrome.

Application of proteomics in reproductive immunology

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Proteomics refers to the identification and quantification of all proteins derived from the genome, tissue, cells or biological fluids. Recently, proteomics have been noticed as a new technique for investigation of the possible molecules associated with pregnancy related disorders. Investigation of the pregnancy related fluid or tissues may provide us more information about the biology of a normal pregnancy and also etiology and pathophysiology of pregnancy related diseases such as pre-eclampsia and miscarriage. Moreover immuno-proteomics may also provide a new investigation tool to detect targets of auto-antibodies on human pregnancy related tissues. Indeed proteomics can serve as a robust 'shortcut' to obtaining information unlikely to be garnered using traditional approaches. Early diagnosis together with improved understanding of underlying immunological mechanisms can enhance outcomes and increase effective management and therapeutic options for pregnant women. This review is focused on the recent published papers regarding the use of proteomics technique in reproductive immunology research. Moreover our recent published papers will present.

Key-words: Proteomics, Reproductive immunology

Performance of atopy patch test for the diagnosis of food allergy in children with atopic dermatitis (6 months to 12 years)

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Background: Food allergy has an important role in exacerbation and recurrence of atopic dermatitis (AD). Because the majority of food reactions are not IgE mediated, recently atopy patch test (APT) has introduced as a diagnostic tool for identification of delayed reactions in food allergy.

Patients and Methods: 42 children 6 months-8 years of age had been studied. Mean SCORAD index (an accepted scoring system for estimation of the severity of AD) for the patients was 39.3 ± 20. Skin prick test (SPT), specific serum IgE (ssIgE), and APT were performed for 5 main food allergens (cow's milk, yolk, egg white, wheat and soy), then oral food challenge was conducted as the gold standard test for diagnosis of food allergy.

Results: the accuracy of APT has a significant conformity coefficient with gold standard for all 5 allergens. Conformity coefficient for ssIgE and SPT were not statistically significant except for SPT of egg white. Combining APT with ssIgE and SPT increased the efficacy of diagnosis of allergy to cow's milk, yolk, wheat and soy, but no significant difference was found for egg white. The results of APT were significantly correlated with the results of food challenge for egg white in children ≤ 1 year compared with children > 1 year ($p < 0.5\%$) , but for other food allergens the differences were not significant. There was no significant difference for APT results between groups by means of SCORAD Index except for soy that the accuracy was increased in severe AD. APT had no important side effect that needs to treatment.

Conclusion: APT is a simple and noninvasive test and its accuracy is more than ssIgE and SPT Combining APT with ssIgE and SPT may decrease the need for food challenges. APT is recommended for routine investigation of food allergy.

Keywords: Atopy patch test, food allergy, atopic dermatitis, children

Stem cell basis of Endometriosis

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Endometriosis is a chronic inflammatory gynecological condition characterized by the growth of endometrial glands and stroma outside the uterine cavity. The most common symptoms are severe dysmenorrhea, dyspareunia, pelvic pain, and infertility. The pathogenesis of endometriosis is multifactorial and genetics, environmental factors and immune system have been proposed to be involved in initiation and progression of this disease. A steadily growing body of evidence supports the fundamental role of endometrial stem/progenitor cells in pathogenesis of endometriosis. Human endometrium is a fully dynamic tissue with cycling changes of regeneration and regression. It is believed that stem cells residing in this tissue are responsible for this remarkable regenerative capacity; the same cells may also have potential capacity to develop endometriosis if seeded in the peritoneum following retrograde menstruation. Here, we overview current understandings of stem cells basis of endometriosis with emphasis on our recent findings on differential characteristics of endometrial/menstrual blood stem/stromal cells of patients with endometriosis in terms of morphology, marker expression, proliferation capacity, invasion and adhesion properties and ability to express certain immunomodulatory molecules.

Keywords: Endometriosis, Endometrium, Stem cells, Menstrual blood

Combined and other well defined syndrome with immunodeficiency

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Defects in one or more components of the immune system can lead to serious and often fatal disorders, which are collectively called immunodeficiency diseases. Because of the central place of Tcells in immune responses, T-cell deficiencies not only affect T-cell effectors of the immune response, but also other effector cells that are activated by T cells.Profound T-cell deficiencies thus usually present as 'combined' immunodeficiencies of the different arms of the immune response. Severe combined immunodeficiency (SCID) is a life-threatening syndrome of recurrent infections. It is the prototype of the primary immunodeficiency diseases Unless immunologic reconstitution is achieved through stem cell transplantation (SCT), death usually occurs during the 1st year of life and almost invariably before 2 yr of age.
A number of syndromes escape formal classification but are otherwise recognizable by particular clinical or immunological features. These diseases were classified as other well defined syndrome with immunodeficiency to reflect the immunological similarity between the disorders included in this group and those in "Combined immunodeficienies".
T-cell defects are generally more severe than B-cell defects characterized by recurrent opportunistic infections–eg, by *Pneumocystis carinii*, cutaneous anergy, growth retardation(FTT) chronic diarrhea and malabsorption, increase susceptibility to GVHD, potentially fatal reactions to live viral or BCG vaccinations and an high risk of malignancy; allergy, autoimmunity and lymphomas are other important characteristics of T-cell immunodeficiency, occurring with a higher frequency than in healthy individuals
Iranian Primary Immunodeficiency Registry (IPIDR) was established in 1999. since 2006

to 2013, 565patients were included and grouped according to the updated classification of PIDs. Statistical data from these patients showed that combined immunodeficiencies represent 11% and other Well-defined syndromes with immunodeficiency 12% of all primary immunodeficiency diseases. The most common syndromes were Wiskott-Aldrich syndrome, Hyper-IgE syndrome and DNA repair defects (Ataxia telangiectasia). Increasing the awareness of risk factors and the clinical presentations of PID among physicians can lead to decrease of delay diagnosis of PID and better outcome for patients.

Autoimmune Aspects of Type 2 Diabetes Mellitus

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Historically, type 2 diabetes (T2D) was considered a metabolic disease of ageing. However, recent discoveries have demonstrated the role of chronic systemic inflammation in the development of insulin resistance and subsequent progression to T2D. Over the years, investigations into the pathophysiology of T2D have identified the presence of islet-specific T cells and islet autoimmune disease in T2D patients. Moreover, the cell-mediated islet autoimmunity has also been correlated with the progressive loss of β-cell function associated with T2D disease pathogenesis.

Autoimmunity is a well-known pathogenic component in type 1 diabetes (T1DM). The assumption that the pathogenesis of type 2 diabetes (T2DM) also encompasses autoimmune aspects is recognized increasingly, based on the presence of circulating autoantibodies against β cells, self-reactive T cells, but also on the glucose-lowering efficacy of some immunomodulatory therapies in T2DM. The identification of these autoantibodies in elderly patients with slowly progressive manifestation of diabetes led to the introduction of a distinct clinical entity termed latent autoimmune diabetes of the adult (LADA), which combines features of both T1DM and T2DM. The autoantibody cluster differs in patients with LADA from patients with T1DM, but their presence indicates steady progression towards β-cell death and subsequent need for initiation of insulin treatment in a shorter period of time compared to autoantibody-negative T2DM patients.

Autoimmune aspects in T2DM are not solely restricted to autoantibodies and thus LADA. They include the self-reactive T cells or defects in regulatory T cells (Tregs), which have been detected in autoantibody negative T2DM patients as well. One contributor to the autoimmune activation in T2DM seems to be the chronic inflammatory state, characteristic of this disease. Upon inflammation-induced tissue destruction, cryptic 'self' antigens can trigger an autoimmune response which in turn accelerates β-cell death. Both innate and adaptive immune system components, specifically macrophages and self-reactive T cells, contribute to an increased secretion of inflammatory cytokines involved in inflammatory and autoimmune processes. However, the extent to which inflammation overlaps with autoimmunity is not known. This review focuses on autoimmune involvement in T2DM, with an emphasis on LADA and the humoral immune response, on the involvement of chronic inflammation in autoimmunity, and specifically the role of B and T cells as links between inflammatory and autoimmune reactions.

The Role of Biomarkers in Asthma Management

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Biological markers are already used in the diagnosis and treatment of cardiovascular disease and cancer. Biomarkers have great potential use in the clinic as a noninvasive means to make more accurate diagnoses, monitor disease progression, and create personalized treatment regimes. Asthma is a heterogeneous disease with several different phenotypes, generally triggered by multiple gene-environment interactions. Pulmonary function tests are most often used objectively to confirm the diagnosis. However, airflow obstruction can be variable and thus missed using spirometry. Furthermore, lung function measurements may not reflect the precise underlying pathological processes responsible for different phenotypes. Inhaled corticosteroids and β2-agonists have been the mainstay of asthma therapy for over 30 years, but the heterogeneity of the disease means not all asthmatics respond to the same treatment. High costs and undesired side effects of drugs also drive the need for better targeted treatment of asthma. Biomarkers have the potential to indicate an individual's disease phenotype and thereby guide clinicians in their decisions regarding treatment. Biomarkers of airway inflammation which may help us to identify, monitor, and guide treatment of asthmatics can be obtained from multiple physiological sources, including sputum, exhaled gases, exhaled breath condensate, serum, and urine. The limitations and benefits of using biomarkers in a heterogeneous disease such as asthma should be more investigated in the future.

Innate Immunity and Pattern Recognition Receptors (PRRs): Recent Developments and New Insights in Veterinary Immunology

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The innate immune responses are the first line of defence against invading pathogens. According to the recent development and understanding of immune system, we now know that the importance of innate immunity in not less and somehow more pronounced than acquired immunity. This notion is based on the discovery of Germ-line-encoded receptors recognizing conserved molecular motifs from both exogenous and endogenous sources which are collectively called pattern recognition receptors (PRRs). PRRs can be broadly classified into different classes: Toll like receptors (TLRs), nucleotide-binding leucine-rich repeat-containing receptors (NLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), C-type lectins and inflammasome complexes are amongst them. The importance of PRRs in the field of veterinary immunology is beginning to emerge. Together these receptor families provide an extensive repertoire of sensors responsive to activating ligands from exogenous sources, such as pathogens and allergens, as well as endogenous danger signals. Receptor activation results in the initiation of a pro-inflammatory immune response that enables the resolution of infection. Understanding the inner workings of the innate immune system is a fundamental requirement in the search to understand the basis of health and disease in human and animals. The development of effective vaccines and adjuvants, the treatment of pathogenic infection, the generation of therapies for chronic and auto-inflammatory disorders, and the ongoing battle against cancer, diabetes and atherosclerosis will all benefit from a greater understanding of innate immunity. The rate of knowledge acquisition in this area has been

outstanding. It has been underpinned and driven by the use of model organisms. Information obtained from genetically modified laboratory animals and through the use of forward genetics has resulted in discoveries that have opened our eyes to the functionality and complexity of the innate immune system. With the current increase in genomic information, the range of innate immune receptors and pathways of other species available to study is rapidly increasing, and provides a rich resource to continue the development of innate immune research. Here, I will address some of the highlights of cross-species study in the innate immune field and consider the benefits of widening our understandings and knowledge for the benefit of veterinary immunology.

Update on Classification of Predominantly Antibody Deficiency

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Primary antibody deficiencies (PAD) are the most common types of primary immunodeficiency diseases, ranging from a severe reduction of all serum immunoglobulin classes and absent number of B cells to a selective antibody deficiency with normal serum immunoglobulin. Recurrent infections, chronic inflammation, autoimmunity, and cancers are the main manifestations of the affected patients. Recent advances in understanding of the genetics of B cell development have led to identification of the genes involved in PAD. Several gene mutations have been identified in association with defects in early B-cell development, including BTK, IGA, IGB, λ5, μ heavy chain, BLNK, PIK3R1, and the E47 transcription factor, which lead to low number of B cells and agammaglobulinemia. Indeed a number of genes that play a key role in class-switch recombination (CSR) and somatic hypermutation (SHM) are CD40L, CD40, IKBKG, AID, and UNG, which their mutations lead to low serum levels of IgG, IgA, and IgE in association with normal or increased IgM levels. And finally, terminal stages of B cell development are controlled by TACI, BAFF-R, TWEAK, MSH5, CD19, CD20, CD21, and CD81, which lead to hypogammaglobulinemia. As differential diagnosis among subgroups of PAD is important, a clear algorithmic approach to a patient with hypogammaglobulinemia is needed.

Immunological characteristics and applications of chicken IgY

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In recent years the generation and application of avian antibodies in wide variety of life science is increasing. Many researches carried out to characterized biochemical and immunological features of IgY.
IgY is the predominant low molecular weight serum immunoglobulin isotype in reptiles, amphibian, long fish, birds and egg yolk proposed by Leslie and Clem 1969.
phylogenetic studies have shown that IgY has similarity with both mammalian IgG and IgE. It considered being evolutionary ancestor to mammalian IgG, IgE and also to IgA. Among three avian isotypes (IgY, IgM and IgA) IgY has high concentration (5- 15 mg/ ml) in laying hens.

Although IgY is essentially an Ig with characteristic and functions similar to mammals IgG, it has slightly different structure provides its distinct properties and biochemical behaviors.
Several mechanisms have been proposed to express how IgY reacts with specific pathogen, but the exact mechanisms have not been determined.
Existence of differences in structure and biochemical behavior provides significant advantages to the application of IgY in many areas of research such as diagnosis and antibiotic alternative therapy to control of enteric and non enteric infectious diseases of either bacterial or viral origin in human and animals.
The advantages of chicken antibodies over mammalian antibodies include: (a) reduction in animal use, since chickens produce larger amounts of antibodies than laboratory animals; (b) the elimination of painful blood collections in animals; (c) the utility of IgY in many immunological assays without loss of specificity and sensitivity; (d) the considerably lower cost of feeding and handling of chickens than mammalians; (e) crude egg may be used as an antibody source.

Environmental Pollution and Cancer

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Any undesirable changes in the physical, chemical or biological characteristics of any component of the environment (Air, Water, and Soil) that can cause harmful effects on various forms of life or property is called Environmental Pollution. Among the various environmental pollution that contribute to causing health problem especially cancer, air pollution is leading cause of cancers. It is estimated that up to 4% of all cancers account for air pollution. This estimate my not shows actual number because cancer takes decades to develop its clinical manifestation.
What is air pollution? It is an atmospheric condition in which certain substances are present in excess concentration, which can cause undesirable effects on man and his environment. These substances include gases, particulate matters, radioactive substances etc.
The substances that contribute the most to increasing incidence of cancers in highly polluted cities are: 1) Particle materials especially those which are called fine particles (PM 2.5) and ultra fine particles (PM 0.1). 2) Gases and agents from cars exhaust and manufactory plants, tobacco smoke, coal combustion and fossil fuel which include: SO_2, SO_3, NO, NO_2, NO_x, CO, and hydrocarbons are also considered major polluting elements of air pollution, which are highly toxics and contribute to lung cancer, asthma, and allergies. In addition to out door pollution, evidence suggest there are other sources of compounds present in polluted air in indoor air: tobacco smoke and indoor emission from use of smoky coal fuel which are source of polycyclic aromatic hydrocarbons (PAHs) also cause DNA oxidative damage which leads to mutagenesis and genotoxicity hence conversion of normal cells to tumor cells.
Large cohort studies in the United States as well as Europe suggest that these air pollutant agents may increase lung cancer risk. Fine particle materials as well as particles from diesel fuel and benzene consuming car exhaust induces oxidative damage to DNA specially in lymphocytes, cause mutation of the tumor suppressor genes; thus, leading cancer incidence.
Evidence suggests that people exposed to urban air pollution, have high level of 8-oxo-dG (7,8 dehydro 2-deoxyguanosine) product of oxidative damage to DNA of lymphocytes. This agent is highly mutagenic. Another deadly agent found abundantly in highly polluted and industrial

cities, are free radicals, which causes breakage of DNAs and RNAs of the cells. Until now scientists knew that air contained free radicals but they had assumed that these agents are nit stable and dissipated in short time, this is not true for those types of free radicals that bind to airborne particles and contain heavy metals. They persist for indefinite amount of time even permanently. Damage done by free radicals is well known (carcinogenic).
What we can do to prevent the detrimental effect of the DNA oxidative damage? Taking antioxidant food supplements and preferably consuming fresh fruits and vegetables rich in anti oxidant regularly, especially in time that air pollution is high, may help to overcome or subside the danger of oxidative agents inhaled to certain extent. Generally, vegetables and fruits that have orange, red, and dark red color are rich in stable anti oxidant.

Quantitative and Qualitative Evaluation of Gene Association Studies, Important Points for interpreting and designing of Genetic Association Studies in Complex Diseases

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Studies of human genetics have been us for many years and offer the fundamental insight to find the etiology of the diseases. There are some different strategies to determine whether genetic markers influence the trait of interest. Since 2004, Genome Wide Association Studies (GWAS) determined a large number of single Nucleotide Polymorphisms (SNPs) that is associated with many complex diseases. After this transition to GWAS, the major problem is interpretation and discrimination of true-positive from false-positive results. The principal purpose of replication studies is to evaluate the positive finding of the previous GWAS and other replication studies in each population. There are several components that are important for interpreting and designing of a genetic association study: power of the study design, prior probability, statistical significance, systematic bias, functional data, reporting the results and etc. The major goal of this lecture is determination explanation of the key components that require for designing and interpreting of genetic association studies.
keywords: Replication Study, Complex Diseases, Gene Association.

Memory T cells and their implication in vaccine design

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Among the major features of the adaptive immune system is to develop immune memory which enables it to react to the previously encountered specific antigens (Ag) with high efficiency, strength and speed. This protective long-lived memory can persist in the absence of re-exposure to Ag. The relevance of memory T cells in vaccination is evident not only in the state of chronic diseases but also in conditions where neutralizing antibody responses target variable antigens and development of a robust T cell immunity is desirable. Memory T cells are diverse in terms of their effector functions, homing, and proliferative capacity. Key phenotypic properties of circulating memory T cells based on the CD45RA, CD45RO, CCR7, CD62L, CD27 and CD28 expression defines T_{CM}, T_{SCM}, T_{EM}, T_{TM} and T_{TDEM} subsets. Expression of CD69 and CD103 delineate tissue resident memory T (T_{RM}) cells. Memory T cell subsets may produce similar types of recall cytokines, such as IL-2, IFN-γ and TNF-a, but they differ in the extent and the quality of these responses which together with the differential localization of

these subsets in the body, affect vaccine efficacy. Therefore, generation of a persistent pool of appropriate memory T cell subset localized at the correct anatomical site for optimal pathogen clearance is essential and is a major challenge in the development of T cell-inducing vaccines. Timing, mechanism, route and the type of antigen as well as dose and nature of adjuvants delivered, have tremendous effect on the phenotypic and functional skewing of memory T cell subsets after vaccination. Moreover, specific targeting of appropriate antigen presenting cells and co-stimulatory molecules are a trend in vaccine design to enhance optimal temporal and spatial T_{RM} generation. A better understanding of the T_{RM} differentiation and homeostasis and designing vaccines that imprint T cells with the ability to home to the desired tissues will be a critical turning point in the development of both preventive and therapeutic vaccines.

Macrophage Defense against *Leishmania* Infection: New Aspects

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Multiple cellular components are involved in the immune response against leishmaniasis but macrophage play critical roles in the initiation, development and maintenance of a protective immunity. Macrophages play a dual role in leishmaniasis: they work as targets of the parasite and extend the infection when alternatively activated, but produce potent effectors molecules against the *Leishmania* parasite and control the disease when classically activated.
Mannose-fucose receptor ligation has been shown to promote inflammatory responses but *Leishmania* promastigotes avoid using MR during their invasion of macrophages in order to enhance their intracellular survival. *Leishmania* infected macrophage do not secrete IL-12 and hence unable to stimulate Th1 cell response. Interestingly macrophages from both the susceptible and resistant strains of mice display similar degree of impairment in IL-12 production after *L.Major* infection.
Dysregulation of macrophage responses during leishmaniasis can lead to chronic disease and appropriate activation of macrophages is critical for eliminating *Leishmania*. There is some evidence that chitin microparticles (CMPs) are potent stimulators of macrophages and can be considered as immunomodulators. Chitin fragments are recognized by TLR2, dectin-1 and mannose receptor on the surface of macrophages.
For the first time we reported that chitin micropaticles have immunomodulatory effects on *L. major*-infected macrophages in-vitro. CMPs reduced the in-vitro parasite infectivity of macrophage by 12%. This inhibitory effect was not directly related to the increased biosynthesis and release of nitric oxide. We observed a significant increase in the level of TNF-α secretion and this overexpression of TNF-α did not impair cell viability. When in vivo experiments have been performed in order to confirm the triggering role of CMPs on macrophage activation against *Leishmania* infection, CMPs induced regulated Th1 response via IFN-γ/IL-10 axis in *Leishmania Major* infected Balb/c mice. It has been shown that in mouse strains that are genetically resistant to *Leishmania* infection, IL-10 is normally produced in the lesion site and IL-10/IFN-γ producing cells consist approximately 25% of the total IFN-γ-expressing population of CD4+ cells recruited to the lesion. Reappraisal of our current understanding of the role of IL-10 in the immunobiology of leishmaniasis is required. Unbridled Th1 and /or Th2 responses in Leishmaniasis are harmful.
The Th1/Th2 paradigm of resistance/susceptibility to *Leishmania* infection is an oversimplification of a complicated network of regulatory/counter-regulatory interactions for removing the parasite. Macrophage modulator may be influence inflammatory pathways to removing the parasite and even lead to tissue repair.

Keywords: Macrophage; Leishmania Major; Chitin

Recombinant Single Chain Antibodies and their applications.

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Recombinant DNA technology has provided the production of recombinant antibody (rAb) fragments such as single-chain variable fragment (scFv) antibodies that are composed of variable heavy (V_H) and light (V_L) chains linked by a flexible peptide linker.
Recombinant antibodies have recently shown great promise in the replacement of monoclonal antibodies in different medical areas. Different formats of recombinant antibodies are available. In this regard, scFv antibodies might be addressed as one of the most popular formats.
Advantages of human scFvs over the intact antibodies including smaller size, fast penetration and tight binding to target tissue, fast clearance from the body and therefore better pharmacokinetic properties as well as fully human origin and manipulating by genetic engineering , have offered these antibodies as desirable tools for both the therapeutic and imaging purposes. ScFvs have been engineered into larger, multivalent, bi-specific and conjugated forms for many clinical applications. The role of these antibodies in cancer therapy has been shown and a number of scFvs are used for targeting specific markers on tumor cells and applied in clinical trials of cancer immunotherapy. The scFvs have been also introduced as ideal antibodies for treating of neurodegenerative diseases. The application of scFvs as vehicles to deliver therapeutics is an emerging area for the development of immunotherapeutics in several diseases has been demonstrated.
The unique properties of scFvs offer these antibodies as favorable agents for effective immunological interventions in several diseases.

Update on Classification of Predominantly Antibody Deficiency

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Primary antibody deficiencies (PAD) are the most common types of primary immunodeficiency diseases, ranging from a severe reduction of all serum immunoglobulin classes and absent number of B cells to a selective antibody deficiency with normal serum immunoglobulin. Recurrent infections, chronic inflammation, autoimmunity, and cancers are the main manifestations of the affected patients. Recent advances in understanding of the genetics of B cell development have led to identification of the genes involved in PAD. Several gene mutations have been identified in association with defects in early B-cell development, including BTK, IGA, IGB, λ5, μ heavy chain, BLNK, PIK3R1, and the E47 transcription factor, which lead to low number of B cells and agammaglobulinemia. Indeed a number of genes that play a key role in class-switch recombination (CSR) and somatic hypermutation (SHM) are CD40L, CD40, IKBKG, AID, and UNG, which their mutations lead to low serum levels of IgG, IgA, and IgE in association with normal or increased IgM levels. And finally, terminal stages of B cell development are controlled by TACI, BAFF-R, TWEAK, MSH5, CD19, CD20, CD21, and CD81, which lead to hypogammaglobulinemia. As differential diagnosis among subgroups of PAD is important, a clear algorithmic approach to a patient with hypogammaglobulinemia is needed.

Major Depressive Disorders and Immunological Disturbances

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MajorDepressivedisorderhaveamultifactorialcausesandimpaired Psychoneuroimmunological axis and that is associated with several immunological abnormalities, including decreased lymphocyte proliferation in response to mitogens and other forms of impaired cellular immunity.
Aspects of depression, such as anorexia and general lack of interest, resemble features of sickness behavior. There is some evidence that after episodes of viral infection, individuals describe themselves as more depressed.
Also consistent with this line of reasoning is that administration of cytokines (e.g., IFN-a) to humans can induce depression. Depressed individuals have been found to have higher levels of acute-phase proteins, proinflammatory cytokines (e.g., IL-6), and soluble IL-2 receptor, as well as higher white blood cell counts, as is often seen in the early response to infection. However, antidepressant treatment does not necessarily normalize cytokine profiles even when effective at ameliorating the depression. The situation is more complicated because there is also evidence that depressed patients are immunologically less reactive than controls. They have lower proliferative responses to mitogens and lower NK cell activity.
In addition, although depressed patients have some of the features characteristic of sickness behavior including hypersomnia (e.g., in atypical depression), most depressed patients have difficulty sleeping. Insomnia affects the immune system; particularly, cytokine production is affected and NK cell activity is decreased as a result of sleep deprivation.
Essentially, depression appears to be associated with activation of some aspects of nonspecific immunity.
Evidence indicates that stressful life events can increase the susceptibility to infectious diseases in humans. Increasing interest exists in the possibility that immune activation may contribute to the pathophysiology of depression. For example, elevated serum concentrations of the proinflammatory cytokines IL-1 and IL-6, as well as increased acute-phase proteins, including C-reactive protein, haptoglobin, and acid glycoprotein, have been found in patients with major depressive disorder. The source of immune activation in major depressive disorder is unknown, although studies have shown that both stress and CRH can induce proinflammatory cytokines in the absence of a formal immune challenge.

Recombinant nano-phage immunotherapy of cancer

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Today cancer immunotherapy is a promising approach against cancer. Among variety of immunotherapy approaches such as using DNA plasmids, dendritic cells or xenogeneic endothelial cells that have been used in cancer therapy, bacteriophage display is a powerful technique to administrate the desirable peptide with anti tumor properties to the immune system. Filamentous bacteriophages (M13/fd) were first used for displaying peptides and are still predominant phage strain, however size restriction in display have been reported in some studies. Filamentous bacteriophages are efficiently able to induce both humoral and cellular immune responses, which has made them an attractive tool as an antigen delivery system in vaccination.
Mimotopes, mimetics of epitopes, are developed using combinatorial peptide libraries and can

efficiently induce immune response. The sequence of natural epitopes and their mimotopes can be different and usually mimotopes are more potent, because they are selected from peptide libraries and tested for their potency to induce immune response.. There are few reports on successful use of phages in immunotherapy. I will discuss and elaborate one example recently being performed in our lab . In our laboratory, we isolated EGFR mimotope from a peptide library, using anti EGFR monoclonal antibody ICR62 by phage display technology .We made two recombinant M13 phage particles as a vaccine displaying three tandem repeat of EGFR mimotope and the other one a single EGFR mimotope at N-terminal of major coat protein VIII. We investigated the prophylactic and therapeutic effect of both particles in mice tumor model. The results showed that the mice receiving genetically manipulated phages develop better antibody and survive longer period of time .

Key note lecture: Updates on Hepatitis C virus Vaccines

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Hepatitis C virus (HCV), the leading cause of chronic liver diseases, has infected 3% of global population. Current standard-of-care therapy (administration of INF-α + ribavirin) and newly approved direct-acting antiviral agents (telaprevir and boceprevir) are also inadequate and expensive with a number of side effects. Therefore, introduction of an efficient vaccine remains the most cost-effective and reliable means against HCV infection. Despite a mass of efforts during last 20 years to develop a protective vaccine, none succeeded in providing sterile immunity and thus no approved vaccine against HCV infection is available to date. However, since it is the chronic state rather than the acute phase that result to liver cirrhosis, any vaccine capable of inhibiting or treating the chronic state of HCV infection might be considered as an efficient HCV vaccine. Unavailability and potential dangers associated with using attenuated HCV viral particles for vaccine preparation have resulted in the use of HCV genes and proteins formulated in novel vaccine modalities. Despite difficulties that hampered the development of HCV vaccines (high mutation rate in the HCV genome and incomplete understanding on correlates of immune protection), but availability of new vaccine technologies present a bright future for development of effective vaccines to introduce robust and cross-reactive CD4+, CD8+T-cell and neutralizing antibody responses.
In this lecture, I review the HCV genome, proteins, recent HCV cell culture systems (HCV/JFH1) and animal models, protective correlations of immunity and introduce the current candidate HCV vaccines modalities, specially those in phase I/II clinical trials such as; ChronVacC (plasmid DNA), TG4040 (MVA-based), and GI-5005 (whole yeast-based).

Edible Vaccines

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In spite of health care progress, prevention of infectious diseases is crucial. In addition to common immunization methods, plant based vaccines have been taken into consideration recently. Besides stimulating the humoral immune system, plant based vaccines can also lead to mucosal immune response in the case of oral consumption, which makes it a perfect candidate for prevention of mucosal infections. Using plant systems to produce edible vaccines has various advantages such as being edible (raw or powdered), protection of the protein against gastric acid and gradual release of it in the intestine by cellulose, there is no need for the cold

chain for transportation or storing, there is no need for the injection, the low cost and ease of production and scale up, there is no disease in common between mammalians and plants. The oral consumption of the immunogen produced in transgenic plants can induce the synthesis of mucosal and homoral specific antibodies (IgA and IgG) in different organisms (such as human). The results of the human and swine gastroenteritis, traveler diarrhea caused by *E. coli*, hepatitis B, and rabies in the first phase of clinical trial showed that the vaccine antigens which have been expressed in plants are safe and have the ability to induce protective immunity response in examined organisms. The main key for the vaccine production in transgenic plants is the selection of the plant and its specific edible organs which can express and maintain the vaccine in high proportion such as leaf, grains, beans, oil seeds, fruits, vegetables, hairy roots and alga plant cell culture. In addition to proper organs, proper immunogen selection and gene designing to express in plant system are so vital (reverse vaccinology). The studies in our research group on the immunization of the oral consumption of canola seeds containing the recombinant chimeric protein (originated from entrohemorrhagic *E. coli* and CCHF virus) showed that these candidates could be able to stimulate the humoral and mucosal immune system and develop the specific immunity against the pathogens. In conclusion, consumption of chimeric protein expressed in plants could lead to effective immune response and utilize as an impressive method in immunogen creating.

Para-inflammation

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Inflammation is an adaptive response that can be triggered by severe disturbances of homeostasis such as noxious stimuli, tissue injury and infection. However, tissue stress and malfunction may similarly induce a low-grade inflammation that has been described as para-inflammation in some texts. This low-grade inflammation depends primarily on tissue-resident macrophages and is placed between a normal homeostatic state and a classic inflammatory response. A regulated para-inflammation helps a tissue to adapt to the noxious conditions and restore the functionality of the tissue. However, dysregulated para-inflammation may result in the chronic inflammatory states that are associated with many modern human diseases, such as atherosclerosis and type 2 diabetes.

Transfusion Related Immunomodulation (TRIM)

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Nearly 1 g of foreign antigens are entered in recipients' circulation after blood transfusion. Allogenic blood transfusion (ABT) has two opposite effects: Alloimmunization and Suppression. These effects are affected by many factors like: storage time of blood products, the presence of white blood cells, underlying disease, presence of specific HLA in recipients and donors. TRIM is a transient immunosuppression effect related to transfusion of blood products which increases risk of infections after blood transfusion, cancer recurrent and prolongs kidney graft survival. It seems accumulation of soluble factors in blood units during storage time prepares condition such that induces cellular down regulation of immune system in recipients.

Defects in B-Cell Development

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Primary antibody deficiencies are the most common type of primary immunodeficiencies, accounting for approximately half of all reported cases. Primary antibody deficiencies comprise a heterogeneous group of disorders with low serum Ig titers and/or specific antibody deficiencies. These deficiencies often arise as a result of defects in early B-cell development, class-switch recombination, or terminal B-cell differentiation.
Several genes are responsible for early B-cell development in bone marrow. Mutations in these genes result in severe primary antibody deficiencies, which are characterized by blockade of B-cell differentiation before the production of surface Ig, markedly reduced mature B-cell counts in the peripheral circulation, profound hypogammaglobulinemia, and early onset of recurrent bacterial infections in affected children. In secondary lymphoid organs, class-switch recombination (CSR) and somatic hypermutation (SHM) are the mechanisms necessary for the generation of effector plasma cells secreting high-affinity IgG, IgA, and IgE antibodies. Several genes play a key role in CSR and SHM. Defects in CSR are characterized by low serum levels of IgG, IgA, and IgE leading to recurrent bacterial infections with normal or elevated serum IgM levels. The terminal stages of B-cell development are controlled by different genetic signatures. Mutations in these genes result in primary antibody deficiencies characterized by blockade of B-cell differentiation after the production of surface Ig, hypogammaglobulinemia, and recurrent bacterial infections in affected patients.

Exercise in air pollution and immune function

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An extensive body of literature outlines the strong association between exposure to air pollution and adverse health outcomes. Different groups of individuals may be affected in different ways, and some individuals are more sensitive to pollutants than others. Children and elderly people and those with health problems such as asthma, heart, nerve and lung disease often suffer more from the effects of air pollution. Air pollution exposure associated with increased inflammation and decreased cellular immune function.One pathway implicated in the response to inhaled air pollutants is initiated by the activation of Toll-like receptors (TLRs) that associated with airway, heart and nerve cells culminates in the translocation of nuclear factor-kappa B and other transcription factors to the nucleus, and therefore initiation of altered signaling of proinflammatory mediators that involved in numerous inflammatory diseases such as asthma, sepsis, atherosclerosis and Alzheimer. Studies that have investigated the effects of exposure to air pollutants during exercise have suggested that people exercising in polluted environments are at increased risk of respiratory and cardiovascular diseases. Exercise increase the adverse effect by increasing the dose of pollutants delivered to target sites. However, these studies do not take into account the potential anti-inflammatory effects of exercise, which could inhibit the proinflammatory events induced by air pollution. Regular exercise reduces the risk of chronic metabolic, nerve and cardiorespiratory diseases, in part because exercise exerts anti-inflammatory effects. It seems thatlow-intensity exercise presentsprotective effects from air pollution-induced inflammation, remodeling,oxidative stress cells exposure.

Keywords: exercise, air pollution, immune system

Development of immunological memory in cutaneous leishmaniasis

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Old world cutaneous leishmaniasis (CL) is endemic in Mediterranean countries, Central Asia and Middle East. In Iran, CL is caused by protozoan parasites of *Leishmania major* and *Leishmania tropica*. Life-long immunity is seen in individuals with CL which is related to development of immunological memory in their body. Memory is a characteristic of adaptive immunity developed against pathogens which maintains the ability of the immune system to recognize and react to re-call challenges. Memory T cells are mostly generated following antigen exposure during infancy and youth while their levels subsequently plateau and are maintained throughout adulthood. Later in life, they enter the third stage and show senescent changes. The increase in memory T cell frequency is inversely correlated with decreased susceptibility to a pathogen. In CL, two types of T cells are responsible for development of immunological memory. First, $CD4^+$T cells of central memory (T_{CM}) which express CD62L, CCR7, IL7R and home to the lymph nodes (LN). T_{CM} do not produce IFN-γ but can give rise to IFN-γ-producing effector cells upon the challenge. They proliferate in the LN and adapt an effector phenotype, migrate to the site of infection and mediate protective immunity. Second, $CD4^+$T cells with characteristics of effector T cells ($CD62L^{low}$, IFN-γ^{pos}) which mediate DTH responses and are protective against *Leishmania* parasites. Induction of an effective

memory pool is an important goal of vaccination. Moreover, determining the involvements of memory T cell subsets in life-long protection against CL is an essential factor for the vaccine development.

Biomarkers as non-invasive diagnostic tolls in transplantation status

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Biomarkers are biological molecules with valuable and attractive properties for the accurate and specific diagnosis and prognostication of disease states as well as for determining and monitoring therapy. The definition of valid pre-and post-transplantation biomarkers will facilitate personalized transplantation medicine, leading to long-term graft survival and decreasing numbers of patients on the waiting list. Current methods for diagnosing graft injury require invasive biopsies which detect pathological changes usually at advanced and often irreversible stages of allograft damage. Identification of biomarkers will aid the understanding of underlying mechanisms by indicating damage early post-transplantation when pathological changes are taking place at the molecular level.The application of biomarkers in transplantation medicine is very sensitive to time. Allograft damage progresses with time after transplantation, and Nowadays, biomarker studies increasingly integrate information from multiple platforms, such as genotype analyses of single-nucleotide polymorphisms (SNPs), epigenetic studies and analyses of mRNA, microRNA (miRNA), as well as protein, peptide, antibody and metabolite profiling.

Biomarkers of acute allograft rejection

Advances in immunosuppressive therapy and improved patient monitoring have decreased the incidence of AR in solid organ transplantation. However, the lack of non-invasive biomarkers makes early diagnosis and optimized treatment regimens,very difficult, leading to approximately 10 to 30% of all transplant patients being diagnosed and treated for AR episodes within the first year after transplantation. AR represents a major risk factor for long-term allograft dysfunction.urinary cell and peripheral blood cell mRNA profiles are diagnostic of acute rejection in renal allografts,including; granzyme B, perforin, FoxP3; miRNA: miR155, miR223, CD103, CD103, CXCR3, CXCL9 and CXCL10. Urinary cell transcriptional levels of perforin, granzyme B and granulysin ,KIM-1 ,were found to be diagnostic of biopsy-proven cell-mediated AR in renal transplant patients.

Biomarkers for chronic allograft injury

In an attempt to correlate blood expression signatures with biopsy-proven chronic allograft damage, gene expression panels were identified that predicted mild and moderate/severe chronic allograft damage, Tribbles-1 (TRIB1) was identified to predict chronic antibody-mediated rejection ; including TGF-β , connective TGF. A molecular signature of CD3ε mRNA, IP-10 mRNA, and 18S rRNA levels in urinary cells appears to be diagnostic and prognostic of acute cellular rejection in kidney allografts The envision a future for biomarkers where these can be used to diagnose disease in its early stages, predict prognosis, suggest treatment options and then assist in the implementation of therapies.

Mechanisms of gene regulation in lymphocytes and apoptosis

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The regulation of gene expression is required for the progressive restriction of a pluripotent hematopoietic stem cell to one of the various lineages and it may be mediated, in part, by nuclear transcription factors. Aiolos and Ikaros, lymphoid transcription factors which are belong to a family of zinc finger DNA-binding proteins known to be a master regulator of lymphocyte differentiation. The physiological effect of Aiolos gene inactivation in knock out mice is an increase in B cell precursors, a spontaneous production of autoantibodies and the development of B cell lymphomas. The Aiolos transcription factor has been reported to control T cell death by regulating the expression of Bcl-2, suggesting the possibility that evasion of apoptotic cell death is a common mechanism by which Ikaros family proteins participate in leukemogenesis. It has also been shown that the association of Aiolos and the anti-apoptotic molecule Bcl-x is involved in the control of apoptosis via IL-4 receptor signalling. In this way, we characterize the regulation of lymphoid Aiolos by cloning its promoter and mapping the transcription initiation site. Retardation gels showed binding activity for Ikaros, NFκB and AP4 transcription factors and mutations in their binding sites abolish Aiolos promoter activity. Chromatin immunoprecipitation assay revealed that Ikaros, NFκB and AP4 are bound to Aiolos promoter. The important function of Ikaros and NFκB is underlined by their over expression, drives Aiolos expression in cell lines and B and T cells, while over expression of a dominant negative Ikaros isoform is able to block Aiolos expression.

Depleted Uranium is a threat

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Depleted Uranium (DU) weaponr has been used against Iraq for the first time in the history of recent war .The magnitude of the complications and damage related to the use of such radioactive and toxic weapons on the environment and the human population mostly results from the intended concealment, denial and misleading information released by the Pentagon about the quantities, characteristics and the area's in Iraq, in which these weapons have been used. People always experience exposure to a certain amount of uranium from food, air, soil and water, as it is naturally present in all these components. Food, such as root vegetables, and water will provide us with small amounts of natural uranium and we will breathe in minimal concentrations of uranium with air. The concentrations of uranium in seafood are usually so low that they can be safely ignored. While uranium itself is not particularly dangerous, some of its decay products do pose a threat, especially radon, which can build up in confined spaces such as basements. Uranium in air exists as dust that will fall into surface water, on plants or on soils through settling or rainfall. It will than sink to the sediment in water or to the lower soil layers, where it will mix with uranium that is already present.

New Concepts of Immunopathogenesis of Periodontal Diseases- Role of Langerhans Cells (LCs)

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Periodontal disease is a bacterial infection of periodontal tissue which can lead to bone resorption and in severe cases, causes tooth loss. Both innate and adaptive immune responses develop during the disease process. Excessive bone resorption is frequently associated with periodontal diseases but its precise etiology is not well understood. It is proposed that T cells can facilitate osteoclastogenesis in such diseases, the role of Langerhans (LC)/dendritic cells, the most potent activators of naive T cells, remains unclear. LCs originate from bone marrow precursors, however, it is well established that, the lineage committed to differentiation of LC is less restricted, and under specific environmental conditions, common precursors can give rise to multiple DC subtypes. Oral LCs phenotypically differ from their skin counterpart especially by the expression of LPS receptor/CD14 and the high affinity receptor for IgE (FceRI) of which the latter might enable oral LCs to display more efficient allergen-binding sites and antigen uptake. LCs in the gingival epithelium are very responsive to the accumulation of bacterial plaque. Five times more number of LCs in inflamed gingiva in comparison to healthy gingiva from the same patient has been found. They also play an important role in the presentation of antigen during all phases of periodontal diseases and may represent “key” cells in pathogenesis and development of periodontal diseases. Such patterns suggest an important role of LCs in pathogenesis. Whereas, an increase in the number of S-100 + LCs in the gingival epithelium and also in connective tissue of specimens with periodontal pockets of <6 -mm depth, and was decreased after the nonsurgical phase of the periodontal treatment. Elevated levels of IL-1β, TNF-α, and PGE_2 in chronic periodontitis stimulate DC maturation and migration. It is established that in inflamed gingiva there is a reduction in number of a some kind of LCs which have a protective role in inflammation-driven bone loss initiated by bacterial infection. Through increasing Treg numbers, LCs are proposed to inhibit IFN-γ production and reduce $RANKL^+$ $CD4^+$ T cells in the inflamed tissue. As a result, bone homeostasis is not disturbed by the local inflammation, and alveolar bone remains intact. So it is suggested that in periodontitis probably the number or ration of this kind of LC has been impaired.

Immunology of Stress

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It is believed that there is a relationship between stress and parameters of the immune system. Stressors can trigger *hypothalamic-pituitary-adrenal* (HPA) system activation and release of steroid hormones. This cascade is immuno-suppressive. Stress can affect the immune response via sympathetic fibers descend from the brain into primary (bone marrow and thymus) and secondary (spleen) lymphoid organs. The negative impact of stress on immune system, are accelerated risk of some diseases, such as heart disease, diabetes, osteoporosis, frailty, cancer, viral infections, rheumatoid arthritis, infertility and asthma. Chronic stressors accelerate the risk of developing age-related diseases by ‘premature ageing’ of the immune response. Of

course, stress affects on different individuals by different ways. In fact, a person's subjective representation of a stressor is a determinant of its impact on his immune response. The loss of self-regulation makes immune system weak in stressful conditions. As a whole, stress and depression are associated with increased concentrations of cytokines such as IL-l, IL-6, IL-2, TNF–α. Furthermore, stressors release certain neurotransmitters that suppress brain activity concerned with short-term memory. Nerve cells can not crosstalk with chemical signals correctly. Behavioral changes in stressful circumstance, is due to neurotransmitter alteration in the brain. In addition, the soluble mediators released by immune cells can affect central nervous system function and will change behavior. Hostile behavior has associated with decreased level of prolactin, and increased level of epinephrine, norepinephrine which, have receptor on lymphocytes and monocytes. Therefore, the neuroendocrine hormones alter immune function.

Role of T cytotoxic cells in tumor microenvironment

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Cytotoxic CD8+ T cells (CTLs) are essential part of the specific immune system, which play crucial roles in controlling malignancies. They are considered as one of the most important infiltrating cells into the tumor niche. Anti-tumor activities of CTLs probably occur through their ability to kill tumor cells as well as cytokine secretion, particularly IFNγ. The presence and the increase number of CTLs have been along with favorable outcomes in many tumor types; however, the prognostic importance of these cells in certain cancer remains unclear. There are also some reports indicating that high CTL infiltration is associated with worse cancer condition. It has been shown that similar to CD4+ T cells, CD8+ T lymphocytes may be differentiated into different effector subtypes with various phenotypes and their cytokines expression profile after exposing o antigen. Type 1 CD8+ T (Tc1) cells produce IFNγ and TNFα and destroy their target cells through perforin or Fas mediated pathway, while type 2 (Tc2) preferentially tend to produce IL-4, IL-5 and IL-10, and primarily use perforin route. More recently, a new subset of CD8+ effector cells have been introduced which secrete IL-17 with little or no IFNγ and IL-4 and do not show in-vitro cytotoxic activities. All subtypes have been found in patients with various disorders, but their roles in anti-tumor immune responses are still unclear. Besides, it should be kept in mind that some suppressive subsets with CD8+ phenotype could be detected in cancer patients with considerable frequency, which showed potent inhibitory capacity. These results highlight a novel role for tumor-specific CTLs in tumor growth and progression which should be taken into account especially in the context of cancer immunotherapy.

Intratumoral B cells: the bright and the dark sides

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Tumor microenvironment contains a mixture of different immune cell types including cytotoxic and helper T cells, dendritic cells, myeloid derived suppressor cells and B cells. Most studies focused on the role of T cell subsets in the tumor microenvironment while the role and function of intratumoral B cells have generally been ignored. In comparison with T cells,little information about the immunophenotype and function of B cells present in different tumors sites has been released. As every other part of the tumor microenvironment, B cells may representtwo opposing faces in response to tumors.B cells can directly kill tumor cells;they can amplify and maintain Th1 and Th2 responses through IFN-γ and IL-4 production. In addition B cells may have a role in cross presentation of antigens to $CD8^{+}$T cells. On the other hand, several studies showed a negative role for B cells in response to tumors. In many cases in mouse models of various tumors, B cell deficiency or depletion had negative effect on tumor growth and metastasis. It has been investigated that B cells can mount these inhibitory effects through IL-10 or TGF-β production resulting in suppression of Th1 responses while inducing and augmenting regulatory T cell compartments. Some studies pointed that tumors can induce regulatory functions in B cells however much remained to be elucidated about this mechanism. The journey through understanding both bright and dark sides of B cell function in tumor immunology has just been started and much remained to be discovered.

Pathogenesis of inflammatory myopathies

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The inflammatory myopathies are a group of disorders sharing the common feature of immune-mediated muscle injury. Clinical and histopathological distinctions between these conditions suggest that different pathogenic processes underlie each of the inflammatory myopathies.
The most common of these disorders include: Dermatomyositis, Overlap syndromes, Inclusion body myositis and Polymyositis. The precise pathogenic mechanisms responsible for dermatomyositis (DM) are unknown, and several different models have been proposed. One model, focuses on a central role for type 1 interferons in causing capillary, myofiber, and keratinocyte injury. Challenges to understanding the pathogenesis of PM include the very heterogeneous nature of this group of patients and the difficulties among experts in agreeing upon diagnostic criteria.

Immune evasion by Staphylococci

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Staphylococcus epidermidis and its more virulent relative, *S. aureus* are the most common causes of (device-associated) nosocomial infections. However, unlike *S. aureus*, *S. epidermidis* mainly causes chronic rather than acute infections due to its biofilm formation ability and while *S. aureus* uses various strategies to evade the challenges posed by human immune system, *S. epidermidis* must rely primarily on its ability to form a biofilm, cell-surface polymers and short amphipathic phenol-soluble modulins. Here, we review the recent advances in our understanding of the various mechanisms employed by *S. aureus* and *S. epidermidis* that allow them to avoid innate and acquired immunity

Allergy & Asthma

Oral Presentations:

2997O

Persistance of milk allergy beyond 5 years of age

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Background: Milk may play an important role in the development of allergies especially in children. The significant changes in diet over the past 20-40 years may have contributed to the increased incidence of allergies in children Our aim was to study cow's milk allergy (CMA) in children beyond five years o f age and to determine the effect of its avoidance in children suffering from various forms of allergic ailments. **Methods:** The study included children (6 months onwards) and selected adults having cutaneous, gastrointestinal and respiratory manifestations Children who had attended allergy centre at Panchkula were examined for different forms of allergies250 patients were selected for the study who showed allergy to cow's milk. The skin prick tests result were confirmed with in-vitro testing method (ELISA) which showed confirmation in 80% results. **Results:**The results are based on Children included in this study with mean age at first symptoms of 6 months. The patients were selected from the Out Patients Department of of ENT AND ALLERGY CENTRE (INDIA) PANCHKULA .The study period included between 2010-2012 and follow up .The symptoms were immediate in 80% with cutaneous gastrointestinal and respiratory manifestations' family history of atophy was identified in 50%.The majority presented increased serum IgE, allergen specific IgE and positive skin prick test to milk. During CM elimination diet there was lot of improvement in the symptoms and signs of the patients (60%) which was confirmed by clinical examination, skin prick testing and in-vitro tests**. Discussions:** Many Children continue to have chronic symptoms even the original problem disappeared. This means some children may have an allergenic tendency that persists.

Keywords: Milk Allergy, Children,Prick test,Specific IgE

3071O

Chemotaxis of iNKT cells from pollen sensitized patients toward pollen lipids

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Background: Allergic rhinitis is a major health problem worldwide and plant pollens are the most important triggers of symptoms. Pollen grains are a rich source of different lipid structures that may modulate some local immune responses. Recent finding about the importance of iNKT cells in allergic responses and recognition of foreign lipid antigens by these cells raised the possibility that pollen lipids can act as a chemoattractant for iNKT cells. The aim of this study was to evaluate the role of pollen's lipid in recruitment of iNKT cells from pollen

sensitized patients in vitro.**Methods:** PBMCs were separated from 9 allergic rhinitis patients (F/M ratio 50.8, mean age 24 years) sensitized to pollens and 7 healthy controls (F/M ratio 50.8, mean age 26 years). Pollen grains from five allergenic weeds were mixed and an aqueous and a crude lipid extracts were prepared by Folch method and were used as a chemoattractant in the lower chamber of Transwell. RPMI was used as negative control.**Results:** The mean percentage of iNKT cells in the lower chamber of Transwell was 10.1,15.2 and 10.6 percent for RPMI, aqueous and lipid extracts respectively. There was no significant difference in the level of iNKT cells migration between different extracts and between allergic and non allergic groups.**Conclusion:** Our results showed that crude lipid extract of pollen cannot recruit iNKT cells but as shown in other studies some particular pollen derived lipids may activate and recruit iNKT cells to the site of allergic inflammation.

Keywords: chemotaxis, iNKT, pollen

2546O

Comparison of the frequency of R381Q functional variant of IL-23 receptor gene, in asthmatic patients and control group

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Background: Asthma is a common chronic inflammatory disease of the airways.Th17,Th2 derived cytokines (such as IL-23, IL-4, IL-5, IL-13) are involved in pathophysiology of asthma. IL-23 is a pro-inflammatory cytokine mainly produced by dendritic cells and monocytes and active macrophages and is required for effector functions and differentiation of naive $CD4^+$ T cells into Th17 cells. IL-23 identified as major factor involved in development of inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis, psoriasisand asthma. Engagement of IL-23 with IL-23R leads to activation of JAK/STAT pathway and further activation of NF-kB and downstream signaling events. A previous study showed that IL-23R gene has a number of polymorphisms one of these is an effective SNP,R381Q,that results in an arginine (R) to glutamine (Q) substitution in position of 381 (R381Q). It is shown that, this amino acid substitution in IL-23Rwhich makes R381Q variant, reduces Th17 cell differentiation by limiting IL-23–induced IL-17A production. The aim of this study was to determine whether this interleukin-23 receptor (IL-23R) polymorphism increases or decreases susceptibility to asthma in Isfahan population. **Methods:** PCR-RFLP applied to determine R381Q variant of IL-23R in all subjects. PCR products were chosen at random for sequencing. **Results:** No significant differences in the frequency of the R381Q SNP were identified between the asthma patients and healthy controls in Isfahan population. **Conclusion:** The present study suggested that R381Q polymorphism in IL-23 receptor may not be a predisposing or protective allele for asthma in Isfahan population.

Key word: R381Q , IL-23 receptor gene,asthmatic patient

2623O

Probiotic treatment: a good strategy for attenuating of allergic airway responses, an experimental model

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Background: Respiratory effects of Lactococcus lactis (L.L), as a probiotic bacteria, have included attenuating allergic airway responses. After allergen insult, type2 cytokines like IL-33 and TSLP are produced and released from lung epithelial cells and promote airway allergic responses. Chenopodium album (C. album) is a main cause of pollen allergy in desert and semi-desert areas such as Khorasan province. We examined gene expression of innate cytokines in response to probiotic treatment.**Methods:** Forty female BALB/C mice, were divided in five groups. Recombinant form of C. album allergen number 2 (rChe a2) was used to induce airway allergy in all groups. Sensitization was confirmed by measuring serum specific IgE. One group was treated sublingually with PBS (control group) and the other four treated with rChe a2, L.L, L.L mixed with allergen and L.L expressing rChe a2. Animal were sacrificed and spleen and lungs were removed. Splenocytes were cultured and levels of IL-4, IL-10, TGF-β and IFN-γ on supernatants were measured using ELISA technique. RNA was extracted from lung tissues and SYBR Green-based Real-time PCR for relative quantification of IL-33 and TSLP genes expressions were done. MHPRT was selected as reference gene. Data were analyzed using SPSS 13. **Results:**Supernatant levels of IL-4 and IL-10, type-2 cytokines, in control group (PBS-treated) were respectively 2.658 (p=0.09) and 1.321 (p=0.22) times higher than treated groups but levels of INF-γ, type-1 cytokine, and TGF-β were respectively 1.380 (p=0.22) and 1.173 (p=0.24) times lower. Fold change expression of IL-33 and TSLP genes were respectively 1.8 (p=0.19) and 2.0 (p=0.11) times higher in allergic group in compare to treatment groups. Based on dR, relative quantity expression of IL-33 and TSLP genes were respectively 1.103 and 1.125.**Conclusion:**Although data had a variation between treated groups but generally in our study, probiotic therapy caused to a reduction in type2-cytokines responses. Lactococcus lactis therapy was more effective when it was used alone than mixed with allergen.

Keywords: Probiotics, Allergy, IL-33, TSLP, Chenopodium album

2245O

Comparison of indomethacin and aspirin effects on airway responsiveness to histamine in an experimental model of allergic asthma in guinea pig

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Background: Asthma is currently a common respiratory problem worldwide. Aspirin-induced

asthma (AIA) refers to the development of acute bronchoconstriction, profuse rhinorrhea and skin flushing in about 10% of asthmatic individuals following ingestion of aspirin or aspirin-like drugs. The biochemical pathways involved in AIA are not fully established. It seems that inhibition of cyclooxygenase (COX) triggers specificbiochemical reactions which lead to AIA attacks. This study aimed to compare the effects of these non-steroidal anti-inflammatory drugs on airway responsiveness in an animal model of allergic asthma. **Methods:**18 Dunkin Hartley guinea pigs (250-300 g) were randomly divided into three groups: control, indomethacin and aspirin. All animals were sensitized by i.p. injections of ovalbumin (150µg/ animal) and AL $(OH)_3$ (150mg/ animal) on days 1, 5 and 8 and then challenged by ovalbumin 0.5% on day 18.The animals received three oral doses of normal saline, indomethacin (10mg/kg) and aspirin (200mg/kg), respectively, within 24 hrs before ovalbumin challenge. On day 19, animals were euthanized and the responsiveness of isolated tracheas was assessed using cumulative doses of histamine in organ bath.**Results:**At all histamine concentrations, indomethacin markedly increased trachea responsiveness to histamine and E_{max} values for indomethacin group (4.5 ± 0.4 g tension) were greater than those of aspirin and control groups ($P< 0.05$).**Conclusion:**It was included that indomethacin (but not aspirin) can exacerbate the asthmatic conditions in this animal model through inhibition of COX enzymes or other pathways that maybe different from those that aspirin acts on.

Keywords: aspirin, indomethacine, AIA, airway responsiveness, allergy

2435O

Association between serum level of IL-17F and lung function impairment in patients with asthma

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Background: Asthma is the most common serious chronic lung disease in which the concordance and persistence of the inflammatory response is triggered by a variety of inflammatory cytokines. The Th17 cytokine, IL-17F, plays an imperative role in asthma by inducing several inflammatory cytokines in bronchial epithelial cells. We proposed that overexpression of IL-17F is responsible for lung function impairmentin patients with asthma.**Methods:**The study population consisted of 44 asthmatic patients and 44 non-asthmatic controls. Diagnosis of asthma and its severity was carried out according to GINA guidelines. Two milliliter of venous blood was collected from all subjects. Serum was isolated by centrifuging the blood samples at 2000g for 10 min and finally IL-17F serum level was evaluated using ELISA method. **Results:**Our results showed a significant difference between serum level of IL-17F in asthmatic patients compared to healthy controls ($p< 0.001$). While the mean ± SEM values of IL-17F in asthmatic patients was 3526.41± 573.81 pg/ml, it was measured as 224.29 ± 23.84 pg/ml in healthy controls. Furthermore, a negative correlation was observed between serum levels of IL-17F and FVC[L] ($r= - 0.387$, $p = 0.010$).**Conclusion:**Nowadays, measurement of cytokines serum levels is used to evaluate asthma pathogenesis and to indicate pharmacological response to therapeutic interventions. Our results showed a strong association between higher level of IL-17F and lung function impairment in asthmatic patients. The proinflammatory nature of

the evaluated cytokines and their influence on arousing the susceptibility to asthma is the best explanation to our results.

Keywords: asthma,ELISA, lung function impairment, cytokine, IL-17F

2482O

The study of association between rs7216389-T mutation and age onset and severity of childhood asthma in Iranian asthmatic child

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Background: The association of rs7216389-T mutation and childhood asthma has been confirmed in numerous studies and it seems that this relationship be ethnic dependent. Because, no study had been performed in Western Mediterranean nations, we studied the frequency of rs7216389-T mutation in Iranian asthmatic child.**Methods:** This study was performed on 87 under 18 years old asthma patients and 115 persons without any asthma and allergy history. Sampling was performed in Hazrate Rasool asthma and allergy department during 6 months. After DNA extraction, sample's genotypes were determined by two different methods: PCR-RFLP and high resolution melting (HRM) curve analysis and results were analyzed by SPSS version 16 finally.**Results:** In patients, the genotype of 11 persons was normal homozygote (CC), 35 persons was CT and 41persons was TT (mutant homozygote). In control group, the genotype of 34 persons was CC, 63 persons was CT and 18 persons was TT. Two groups were compared by Chi-square test and our results showed that the frequency of the rs7216389-T mutation in two groups was significant (P=0.000). Patient's asthma severity was determined according to the GINA protocol and patients were divided to four categories: 1) 33 persons (37.9%) were mild intermittent, 2) 23 persons (26.4%) were mild persistent, 3) 13 persons (14.9%) were moderate persistent and 4) 18 persons (20.7%) were severe persistent. **Conclusion:** Our results showed that rs7216389-T mutation is correlated with asthma in Iranian asthmaticchild (P=0.000). Also, no relationship was observed between the asthma age onset and its severity with rs7261389-Tmutation.

Keywords: child asthma, rs-7216389, real-time PCR, PCR-RFLP

1396O

Prevalence and Risk Factors of Asthma and Allergic Diseases in Primary Schoolchildren Living Bushehr, Iran: Phase I, III ISAAC Protocol

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Background: Asthma and allergic diseases present a major health burden. Information on the prevalence of these diseases indicate increasing in various parts of the world. It was hoped that the study would be helpful to health system policy-makers in planning allergies prevention programmes in the region. **Objectives.** The prevalence of asthma and allergic diseases and the relation of various risk factors among schoolchildren of Bushehr, Iran, was assessed. **Methods.** The ISAAC Phase I and III questionnaires were completed by parents of 1280 children aged 6-7 years and self-completed by 1115 pupils aged 13-14 years. **Results.** The prevalence of atopic eczema, allergic rhinitis and asthma among 6-7 years old pupils of Bushehr, Iran, were 12.1%, 11.8% and 6.7%, respectively. While, The prevalence of these diseases were assessed 19%, 30% and 7.6%, respectively. There was an association between asthma, rhinitis and eczema ($P< 0.05$). Fast food consumption as a risk factor associated with asthma ($P= 0.03$). **Discussion.** The prevalence of asthma and allergic diseases in pupils of Bushehr, Iran, is high. There was also a strong relation between fast food consumption and asthma in schoolchildren.

Keywords. Asthma, atopic eczema, allergic rhinitis, ISAAC, prevalence, children

1741O

Evaluation of newly identified helper T cells specific cytokines expressions in the peripheral blood and sputum samples of patients with Asthma

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Background: Allergic asthma is characterized by airway inflammation, increased mucus production and airway hyperresponsiveness (AHR). Interleukin IL-17 producing T_H17 cell and interleukin IL-22 producing T_H22 cell are described subsets of $CD4^+$ T cells that may have a role in severe asthma. Another newly identified subtype is IL-25 producing T (T_H25) cell, which is capable to amplifying allergic type inflammatory response and T_H9 cells as a significant mediator in allergic inflammations that some evidences have shown its role in accelerating of respiratory system sensitivity may have a role in severe asthma. Innate lymphoid cells (ILCs) are newly identified members of the lymphoid lineage that have emerging roles in mediating immune responses and in regulating tissue homeostasis and inflammation through their cytokine secretion. **Methods:** Total mRNA extracted from whole blood and sputum samples of 23 asthma patients and 23 normal controls, and IL-17, IL-9, IL-22 and IL-25 transcript levels were measured using qRT-PCR. Lineage negative, $CD127^+$, $CD161^+$ ILC counting in blood samples of both studied groups performed with flow cytometry method. **Results:** This study showed a significant increase in transcript levels of IL-17, IL-9, IL-22 and IL-25 in asthma patients. Accordingly, counting of ILCs showed that these cells are increase in these patients as well. **Conclusions:** We showed that the expression levels of IL-17, IL-9, IL-22 and IL-25

genes and the count of ILCs (another source of these cytokines) are increased in asthmatic patients and this could clarify more aspects of the immunopathogenesis of asthma in these patients.

Keywords: Asthma, Th17, Th22, Th25, Th9, ILCs

1524O

Mesenchymal stem cell ameliorates ovalbumin induced allergic asthma in mice

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Background: Allergic asthma is a potentially life-threatening inflammatory disease of the lung characterized by the presence of large numbers of CD4+ T cells. These cells produce the Th2 cytokines that are thought to orchestrate the inflammation associated with asthma. Bone marrow-derived mesenchymal stem cells (MSC) In addition to potential for tissue repair possess potent anti-proliferative and anti-inflammatory effects which support their therapeutic use for immune mediated diseases. In this study, effects MSC on mouse model of allergic airway inflammation were evaluated.**Methods:** Systemic administration of MSC was examined in an Ovalbumin (OVA) induced airway inflammation model. Th2 mediated parameters include IL-5 and OVA specific IgE and lung histopathology were evaluated.**Results:** Results show that systemic administration of MSC suppressed the production of Th2 related cytokine (IL-5) and OVA specific IgE. According to histopathologic study, MSC administration ameliorates the air way inflammation.**Conclusion:** These results indicate that MSC efficiently diminishes bronchial inflammation in an OVA-induced allergic asthma murine model and suggest that MSC has potential therapeutic value for controlling allergic asthma responses.

Keywords: Mesenchymal stem cell, Allergic asthma, Ovalbumin, Mice.

1614O

Role of protein phosphatases inhibitors on the histamine release and the functional desensitization of in human lung mast cells.

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Background: The β_2-adrenoceptor agonist, isoprenaline, is an effective inhibitor of histamine release from human lung mast cells (HLMC). Since phosphorylations of the β_2-adrenoceptors are probably important in inducing desensitization, we sought to investigate the importance of phosphorylation events by targeting protein phosphatases in mast cells. **Method:** To this end the effects of inhibitor of on the functional desensitization of β-adrenoceptor-mediated responses in mast cells were investigated.We investigated the effects of PP inhibitors on the inhibition of histamine release from HLMC, on β-agonists in mast cellsand on desensitization. **Result:** Long-term exposure of mast cells to both isoprenaline and salbutamol substantially reduced the extent to which isoprenaline inhibited histamine release. Pre-treatments of up to 24 h with inhibitors alone had no effect on IgE-mediated histamine release. Shorter (≤

4 h) pre-treatments had little effect on the activity of isoprenaline and salbutamol to inhibit histamine release from mast cells**Conclusion:** Collectively, these data suggest that PP has an important role in regulating mast cell β_2-adrenoceptors.
Key word: Protein phosphatases inhibitors , Mast cells , Histamine

1825O
Interference in TGF-b signaling by WW2/WW3 domains of smurf proteins

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Background*:* ww2/ww3 domains are tandem sequences within Smurf2 molecule which plays an inhibitory role in TGF-β signaling via interaction with smad-7 protein as an inhibitory protein. In our study we expressed these two domains and TAT polypeptide all in tandem so that it could be studied for its effects on TGF-β signaling and arginase gene expression as consequence of that.**Methods***:* the coding sequence of TaT-WW domains, were cloned and purified. Protein evaluation and confirmation were done with SDS-PAGE and Western Blotting. The murine macrophage cell line J774A.1 was cultured with and without tgf-b and tat-ww protein for 20h. RNA from Harvested cells was extracted and reversed-transcript into cDNA. Gene expression of arginase I and GAPDH as housekeeping gene by SYBR GreenI real time PCR was carried out. At the end immunoprecipitation was done to confirm smad7-ww2/ww3 interaction.**Results***:* Sequencing results of the inserted plasmid confirmed that the gene had been inserted in-frame into the vector. Arginase gene expression showed that 9 times down regulation in groups that treated with tat-ww protein in contrast to control group that did not have any tat-ww protein.**Conclusion***:* The expressed protein ww2/ww3 entry into cells by TAT protein and down regulates TGF-β signal transduction and arginase gene expression. Since TGF-b signaling and arginase as a consequence of that have effects on remodeling in asthma and cancer progressing, so these finding can useful in Asthma and cancer therapy because of Arginase effects neutralization.
Keywords*:* TGF-b, Arginase, Smurf2, Smad-7

1809O
Evaluation of IgE, IFN-Y, IL2, TNF-a, CRP variations due to exposure to *Quercusbrantii* L. in rat

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Background: Allergy is amongthose diseases caused by different substances suchas pollen affecting many people worldwide where they are living next to such pollen-distributing plants. *Quercusbrantii* is one of the most common tree growing in different parts of Iran including Ilam. The current study aimed to evaluate the variations of IgE and some cytokines such as IFN-Y and TNF-a In rats exposed to the pollen of this tree.**Metods:** Pollens were collected from different parts of Ilam. Rats included in five groups, optical group, dermal group, nasal group, serological and control group with 5 rats in each group. Pollen essence was extracted

and applied for each group and 2hrs, 24hrs and one week after exposure, serum sample was collected while the reactions of each rat for symptoms of allergy were recorded too. ELISA was employed to measure the TNF, IFN and nephlomety analysis was applied for totalIgE. Data were analyzed by SPSS using ANOVA, Mean evaluation and Pearson correlation. **Results:** Wheal and flares was observed by $75mg^{-L}$ of the pollen subcutaneously eye and nasal allergy symptoms were seen in optical and nasal groups,together with sneezing. Total IgE for the serology group was 16.50. IFN and TNF were also different in case and controlgroups indicating the variation of these immunological properties due to the pollen of this tree. **Conclusion:** The pollen of *Quercusbrantii* is allergeric when it is inhaled or is in contact with skin and also even when it is injected.

Keywords: *Quercusbrantii* l., Allergy, IgE, TNF

2061O

Allergic asthma and expression of T-bet in cell culture containing the porcupine extract

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Background &Objective: Asthma is a chronic inflammatory disease. The Th1 & Th2balance play a major role in allergic diseases. The transcription factors ofT-bet involved in the differentiation of Th1. In this study, the effect of porcupineextract onTh1 levelsin cell culture was examined. **Methods:** The samples, including 20 patients with allergic asthma and 20 healthy. PBMC were isolated of peripheral blood and incubatedwith 400µg/dl of Porcupine extract within cell culture. The Expression levels of T-bet gene were assayed by using Real-time PCR and normalized by control.**Results:** Expression of T-bet gene significantly increased in allergic patients in presence of extracts compared with control group ($p<0.05$). **Conclusion:** The Porcupine extractscauses up-regulation of T-bet gene that results to Th1 differentiation and so can be effective in the treatment of allergic asthma.

Keywords: Allergic asthma, expression, T-bet, Porcupineextract

2051O

High polymorphic human leukocyte antigen genes are associated with susceptibility to chronic rhinosinusitis with nasal polyps and aspirin intolerant asthma

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Background: Human leukocyte antigens class II; *HLA-DR* and *-DQ* have been found as susceptibility loci to asthma in several genome wide association studies. In addition, highly polymorphic *HLA-DR* and *HLA-DQ*are associated with allergic airway diseases and total serum immunoglobin E. A subset of patients with chronic rhinosinusitis and nasal polyps (CRSwNP) show asthma phenotype after ingestion of aspirin regarded as aspirin exacerbated respiratory disease (AERD). Complex interactions between genetic and environmental factors

underlie AERD.Here, we aimed at investigating association of *HLA-DR* and *HLA-DQ* allele and haplotype frequencieswith susceptibility to AERD in patients with CRSwNP.**Methods:**A genetic association analysis in three different groups including 33 CRSwNP patients with aspirin intolerant asthma (AIA), 17 CRSwNP patients without aspirin sensitivity and 100 healthy controlswas performed. *HLA-DRB*, *HLA-DQA1*, and *HLA-DQB1* were genotyped using polymerase chain reaction.**Results:**Minor alleles at following loci were over represented in CRSwNP and AIA comparing to healthy controls: *HLA-DRB1*04* (OR,3.1, 95%CI;1.5-6.4), *HLADRB4* (OR,2.5, 95%CI;1.4-4.5), *HLA-DQA1*0301*(OR,2.9, 95%CI;1.6-5.5), *HLA-DQB1*0302* (OR,5.4 95%CI;2.4-12.5). *DRB1*04/ DQA1*0301/ DQB1*0302* haplotype was higher in CRSwNP and AIA (OR,4.2, 95%CI;1.9-9.2). However, in patients with CRSwNP and aspirin tolerance only *DRB1*15/ DQA1*0103/ DQB1*0601* (OR,3.5 95%CI;1.1-9) was higher comparing to healthy controls. Minor allele at*HLA-DRB1*11* (OR,3.6, 95%CI;1.1-11.1) and *HLA-DQB1*0301* (OR,3.6 95%CI;1.1-11.1) and haplotype of DRB1*11/ DQA1*0501/ DQB1*0301 (OR,6, 95%CI;1.5-23.8) were higher in aspirin non sensitive versus sensitive patients with CRSwNP (all p<0.05).**Conclusions:***HLA-DR* and *HLA-DQ*genes are associated with not only CRSwNP but also to more complicated phenotype of AERD.

Keywords: Human leukocyte, Chronic rhinosinusitis, Nasal polyps, Asthma

2087O

Sublingual immunotherapy and regulatory T cells

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Background: The immunologic mechanisms underlying sublingual immunotherapy (SLIT) are still unclear,but recent evidence suggests a role for regulatory T cells (Treg cells). Our aim wasto evaluate the effect of SLIT on the expression of specific Treg cell markers in house dust mite–allergic patients.**Methods:** Forty poly-sensitized patients with allergic rhinitis, all of them sensitive to mite, aged from 6 to 33 year were enrolled into the study.Twenty one patients in the active group and 19 received placebo via sublingual administration for 6 months. mRNA expression levels of IL-10, TGF-β , FoxP3 and GITR were measured by using real-time PCR before and after of SLIT.**Results:** We controlled allergic rhinitis symptoms in both groups but we could taper drugs only in active group. After 6 months' active SLIT, TGF-β mRNA expression levels were increased compared with pretreatment (P =0.001). TGF-βhad a significant correlation with IL-10 after immunotherapy(p=0.001,r=0.72). There were also a significant correlation between expression of IL-10 (p=0.04,r=0.58) and TGF-β(p=0.014,r=0.68) after immunotherapy with GITR before immunotherapy. In placebo group, there were a significant reduction in GITR (p=0.02) and IL-10(p=0.02) mRNA expression level compared with pretreatment. Also, there was a strong correlation between GITR and FOXP3 after treatment(p<0.0001,r=0.96).**Conclusion:** TGF-β may have an important role in inducing tolerance in the first 6 months of SLIT and activity of Tregs(GITR expression) before immunotherapy has correlate with TGF-β expression after SLIT. Control of disease only with drugs reduces activity of Tregs and IL-10 mRNA expression.

Keywords: sublingual, Immunotherapy, regulatory T cells

3196O
Study of allergenicity properties of recombinant mutant A86Q β-lactoglubolin produced in pichia pastoris

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Background: β-Lactoglobulin (β-Lg) is the most abundant whey protein of the cow's milk. This globular protein of about 18 kDa is folded, forming a β-barrel (or calyx) structure which is a binding site for a wide variety of hydrophobic ligands. β-Lg is also one of the major allergens in bovine milk. Mutation can be an effective method for changing its 3-D structure and allergenisity.**Methods**: A86Q mutant bovine β-Lg was expressed in the yeast pichia pastoris. After purification with Ion exchange and size exclusion chromatography, Binding of IgE from patients with cow's milk allergy has been done to native, recombinant wild-type and A86Q mutant β-Lg using competitive ELISA method.**Results**: The results demonstrated more IC_{50} of fluorescence intensity in competitive ELISA test for recombinant A86Q mutant β-Lg in comparison with native and recombinant wild-type β-Lg.**Conclusion**: The present study showed that this mutation disrupt some epitopes on β-Lg structure and subsequently less allergenicity and immuno-reactivity has been occurred with recombinant A86Q mutant β-Lg.

Keywords: β-Lactoglobulin, allergenicity, mutant, competitive ELISA

Poster Presentations:

3206P
Association of *IL9* gene polymorphisms with asthma in pediatric patients

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Background: Allergic asthma is recognized as a chronic inflammatory disorder of the airways. Interleukin-9, a pleiotropic Th2 type cytokine, seems to play an important role in the pathogenesis of asthma. The 5q31–q33 region is one of the most important loci linked to asthma and atopic disorders that*IL9* gene is located on this region. This study was performed to investigate possible contribution of *IL9* gene polymorphism in predisposition to asthma in pediatric patients. **Methods:** The intronic *rs2069882* polymorphism of *IL9* gene was assessed in 61 Iranian asthmatic children and 63 controls by Real time PCR.**Results:** The frequency of the allele T (g.135229633T>C) was lower in the asthmatic group than in healthy group [p=0.56, OR(95%CI)=0.81(0.44-1.49)], while the frequency of the allele C was higher in the patients

[*p*=0.56, OR(95%CI)=1.24 (0.67-2.30)]. The frequency of the TT genotype was lower in the patients than in the control population [*p*=0.48, OR(95%CI) =0.73(0.34-1.56)], while the frequency the CT genotype was higher in the patients than in the controls [*p*=0.48 , OR(95%CI) =1.30(0.64-2.99)] and the frequency of the CC genotype was higher in the patients than in the controls [*p*=0.63, OR(95%CI)=1.03(0.10-10.70], but none of these differences were significant.**Conclusion:** This study suggested that the *rs2069882* polymorphism within *IL9* was not associated with asthma risk in Iranian population.

Keywords: Asthma, IL9, polymorphism

3102P

Early diagnoses of aspergillosis in patients with asthma by assay of A fumigatus-specific IgE

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Background: Aspergillosis is a large group of fungal infections, that caused by Aspergillus spp. A fumigatus, A flavus, AAspergillus fumigatus is responsible for about 95% of aspergillosis infections in human. . The prevalence of mold allergy has been reported from6-24% in the public population, up to 44% among atopic patients and up to 80% among asthmatic patients. The Aspergillus-associated respiratory disorders may be classified into three clinical categories, allergic brochopulmonary aspergillosis (ABPA), aspergilloma and invasive aspergillosis. Prevalence of ABPA is estimated to be about 1-13% in patient of asthmatic in the world. The aim of this study was isolate aspergillus spp from sputum of asthmatic patients with high level of A fumigates-specific IgE in their serum. **Methods:** Between asthmatic patients that were referred to center of asthma allergy in Tabriz, 26 patients were selected by the clinicians, who had positive skin test to aspergillus antigens and the antibodies of IgG, IgE specific aspergillus were high level in their serum. Sputum specimens of these patients were sent to mycology laboratory for mycological examinations. Positive isolates were identified by slide culture and study of fungi morphology.**Results:**From 26 sputum specimens, 4 samples were posotive for Aspergillus fomigatus, (15.30%), by both methods, culture and direct examination. Also Candida albicanc were isolated in 2 (7.6%) samples.

Keywords: aspergillosis, Aspergillus fumigates, asthmatic patients, Tabriz

3312P

Skin prick test with pollen lipid extracts in allergic rhinitis patients

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Background: Allergic diseases especially allergic rhinitis and asthma are increasing worldwide and represent a major health problem. Plant pollens are the most important source of allergens which triggers allergic reactions. Pollens not only have a very potent allergic proteins and acting as vehicles for foreign protein antigens but also there are different kinds of lipids in their structure that besides their physiological roles for reproduction of plants have some

immunological effects particularly in relation with iNKT cells which recognize lipid antigens and are involved in many immunologic responses such as asthma and allergic diseases. The aim of this study is to evaluate the role of pollen lipid extract in promoting allergic reactions by skin prick test. **Methods:** Mix Aqueous and lipid extract (Folch method) from five different allergic pollen (Poa pratnnse, cynodon dactylon, dactylic glomerata, rumex acetocella and amaranthous retroflexus) were prepared and analyzed by means of Gel electrophoresis and TLC.
skin prick test with both extracts as well as commercial allergenic extract were performed on 45 patients with active allergic rhinitis. **Results:** among 45 allergic patients, 38 cases showed positive skin reaction (>3mm than negative control) to aqueous mix extract and at least one commercial extract. only 8 cases showed skin reaction to lipid extract but just one case had a real positive skin reaction (>3 mm greater than negative controls). **Conclusion:** the result of this study shows that in comparison to allergenic proteins, pollen lipids are not potent triggers of allergic reactions.
Keywords: iNKT, lipid, pollen

2913P

Evaluation of the correlation between childhood Asthma and *Helicobacter pylori* in 5 to 18 year-old children,ShahidBeheshti hospital, Kashan City, central Iran(2011)

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Background: there is an epidemiological correlation between the Asthma prevalence and decreasing HelicobacterPylori (*H. Pylori*), the recent studies indicated. The aim of this study was to survey thecorrelation between *H. Pylori* and Asthma in 5 to 18 year-old children. **Methods:**300 children (5 to 18 year-old) undergoing endoscopy owing to gastro-intestinal problems in ShahidBeheshti Hospital were observed for Childhood Asthma by Gina 2010 questionnaire; in which 24 questions with "yes" and "no" answers were included - identifying patient as Asthmatic with 5 positive answers. In the next stage, the patients were referred to an Allergy & Asthma Specialist for clinical examinations, spirometry, and also post bronchodilator test (Post BD).**Results:**Among the 138 *H. Pylori* positive patients, 8 cases (5.8 %) were asthmatic and 28 cases (17.3 percent) of 162 *H. Pylori* negative patients were asthmatic; statistically significant (P value= 0.002). The correlation between H. Pylori and Asthma was surveyed after controlling the confounding variables, the gender, the age and the familial history. The obtained results for the mentioned variables were significant (P values of 0.004, 0.005 and 0.002, And also the ORMHs of 3.38, 3.24 and 4.06, respectively.**Conclusion:** There is an inverse correlation between *H. Pylori* and Asthma, the obtained results indicated. Performing more studies with larger sample sizes is necessary to confirm these results, clearly.
Keywords:*H. Pylori*, Asthma, Childhood Asthma

2834P

Association analysis of TIM1 -416G>C polymorphism with asthma in Chaharmahal va Bakhtiari, Iran

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Background:TIM1, One of the genefamily members ofT cell immunoglobulin (Ig) domain and mucin domain (TIM) expressing on TH2 cells, promotes producing of Th2 signiture cytokines andincreases aseriesof responsesin this cells which could be one of the causes of asthma.We are determined to investigate whether a TIM-1 promoter single nucleotide polymorphism (SNP), -416G>C, associated with asthma inChaharmahal va Bakhtiari, Iran.**Methods:**Existence of the -416G>C polymorphism was examined using polymerase chain reaction (PCR) and restriction fragment length polymorphism in 130 asthmatic patients.**Results:** The results clearly showed that the -416G>C SNP is significantly associated with asthma susceptibility in this population (p<0.05). **Conclusion:**Our results indicate that -416G>C polymorphism in TIM-1 gene, could be a predisposing factor for asthma susceptibility in Chaharmahal va Bakhtiari.

Keywords:T-cell immunoglobulin and mucin domain molecule-1, asthma, polymorphism

2836P

Association analysis of TIM-1 5383_5397 insertion/deletion polymorphism with asthma and total serum Immunoglobulin E levels Chaharmahal va Bakhtiari, Iran

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Background:A member ofa T cell immunoglobulin (Ig) domain and mucin domain (TIM) genefamily,TIM-1, located in5q31-33 region is shown to be correlated with development ofT helper-2 (TH2) Cellsandallergic diseases. polymorphism insertion/deletion (ins/del) 5383_5397canbechangedlength of TIM-1 mucin domain .The aim ofthis study wasto investigatethe association betweenpolymorphism insertion/deletion (ins/del) 5383_5397 and susceptibility to asthma in Chaharmahal va Bakhtiari,Iran.**Methods:**Polymerase chain reaction (PCR) - polyacrylamide gel electrophoresis (PAGE) was used for investigating thepresence of (ins/del) 5383_5397 polymorphism in 130 asthmatic patientsand healthy controls. Total IgE was measuredbyELISAtechnique in their serum samples as well.**Results:** Significant association betweeninsertion / deletion (ins / del) 5383_5397 polymorphism was found neitherwithasthma nor with levels of total serum IgE in this population.**Conclusion:** We identified no significant association between 5383_5397 ins/del polymorphism with asthma and with total serum IgE levels Chaharmahal va Bakhtiari.

Keywords: T-cell immunoglobulin and mucin domain molecule-1, asthma, polymorphism

2947P

Asthma Economic Costs in Adult Asthmatic Patients in Tehran, Iran

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Background: High prevalence and increasing rate of asthmatic patients around the word emphasis on the high burden of asthma to health care systems of countries and families but we have little data on asthma burden and economic costs in Iran, This study aimed to fill this gap, for the pilot step, we choose one of the referral tertiary centers of diagnosis and treatment of asthma in adult population to calculate direct and indirect economic costs of asthma and their association with some background factors such as concomitant diseases, cigarette smoking and asthma control situation.**Methods:** In this cross sectional study, we surveyed economic costs of 197 adult asthmatic patients. First, we record patients' demographic data, concomitant diseases, asthma control situation and direct and indirect economic costs of asthma. The patients were followed for a period of one-year ±1 month and asthma related costs and control status were registered.**Results:** Total cost of asthma was 591.68 ±483.16 USD, direct costs calculated 371.28 USD and indirect costs 218.92 USD for one patient per one year.**Conclusion:** asthma affects a high economic burden to families and health system of Iran. We suggest that improving asthma management regimens and accessibility to specialized treatment centers result in reducing costs of pharmacy, absence from work and transportation that are the major asthma related costs

Keywords: Asthma, Direct cost, Indirect cost

3273P

Evaluation of allergy and eosinophilia level in peripheral blood of patients with cardiovascular disease in Ilam

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Background: The cardiovascular diseases are one the most common causes of the deaths occurred in developing countries and also they can cause one third of the all deaths in Iran and other countries throughout the world. The risk factors for the cardiovascular diseases are divided into two categories; the variable risk factors and the non-variable risk factors. The variables are the high blood pressure, hyperlipidemia, diabetes mellitus, smoking, sedentary life, obesity, stress etc. while the non-variables include the males, elders, black races and the genetics. Recently many have been performed to assess the relationship between the higher eosinophilia and allergy levels with the incidence, progress and severity of the cardiovascular diseases but the exact correlation between these two still remains to be understood. The current study was designed to measure the levels of allergy and the eosinophilia amongst the patients with cardiovascular diseases in Ilam.**Methods:** This case control study was carried

out employing 59 cardiovascular patients randomly selected among those admitted to the Mostafa Khomeini Hospital of Ilam University of Medical Sciences at 2010 as the case and 55 healthy individuals without any history of allergy and parasitic infections as the control group. A questionnaire including some questions such as age, sex, smoking status,etc. was completed by each participants after a written consent was taken. 7 ml blood was taken from each participant for the CBC measurements and sera were extracted from the rest for IgE using ELISA. Data were analyzed using Epi-Info program in statically software.**Results:** There was a significant relationship for the variables such as the family history of cardiovascular disease ($P<0.001$), diabetes ($P<0.003$), hyperlipidemia ($P<0.0001$), high blood pressure ($P<0.0001$) and physical activity ($P<0.0001$) between the case and the control groups. The mean IgE titer in case group was 95.3 ± 71 and 62.44 ± 49 in control group. The mean eosinophila level in peripheral blood was 3.95 ± 1.057 in case and 1.53 ± 0.57 in control group. The differences between the IgE and eosinophilia levels in the case and the control groups was statistically significant ($P<0.0001$). **Conclusion:** It can be concluded that the higher amounts of IgE and the eosinophilia can be considered in incidence, severity and the progress of different types of the cardiovascular diseases so that the cardiologists can rely on the role of these variables in diagnosis of cardiovasculardiseases.

Keywords: Allergy, eosinophilia, cardiovascular disease, antibody

2670P

Assessment of allergen-specific IgE by immunoblotting method in atopic dermatitis (AD) patients in northwest of Iran

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Background: Stimulating the immune system by Exposure to various allergens to produce specific IgE has a significant role in the pathogenesis of atopic dermatitis. Identifying disease-causing allergens and prevention of exposure to those allergens and immunotherapy will play important role in the treatment of atopic disease (AD). Purpose of this study was to determine the common allergens of northwest of Iran in patients with atopic dermatitis that are resistant to treatment.**Methods:** In this descriptive-analytical study, serum levels of total IgE and frequency of specific IgE were measured by Immunoblot against 20 common allergens in 150 cases of patients with atopic dermatitis attending to dermatology and asthma and allergy clinics from 2010 to 2011. Control group consisted of individuals who had been diagnosed healthy. Mean of the patient's age was 29.02 ± 14.79 years old. In this study, 77 patients (51.3%) were male and 73 patients (48.7%) were female.**Results:** In the ninety percent of patients that were included in this study, total IgE levels were higher than healthy people with mean serum levels of total IgE 227.51 ± 103 IU / ml. In this study, 136 patients (90.6%) had specific IgE for at least one allergen. Most abundant allergens respectively related to: cultivated rye (48.6%), Timothygrass (42.6%), house dust mites (22.7%) dog (16.7%), dog (16.7%), birch (11.3%), Potato (11.3%), horse (10%), hazelnut (10%), cat (10%), soybean (10%), sagebrush (10%). Apple, Carrot, Fish, Wheat, Rice, Cladosporium herbarum, Cow milk, egg white, Alternaria alternate had little frequency. The frequency of positive allergens among the patients who were included in this study was 53.34%, 26.8% and 19.56% respectively in plants and fungus

allergens group, animal allergens group and food allergens group.60% of patients after avoiding of the allergens exposure which they had been sensitized to them, and in some cases immune therapy, were cured. In the control group total IgE serum levels was not increased. **Conclusion:** Identifying the abundant allergens such as Cultivated rye, Timothy grass, House dust mite, birch ,Cat , Horse, Potato ,Dog , Egg white , Cow milk in order to advise patients to avoid them or do immunotherapy and desensitization is useful in this area.
Keywords: Atopic dermatitis, Allergen, IgE

2341P

Unique pattern of sensitivity to *Blomia tropicalis* in malaysian mite-induced allergic rhinitis patients

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Background: Among different allergenic agents, mites are one of the most common ones, especially in tropical climates. We aimedat finding the potential difference between the most common allergenic mite species regarding the results of the routinely used diagnostic methods for Allergic Rhinitis. We also tried to evaluate the effect of topical and systemic medications on the results of these diagnostic methods. **Methods:** Using a detailed questionnaire, 138 mite-induced allergic rhinitis patients (allergic to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, or *Blomiatropicalis* and discontinued one week from drug usage) were scored and categorized for severity of disease based on intermittent or persistent features, type of medication (topical vs. systemic), frequency of use and effectiveness. Results were statistically correlated with average wheal size of skin prick test and serum specific IgE levels (sIgE).**Results:** The average wheal size for *Blomia tropicalis* was larger compared to *Dermatophagoides* species ($p<0.05$) but was inversely correlated with severity of disease ($p<0.01$). The latter may be due to a higher consumption of systemic and topical antihistamines in patients with more severe disease ($p=0.001$). Similarly, the average wheal size for *Blomia tropicalis* was larger in patients with intermittent symptoms compared to permanent ($p=0.005$). The levels of sIgE were not affected by medication usage and may be the factor for wheal size after discontinuation of medication for a week.**Conclusion:** Compared to *Dermatophagoides* species, the pattern of sensitivity to *Blomia tropicalis* to skin prick test was significantly different in allergic rhinitis patients. This emphasizes the importance of using this allergen in the panel of diagnostic tests for allergy. Additionally, further placebo controlled studies are necessary to evaluate whether the routinely employed one week of medication-free phase is adequate before administration of skin prick test in mite-induced Allergic Rhinitis patients.
Keywords: Allergic Rhinitis, Mites, Prick Test, IgE, Antihistamines

2711P

CXC- type chemokines in pediatric food allergic patients

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Background: However food allergies (FA) are frequentin approximately 0.5-3.8% of children under 3 years, 0.1-1% of adults also experience FA. Chemokines play fundament of part (s) in various allergies, including FA. In the present study, we asked whether if CXCL1, CXCL9, CXCL10 and CXCL12 serum levels were altered in FA. We also sought to examine of these chemokines concerning type of allergen used by FA patients.**Methods:**In the present investigations, we enrolled 83FA patients along with 1 0 0 healthy controls. The serum concentrations of CXCL1, CXCL9, CXCL10 and CXCL12 measured by enzyme-linked immunosorbent assay(ELISA). Statistical analysis of data was performed by SPSS software package version 18. Differences were considered significant if $P<0.05$.**Results:**Result of this study demonstrated that CXCL1, CXCL9, CXCL10 and CXCL12 concentrations were elevated in patients sufferings from FA in compare to controls. We also showed that both type and source of allergens are important in chemokine production. Our findings showed that food elimination caused decreased levels of CXCL9 and CXCL10 while CXCL1 and CXCL12 concentrations were not altered following food elimination. **Conclusion:**With regard to these results, chemokines could probably be involved in pathogenesis of FA as useful biological marker. It is also possible to expect the severity of FA according to the serum concentrations CXCchemokines.

Keywords: Food allergy, CXC chemokine, CXCL1, CXCL9, CXCL10

2607P

Investigation of pistachio allergens

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Background: IgE-mediated allergic reactions in pistachio appear to be occurring more frequently; however, little is known about its allergenic proteins. Tree nut allergy is a common disorder with a prevalence rate of about 1% in westernized countries. Pistachio (*Pistacia vera*) is a tree nut that has been reported to cause IgE-mediated allergic reactions. In present investigation we aimed to identify the pistachio allergens. **Methods:** Pistachio proteins were extracted and separated by SDS-PAGE. We studied clinical allergenic of pistachio in some Iranian hospitals. **Results:** Pistachio allergenic is caused by pollens in the pollen season, ripe fruits at harvest time and also by eating pistachios. Some of pistachio allergens have been isolated and many of them are well-known such as: Pis v 1, Pis v 2, Pis v 3 and Pis v4, also researchers believe that LTP allergen is present in pistachios. **Conclusion:** Understanding the characteristics of pistachio and other nuts could help the possible interaction between nut trees and this is the first step toward better diagnosis and treatment of allergic individuals. Determining the allergen amounts in different varieties of pistachio and nuts could help the

biotechnologists and horticultural breeders for releasing cultivars with less allergens.
Keywords: Allergy, Pistachio, Pis v 1, Tree nut

2493P

The +4259 G>T of TIM-3 polymorphism are associated with asthma susceptibility in the Isfahan population

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Background: Asthma is a common chronic inflammatory disease of the airwaysthat caused by a combination of genetic and environmental factors.The family of T-cell immunoglobulin domain and mucin domain (TIM) proteins is identified to be expressed on T cells. A member of the TIM family, TIM-3 is selectively expressed on the surface of differentiated T helper 1 (Th1) cellsand regulates TH1 proliferation. There are now a large number of genetic studies that have investigated the possible association of various TIM3 polymorphisms with different diseases including asthma.The aims of this study were to examine whether genetic variation in the exon region of TIM3 influenced risk for asthma. **Methods:** Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were used to determine polymorphism of tim 3, in all subjects. PCR products were chosen at random for sequencing. **Results:**The genotype and allele frequency of +4259G>T polymorphism were significantly different between asthma patient and control individuals. **Conclusion:**Our results support an association between the +4259G>T polymorphism of the TIM-3 gene and asthma. These data suggest that polymorphism in the TIM3exon region play an important role in susceptibility to asthma.
Keywords: Asthma,Tim3, Polymorphism

2652P

Investigation of interaction between aspartame, an artificial sweetener, and DNA

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Background: Aspartame (APM) is an artificial sweetener. APM is found in many products, including soft drinks, chewing gum, candy, yoghurt, and some pharmaceuticals such as vitamins and sugar-free cough drops.The dangers of aspartame poisoning have been a well guarded secret since the 1980's. The research and history of aspartame is conclusive as a cause of illness and toxic reactions in the human body.Analysis of more than 500 reports

showed the problems associated with aspartame were divided into two categories. Two-thirds of the people experienced neurological problems while one-quarter of the people experienced gastrointestinal problems. The most common aspartameallergy symptoms are headaches, dizziness, nausea, and vomiting. Researchers have been reported that a small proportion of the APM (10-12% of the intake) might be absorbed without metabolization. Accordingly,we searched about the whole APM molecule. In this study, we investigated the interaction of mentioned molecule with DNA.**Methods:** Using several methods including: Viscosity (The DNA concentration was fixed while varying the APM concentration, and flow time was measured with a digital stopwatch,circular dichroism(CD) CD spectra of DNA in the presence and absence of APM were obtained at 25˚C using 1 cm path length quartz cuvette)and UV absorption techniques (by keeping the concentration of APM constant while varying the DNA concentration).**Results:** Hypochromism in the UV absorption spectra were observed upon addition of DNA increasing concentrations to the APM.The intrinsic binding constant (Kb) value was calculated to be 5×10^{4} M^{-1}. CD spectrum of DNA with APM shows little perturbation of the two bands. No changes were observed in viscosity of the ct-DNA upon addition of APM.**Conclusion:** According to results of Viscosity, CD and UV absorptionmeasurements,we suggest that APM interacts with calf thymus DNA via groove binding mode. One may assume that, the allergy symptoms of aspartame could be due to its interaction with DNA molecule.

Keywords: ct-DNA interaction; Artificial Sweeteners; Aspartame (APM); Groove binding

2501P

Skin prick test for determination of sensitivity to food and respiratory allergens in patients with symptoms of chronic urticaria referred to afzalipoor hospital clinic

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Background: Urticaria is an allergic reaction that leads to release histamine and other inflammatory mediator of mast cells and basophils. The clinical signs of urticarial is manifested-by wheals with central pallor, erythema and itch for <24 hours. Two forms of disease, acute, less than 6 months, and chronic, more than 6 weeks, have been described. The aim of the present study was to investigate the sensitivity to food and respiratory allergensby skin prick test in patients with symptoms of chronic urticaria have to be taken a step toward proper treatment for these patients.**Methods:**In a retrospectively study, the history of 20 patient's forms with the results of of skin prick test was reviewed. All histories of disease were recorded for four years using estallergea prick test allergens. Swelling at least 3 mm and erythema at least 10 mm were reported as positive test and for the positive control used of histamine and saline were used as negative controls. Percentage susceptibility to respiratory allergens and food allergens and both were analyzed by the software SPSS 20.**Results and Conclusion:**Twenty patients with chronic urticaria symptoms were referred to the Afzalipoor Allergy Clinic for 4 years 1386-1390. The prick test was done for respiratory and food allergens. The results shown that the percentage of susceptibility of individuals toward to respiratory and food allergens.45% are allergic to respiratory allergens and 5% are allergic to food allergens and 5 percent are allergic to both types of allergens.

Key word: Skinprick test, Sensitivity to food, Chronic urticaria

2364P

Expression of IL-1β and caspase-1 activity can be involved through IPAF inflammasome in experimental model of allergy

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Background: Recent studies indicate IL-1β plays an important role in development of airway hypersensitivity responses. So, this study was designed to investigate IL-1β attending and it's upstream pathway in mouse model of allergy. **Methods:** Female 6-week-old BALB/c mice were sensitized by intraperitoneal (i.p.) injection of a recombinant allergen adsorbed to alum gel suspension on days 0, 7, 14 and 21. Some mice received PBS in alum by the i.p. route at the same time points as negative control. The allergic status was confirmed serologically by testing for specific IgE. At the end of experiment, mice were euthanized and blood was obtained by cardiac puncture and lungs were recovered. Total RNA was extracted and expression of IL-1β was evaluated in mRNA and protein level by semi-quantitative RT-PCR and ELISA, respectively. In addition, caspase-1 activity was measured by flurometric assay. **Results:** Sensitized mice developed significant ($p<0.05$) high levels of specific IgE in sera. In contrast, no specific IgE production could be observed in control mice receiving PBS in alum gel suspension. IL-1β production in mRNA and protein level was significantly higher than control group as well as caspase-1 activity. In addition, expression of genes involved in inflammasome pathway such as NALP3, ASC and IPAF showed that only IPAF expression is increased in sensitized mice compared with control mice ($p<0.05$). **Conclusion:** Obtained results indicate that caspase-1 activation has a rising effects on IL-1β level and IPAF inflammasome pathway may be involved in IL-1β production in allergy.

Keywords: Allergy, IL-1β, Caspase-1, Inflammasome

2494P

Characterization of phosphodiesterases 7 and 8 in human basophiles

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Background: Cyclic nucleotide phosphodiesterases (PDEs) comprise 11 different families of enzyme that metabolize the second messengers adenosine 3', 5'-cyclic monophosphate (cAMP) and guanosine 3', 5'-cyclic monophosphate (cGMP) to their respective inactive 5'-monophosphate. Of these families, the cAMP-specific PDE, PDE4, is the most important PDE regulating inflammatory cell activity, although alternative cAMP hydrolyzing PDEs, such as PDE7 and PDE8, may have subsidiary roles. The aim of present study was to try to characterise cAMP-specific PDE7 and 8 in human basophiles. **Methods:** Basophiles were purified (95-100% purity) by immunomagnetic bead separations. Mononuclear cells (MNC) were also isolated and used as comparative controls. Western blots were performed on purified preparations of basophiles (≥95%) using antibodies for PDE7 and PDE 8 with recombinant proteins used as positive controls in the blots. **Results:** Immunoblotting experiments using PDE7A–specific antibody indicated that basophiles does not express PDE7A and PDE8 at the protein level. In these same immunoblotting experiments, PDE7A was detected in Hut78

cells, a T cell line. The recombinant protein, PDE7A2, which was used as a positive control in these experiments, has a slightly lower molecular weight than the splice variant, PDE7A1. **Conclusion:** We conclude that basophiles do not express.These experiments indicate that PDE7 or PDE8 are not the PDE expressed by basophiles.

Keywords: Phosphodiesterases7, Phosphodiesterases 8, Basophiles

2280P

How the immune and stress factors response to severe exercise in pediatrics?

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Background: Evidence indicates that increase of stress factors due immobility, immune suppressionand cause to impairment learning and performance levels in pediatrics. The aim of this research was investigation response of the immune and stress factors of pediatrics to Severe exercise.**Methods:** Eleven healthy non-active pediatrics boys with the age range of 8-10 years old volunteered to participate in the research after having expressed their consent through a consent form. Blood and salivary sample were collected in the three stages; pre, immediately after, and 1h after Graded exercise test (grade: 5%, speed: 12 km/h, time: 20 minutes). ELISA method was used for measurement of salivary immunoglobulin A, cortisol serum levels.Also,one-way ANOVA and Pearson correlation coefficientmethods used for statistically analysis.**Results:**Cortisol serum and S-IgA concentrationswere significant increased immediately after of exercisecompared with baseline conditions($p< 0.05$).The levels ofS-IgA and cortisol serum, 1 h after the sever exercise compared to baseline conditions showed a significant increase($p< 0.05$).Immediately after of sever exercise,a Significantpositive correlation observed betweencortisol serum and S-IgA ($p=0.05$).**Conclusion:**This study indicates that sever exercise significantly increasecortisol and S-IgA levels in non-active pediatrics, so that may lead to impairment in the immune system. These physical activities do not recommend for Pediatrics.

Keywords: Stress factors,Sever exercise,Immune system

2234P

YKL-40 in asthma and its correlation with different clinical parameters

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Background: It has been suggested that elevated serum level of YKL-40 could be a marker of asthma and its severity. Along with few published studies, we investigated its correlation with asthma and its severity as well as spirometric indices. **Methods:** 114 patients with asthma and 114 healthy controls underwent the assessment of serum level of YKL-40 (by ELISA) and spirometric indices. Pearson's coefficient determined the correlation between the variables and multivariate linear regression analysis was used for adjusting the effect of different probable

confounding factors.**Results:** Serum level of YKL-40 was significantly higher in the asthmatic patients compared to that in healthy people (P<0.001). We also found a significant correlation between YKL-40 serum level and spirometric indices even after adjusting the effects of other variables.**Conclusion:**We report for the first time in an Iranian population that YKL-40 may be a good diagnostic marker of asthma.

Keywords: Asthma, YKL-40

2285P

The effect of training and breathing exercises on anxiety sensitivity, negative emotion, alexithymic and quality of life of patients with asthma

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Background: By Improving People's Physical, Mental And Social Conditions, Exercise Intervention Can Play A Crucial Role In Improvement Of Quality Of Life And Mental Disorder Of Different People. The Aim Of This Research Is To Study The Effect Of A Course Of Training And Breathing Exercises On Anxiety Sensitivity, Negative Emotion, Alexithymic And Quality Of Life Of Patients With Asthma.**Methods:** This Study Has Been Conducted Using Pretest, Posttest And Follow-Up Test On A Group Control. Our Sample Included 38 Patients With Asthma Which Was Chosen Using Available Sampling And Was Randomly Dividend To An Empirical Group Of 18 And A Control Group Of 20 Subjects. Gathering Data, We Used Standard Questionnaires Of Quality Of Life, Alexithymic Of Toronto, Positive And Negative Affect Scale And Anxiety Sensitivity Index Which Were Filled Out By Subjects In Pretest, Posttest And Follow-Up Test. Training And Breathing Exercises Were Applied Under The Supervision Of Sport Specialist And Physician For Four Weeks, Three Times A Week, Each Session Being 45 Minutes. Descriptive Statistics (Mean And Standard Deviation), Inferential Statistics (Paired T-Tests And Wilcoxon)And Spss Software Were Used To Analyze The Data.**Results:** The Mean And Standard Deviation Of Research Components Were Increased Significantly Before And After The Training And Breathing Exercises.**Conclusion:** Regular Training And Breathing Exercises Have Positive Effect On Different Aspects Of The Quality Of Life Of Patients With Asthma And It Is Also Effective In Reduction Of Mental Disorders Such As Anxiety, Negative Emotion And Alexithymic. Thus It Is Recommended That Patients With Asthma Exercise Under Medical Supervision.

Keywords: Quality of Life, Alexithymic, Anxiety Sensitivity, Negative Emotion, Asthma

2334P

The SDF-1α 3`A Genetic Variation Is Correlated with Susceptibility of Asthma in Iranian Patients

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Background: Chemokine/receptor axis is a predominant actor of clinical disorders. They are key factors of pathogenesis of almost all clinical situations including asthma. Correspondingly, CXCL12 is involved in the immune responses.Therefore, this study was designed to explore the association between gene polymorphism at position +801 of CXCL12, known as SDF-1α 3`A, and susceptibility to asthma in Iranian patients. **Methods:** In this experimental study, samples were taken from 162 asthma patients and 189 healthy controls on EDTA.DNA was extracted and analyzed for CXCL12 polymorphisms using PCR-RLFP. The demographic information was also collected in parallel with the experimental part of the study by a questionnaire which was designed specifically for this study. **Results:** Our results indicated a significant difference ($P< 0.0001$) between the A/A, A/G, and G/G genotypes and A and G alleles of polymorphisms at position +801 of CXCL12. We also showed an elevated level of CXCL12 circulating level in Iranian asthma patients. **Conclusion:** Our findings suggest that SDF-1α 3`A (CXCL12) polymorphism plays a role in pathogenesis of asthma. It can also be concluded that circulatory level of CXCL12 presumably can be used as one of the pivotal biological markers in diagnosis of asthma.

Keywords: Asthma, Chemokine, CXCL 12, SDF-1α

2614P

An efficient method for purification of non-specific lipid transfer protein from rice seeds

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Background: The small basic lipid transfer proteins (91-95 amino acids, 9 kDa, PI 8.5) are ubiquitously expressed in the plants which are known for their capability of binding and transferring different types of lipids between biological members. LTPs have been recently introduced as major allergens in food allergy and are clinically important target molecules for allergy diagnosis and therapeutic intervention. To use the nsLTP for the use of recombinant nsLTP in allergy diagnosis and preclinical allergy studies, the preparation of high pure nsLTPs in biologically active form is required. Because production of nsLTPs using recombination, RP-HPLC or subsequent chromatographic steps is onerous, we have developed a relatively simple and efficient method for the purification of rice nsLTP.**Methods:** Proteins of rice seeds extract was precipitated by 40-80% ammonium sulfate. The precipitated proteins was heated up to 90°C for 30 min. After centrifuge, the clear supernatant was applied on a pre-equilibrated DEAE-Sepharose column (pH 8.5). The high pure LTP was obtained in column washing step. The purity of protein was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Also, tyrosine fluorescence and ligand binding capacity of LTP were determined by spectrofluorimetry.**Results:** SDS-PAGE analysis showed over 99% purity. Since LTP lacks tryptophan, its fluorescence maximum intensity was observed at wavelength 305 nm. Also, high ANS binding capacity of LTP was comparable with previous studies.**Conclusion:** The obtained LTP is high pure and biologically active. Hence, this simple and efficient method can be used to purification of nsLTPs.

Keywords: non-Specific Lipid Transfer Protein, Food Allergy, Chromatography

2628P

Preliminary structural analysis of rice-nsLTP2 for the investigation of the antigenic properties

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Background: Lipid transfer proteins, also known as LTPs, are a group of highly-conserved proteins. Due to the resistance to temperatures and protease digestion, LTPs are the most important food allergens- especially in the Mediterranean areas. However, the stable structure of nsLTPs can prevent the oxidation and degradation of drugs and are potential as drug carriers. In this study, antigenic properties of rice-nsLTP2 were predicted using web-based servers. Moreover, we discussed about the effects of mutagenesis on the antigenic properties and lipid transfer activity of this protein. **Methods:** The evaluation of antigenic properties of rice ns-LTP2 was carried out using Madrid University database (http://imed.med.ucm.es/Tools/antigenic.pl). Studies using another server (http://www.imtech.res.in/raghava/propred) represent as more specific amino acids involved in the interaction between MHC-I T cells and the antigenic peptides.**Results and Conclusion:** Bioinformatics study of LTP showed four antigenic determinants in it sequences and the largest part exists in the sequence of signal peptide. Moreover, the average antigenic propensity for this protein is 1.0794. A more detailed examination of antigenic properties of LTP with the second server revealed that the key amino acids involved in the interaction between T cells and the peptide are including 3 sequences from 6-15, 20-28 and 54-62 residues. Our results revealed that the efficacy of nsLTP2, as a drug delivery system, could be improved by appropriate manipulation at specific amino acid sequence and omission of the allergenic sites.

Keywords: Rice-nsLTP2, Bioinformatics, allergen, drug carrier

2751P

An investigation of the prevalence of latex allergy among the personnel of Gorgan's medical educational centers

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Background: Latex allergy is the major problem among healthcare personnel who use gloves regularly. Latex gloves are the cause of 90 % of all latex allergy cases. Latex allergy is three times more prevalent in healthcare personnel than in general population. With regard to its high prevalence and relationship with diseases such as occupational asthma, it is essential to determine the prevalence of latex allergy among healthcare personnel. Therefore, this research aims at measuring the prevalence of latex allergy among the personnel of Gorgan's medical educational centers.**Methods:** In this descriptive cross-sectional study, 130 personnel of Gorgan's medical educational centers were included with informed consent using the census sampling method. The project was implemented using a standard questionnaire.The data were collected using a questionnaire and analyzed using SPSS 16, Pearson's correlation coefficient test, chi-square test and t-test ($p<0.05$ was considered significant). **Results:** The participants consisted of 130 individuals, the majority of whomwere female (78.5%). The participants'

average age was 32.8±8.57. Hand dermatitis, food allergy, reactive airways disease, and latex allergy were observed in 33.1%, 76.9%, 10%, and 20% of the cases, respectively. A significant statistical relationship was observed between the prevalence of latex allergy and the variable of family history of the disease (p=0.019).**Conclusion:** Given the high prevalence of latex in this study, all employees with suspected latex allergy are recommended to be examined and provided with hypo-allergenic and latex-free gloves if they are definitely diagnosed.

Keywords: Latex, Allergy, Prevalence

2772P

Saffron- induced life threatening angioedema, a case report

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Background: Saffron (Zaaferan) is the most expensive spice in the world.it contains a profilin that may cause allergic reactions in atopic subjects. We describe a potentially fatal adverse event after saffron ingestion. **Methods:** The case was 45 years old female admitted to our ED as she complained of hoarseness and dyspnea due to recent saffron eating accidentally added to her food. As she had a progressive angioedema leading to apnea, Rapid sequence intubation (RSI) and supportive ventilation was performed. The electrocardiogram (EKG) was negative for any myocardial abnormality. Laboratory tests were normal. The symptomatology was completely relieved some hours later, as rapid conservative treatment with Epinephrine (0.1ml of 1:000 in 10ml NS, IV), Ranitidine (50 mg IV), and chlorpheniramine (50 mg IM) and supplemental oxygen and ventilation. We found that she had a previous history of skin reaction to saffron from 35 years ago. **Results:** We searched PubMed and EMBASE (through January 2014) for previous reports of saffron-related angioedema .However; there are no reports of this potentially fatal adverse event after saffron ingestion. To our knowledge this is the first case reported of saffron life-threatening anaphylaxis in humans. **Conclusion:** Aserious life-threatening angioedema can occur after saffron eating and Clinicians should be aware of this possibility.

Keywords: Saffron, Adverse effect, Angioedema

1882P

Polymorphismin TIM-3 are not associated with asthma in the Isfahan population.

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Background:Asthma is a complex disease, caused by a combination of geneticand environmental factors. T cell immunoglobulin domain and mucin domain (Tim) genes are located in chromosome 5q31-33, a region repeatedly linked to asthma or asthma-related phenotypes in several populations.T-cell immunoglobulin mucin-3 (TIM3) is a TH1-specific surface protein that regulates TH1 proliferation. TIM3 and its genetic variants have been

suggested to play a role in regulating allergic diseases andasthma.Polymorphisms in the TIM3 promoter region have been reported to be associated with allergic phenotypes in several populations. The aims of this study were to examine whether genetic variation in the promoter region of TIM3 influenced risk for asthma.**Methods**:In order to evaluate the effects of the promoter polymorphism in the TIM-3 gene on asthma susceptibility in a Isfahan population, promoter polymorphism, -574G>, were genotyped in 130 unrelated asthma patients and 80 healthy controls by using ARMS-PCR.**RESULTS:**The genotype and allele frequency of -574 G>T polymorphism were not significantly different between asthmapatient and control individuals.**Conclusions**:Our result indicate the polymorphismof -574 G>T in the TIM3 promoter region is not associated with asthma in Isfahan asthma patient. These data suggest that polymorphism in the TIM3 promoter region are unlikely to play an important role in susceptibility to asthma.

Keywords: Asthma, SNP, TIM-3

1640P

Effect of cocoa on IL-5 and IgE synthesis in mouse model of allergic asthma

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Background: Allergic asthma is an inflammatory disease of the lung characterized by the presence of large numbers of CD4+ T cells. These cells produce the Th2 and activated Th2 cells produce cytokines such as interleukin (IL)-4, IL-5, IL-10, and IL-13 which are important in switching antibody production from B cells to predominantly IgE production against the allergen, as well as stimulating mast cells and eosinophils. Cocoa is a rich source of polyphenols, particularly flavonoids such as epicatechin andcatechin as well as procyanidins, the polymers derived from these monomers. Chemically, they have a polyphenolic structure showing antioxidant activities. In addition to its antioxidant properties, cocoa has immunoregulatory effects on innate and acquired immunity.**Methods**: Orally administration of cocoa was examined in an Ovalbumin (OVA) induced airway inflammation model. Th2 mediated parameters include IL-5 and OVA specific IgE were evaluated.**Results:** Results show that orally administration of cocoa reduced the production of Th2 IL-5 and OVA specific IgE, significantly.**Conclusion:** These results indicate that cocoa efficiently diminishes activation of Th2 cells an IgE production in mouse model of allergic asthma and suggesting a potential role for cocoa flavonoids in the prevention or treatment of allergic diseases.

Keywords: Cocoa, Allergy, Ovalbumin, IL-5, Mice.

1891P

Asthma: change of eosinophiles, platelets count, as an inflammatory marker?

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Background: Asthma is the most common chronic airway inflammatory disease. Airway inflammation asthmatic is due to recruitment activated inflammatory cells.This study investigated the relationship between the count of peripheral blood eosinophils, platelets and

markers of disease activity in asthmatic patients. **Methods:** This study were performed on 37 asthma patients, aged 7 - 81 years and female/male: 20/17 (54.1% and 45.9%, respectively). For all patients did spirometry test, CBC and blood smear for counting eosinophils and platelets. Based on medical history, physical examination and spirometry test, severity activity disease was defined as a score between 1-5. (Intermittent MILD = 1, persistent MILD = 2, MODERATE = 3, SEVER = 4, MOST SEVER = 5)**. Results:** Based on analysis was reporetd that there are the positively correlation between eosinophil count and severity of asthma ($r = 0.121$, $p < 0.004$), whereas an inverse relationship between platelet count and severity of disease ($r = -0.132$, $p < 0.001$). Eosinophil count was also an inverse relationship with lung respiratory function for example (FEV1%), ($r = -0.104$, $p < 0.0001$) and there is a negative correlation between platelet count and FEV%, ($r = -0.071$, $p < 0.0001$). Also, there are a significantly inverse relationship between age and patients FEV1%, ($r = -0.328$, $p < 0.0001$) , a direct correlation between age and score activity disease, ($r = 0.286$, $p < 0.0001$) and indirect relationship between WBC and severity of disease, ($r = -0.076$, $p < 0.0001$). **Conclusion:**The result of study suggest that the peripheral eosinophil count reflects asthmatic activity, and possibly the degree of inflammation in the airways and it can be used as clinically diagnostic marker for asthma severity, whereas platelet count can not be used.

Keywords: asthma, eosinophils, platelets, FEV1

1453P

Hair comb allergenesity used for the scalp skin

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Background: Dandruff (Pityriasis capitis) is a non-inflammatory form of seborrheic dermatitis. People reporting dandruff often has seborrhoeic dermatitis, but can also have other diseases. **Methods:** In this study, relation between type of comb and allergic inflammatory response and dandruff appears in skin and hairs were studied.**Result:** Results showed that plastic comb was allergen and this was high in men. **Conclusion:** The most common treatment for dandruff is the use of shampoo formulations and avoid of allergen with suing of wooden comb.

Keywords: Dandruff**,** seborrheic dermatitis, Allergen, electrostatic, comb

2055P

Role of tonsils in immunological activity

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Backgrounds: Tonsils are part of waldeyers ring; many studies indicate that inflammation and hypertrophy of tonsils are caused by hypofunction of local or systemic immunity. Tonsillectomy is the most common operation in children but the exact reasons of tonsillar hypertrophy remains unknown. Some researches have shown that allergy may be at risk factor for tonsillar hypertrophy and the importance of allergy of the upper respiratory tract is however

one of the most controversial problems in allergology. The goal of this study is to investigate the rate of tonsillar obstruction in hypertrophy of tonsils.**Methods:**A total of 125 children with upper airway obstructive symptoms were evaluated for their tonsil hypertrophy. For tonsillar size, several grading systems have been adopted in previous studies. Among them, the most well-known and accepted grading scale protocol of severity of palatine tonsillar obstruction was proposed by L. Brodsky.**Results:**In this study 55 girls and 70 male were participated with a mean age of 7.5 ± 2.3 years. Physical oropharyngeal examination showed 4.9% of patients suffered from grade 1, 20.6% grade 2, 39.9% grade 3 and 34.5% grade 4 tonsillar enlargement. **Conclusions:**Tonsils are inductive sites for humoral and cell-mediated immune responses. The degrees of nasopharyngeal obstruction caused by tonsil hypertrophy were associated with the tonsil disorders. Then tonsillar size examination can be an indicator for knowing the rate of immunological activity.

Keywords: Tonsillar obstruction, Tonsillar size, Immunological activity, Allergy

1703P

Association between the concentration of serum immunoglobulin E and allergic rhinitisin Chaharmahal va Bakhtiari province

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Background: Allergic rhinitis, a chronic inflammatory disease of the upper airways, has a major impact on the life quality of patients and is a socio-economic burden. The inflammatory response in the nasal mucosa includes an immediate IgE-mediated response by mast cell So that the late phase of response is characterized by recruitment of eosinophils, basophils. IL-18 is a member of the IL-1 family and originally is described as an IFN-γ –inducing factor (IGIF), also it is known for influencing the balance of Th1/Th2 immune response. In present study this problem is investigated that whether immunoglobulin (Ig) E levels in serum are associated with allergic rhinitis.**Methods:** Genotyping for the some SNPs of IL18 promoter was performed using 133 patients with AR and 62 healthy control volunteers then serum levels of total IgE were determined by ELISA method. Statistical analyses were carried out using the SPSS version 11.5.**Results:** level of total IgE in Serum were significantly greater in allergic rhinitis patients than controls (P=0.017). In addition, the total serum IgE level of the individuals with heterozygous genotypes of IL18 (-133C/G, -607A/C) were significantly higher regarding other investigated polymorphisms (P<0.05).**Conclusion:** According to the results of this study, it seems that the IL18 gene polymorphisms (-133C/G, -607A/C) is associated with IgE levels and susceptibility of allergic rhinitis.

Keywords: Allergic rhinitis ,immunoglobulin (Ig) E , (interleukin-18) IL-18

1745P

The effect of the extract of *Zataria multiflora* on total and differential White Cell count in Balf of ovalbumin sensitized guinea-pigs

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Background: The results of previous studies demonstrating anti-inflammatory and analgesic effects for Z. multiflora may indicate its preventive effect on airway inflammation in asthma disease. **Method**: Five groups of guinea pigs sensitized to ovalbumin were given drinking water alone , drinking water containing three concentrations of *Z. multiflora* and dexamethasone. Total and differential WC count in Balf of sensitized and control guinea pigs were evaluated .**Results**: Total and most differential WC count in Balf (broncho-alveolar fluid) were increased but lymphocytes decreased in sensitized animals compared to controls. Treatment of S animals with dexamethasone and two higher concentrations of the extract significantly improved total and differential WC count. Treatment of S animals with two high extract concentration also significantly improved total WC , lymphocyte and monocyte counts . Although the effect of low extract concentration on all parameter was lower than that of dexamethasone ,the effect of high extract concentration on some parameter was greater than dexamethasone. **Conclusion**:These results showed that the extract of *Z. multiflora* caused reduction of total and most differential WC count but increased lymphocyte in the balf of sensitized guinea pigs.

Keywords: Zataria multiflora; asthma; white cell; Balf; sensitization

1958P

Efficient expression of a soluble lipid transfer protein (LTP) of *Platanus orientalis* (Pla or 3) using short peptide tags, and structural comparison with the natural form

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Background: Successful recombinant allergen-based immunotherapy has drawn a great deal of attention to using recombinant allergens for new therapeutic and/or diagnostic strategies. The *Escherichia coli* (*E. coli*) expression system is frequently used to produce recombinant allergens; however, protein expression in *E. coli* often results in inclusion bodies.**Methods:** Here, we focused on the expression of two recombinant soluble forms of Pla or 3 using Solubility-enhancing peptide tags, HIV-1 TAT core domain and poly-arginine-lysine: rTAT-Pla or 3 and rPoly-Arg-Lys-Pla or 3. The structural characteristics and IgE-reactivity of purified recombinant proteins were compared with natural Pla or 3 (nPla or 3) isolated from *Platanus orientalis (P. orientalis)* using circular dichroism (CD) spectra, fluorescence spectroscopy, and immunoblotting. Likewise, intrinsic viscosity and stokes radius of the natural and recombinant Pla or 3 allergens were determined to analyze structural compactness in aqueous media.**Results**: The results indicate high-level solubility and efficient expression of the fusion proteins (rTAT-Pla or 3 and rPoly-Arg-Lys-Pla or 3) compared to the wild type recombinant. Furthermore, the similar structural characteristics and IgE-binding activities of the fusion proteins to nPla or 3 were observed.**Conclusion**: high-level solubility of engineered recombinant *P. orientalis* pollen Pla or 3s, rTAT-Pla or 3 and rPoly-Arg-Lys-Pla or 3, beside their similarities to the natural molecule in secondary structure and IgE-binding suggest promise for reliable allergy

diagnosis and treatment strategies.

Keywords: Allergy; Circular dichroism spectra; Fluorescent spectra; Inclusion bodies; Lipid transfer protein (LTP); Protein expression

2095P

Study of allergenicity of petal and stamen in older ontogenical stage of *Cytisus scoparius L.* in guinea pig

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Background: Plant cultivation of *Cytisus scop*arius L. due to the perfume and the smell of it has increased in cities green space. The petals and stamens in aromatic plants are important allergenic agents. The aim ofthis study is examination study of allergenicity of petalandstamen in older ontogenical stageof *Cytisus scoparius*L. in guinea pig. **Methods:**In this experimental study, 18 guinea pigs from hartly stirps were randomly selected and divided into three equal groups. The first group phosphate buffered saline, second group older petal extract and in the third group extract of older petal and stamen were injected intraperitoneally. Plant extracts were prepared with 16% concentration. This injections within 4 weeks, once per week, and subcutaneous injection was performed in the fifth week. Finally, a week after the last injection blood sampling directly from the heart of animals, was taken and the number of eosinophils, immunoglobulin E and blood sugar levels were measured in experimental groups.**Results:**Skin test in both groups treated with Cytisus scoparius L. significantly increased compared to controls ($p<0.001$). in groups which treated with older petal and stamen showed significant increase serological test, only blood sugar of ($p<0.05$). Electrophoretic profiles was observed 3 protein bands in the range of 46 to 85 kD in both groups treated with Cytisus scoparius L, in which these bands were much more in group with older petal and stamen. **Conclusion:** the obtained results showed that the allergenicity of petal with stamen is more than petal.

Keywords: Allergenicity, *Cytisus scoparius*L , IgE, guinea pig

1447P

Allergy to pets in small animal hospital staffs in Tehran

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Background: Veterinarians and coworkers are potentially exposed to various occupational hazardous agents such as allergens shed by animals, microbial and chemical agents. Exposure to animal-derived allergens is well known to induce immediate (IgE-mediated) sensitization and development of respiratory, eye and skin symptoms, and allergic asthma. We carried out

a survey for the first time among all the staffs working in the main small animal hospitals in Tehran to find determinants associated with this kind of allergy.**Methods**: We carried out a survey among all the staffs working in 3 main small animal hospitals. Serum samples were obtained from symptomatic individuals and total serum IgE were determined by Immuno CAP and specific serum IgE Abs to cat, dog, mixed rodent and mixed feather allergens was evaluated applying ELISA test. Chi-square and Pearson correlation tests were used to analyze the data using SPSS software.**Results**: Among 92 staffs working in small animals hospitals, 42 (45.6%) individuals had positive allergic symptoms. Specific IgE test show that6(14.3%) were positive for dog,9(21.4%) for cat 11.9%(5/42). No positive individual was detected for allergy to mixed feather specific IgE test.All the cat IgE positive individuals had sneezing symptom while this frequency among negative individuals was 60.6% (p=0.008). Among positive individuals the prevalence of nose discharge was 88.9% and (p<0.0001).**Conclusion**: We show that any of the assessed factors in this study including; sex, age, application period, ward, pet ownership in childhood and current pet ownership and total IgE level or even presence or absence of atopy are not a good indicator for developing allergy to pets in veterinarians working in small animal hospitals. Considering high prevalence of allergy in this investigation we suggest routine examination especially of staffs with rhinitis symptom to avoid possible consequences like asthma.

Keywords :Occupationalallergy ,veterinarians ,Animalallergens ,ELISA

1587P

Prevalence offood and aero allergens in patients with chronic urticaria

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Introduction: Urticaria, also,is a common type of skin condition, triggered by reactions to various allergens. If the urticaria lasts more than 6 weeks,it is called chronic urticaria. Since allergens of various types are perceived to be the main cause of the chronic urticaria. The aim of this study isto examine the impact of two types of allergens—environmental and food allergens—on the outbreak of the chronic urticaria via using the prick test.**:**This is a longitudinal-descriptive study that lasted for duration of two year. The 90 patients,who participated in this study,were all diagnosed with the chronic urticariaand were all from the city of Sari in Mazandaran. Using the prick test as the main allergy test. The results of the prick tests were processed by the SPSS18 software.**Results:**Of the 90 patients who were examined in this study, 71 (79%) were female whereas 19 (21%) were male. The average age was 25+2, of which 5 and 50 were the ages, respectively. the most common environmental allergens were these:the farinae mite ranked the highest (77.8%),followed by the *pteronyssinus* mite (75.6%), candida (67.8%), the T*uscaloosa* allergen (68.9%).Of the most common food allergens reported by this study, these stood out: egg white (47.8%).**Conclusion**:The most common cause of the chronic urticaria in the city of Sari, as suggested by the findings, is reactions to mites. Therefore, identifying the common allergens of every region may better assist the diagnosis, treatment, and prevention of many allergic conditions, especially the chronic urticaria.

Keywords:aeroallergens, food allergens, Chronic Urticaria

1490P

Sensitizing-operation confirmation; Wistar model of Peanut Allergy

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Background: Where possible, animals are being recruited in basic and clinical immunology researches and, for the production of human therapeutics. In the field of allergology, animal-based testings are used for scrupulous recognizing/characterizing/categorizing the potential sensitizing-activity of the predetermined and novel allergenic proteins in any accused food, and so forth.**Methods**: 25 out of 50 male wistar rats, 4-6 weeks old at study initiation, were subjected to sensitization, three times one week apart, with crude peanut extract(CPE). Each sensitization attempt was done over 2 consecutive days;First day: intragastric(ig) administration of 1 mg CPE plus 10 µg Cholera toxin adjuvant/rat.Second day: intraperitoneal(ip) injection of 0.5 µg CPE plus 0.2 mL Aluminium hydroxide adjuvant/rat.In order to confirm the Sensitizing-operation fulfillment, some well-established anaphylactic reaction parameters were measured. **Results**: Allergic responses of the sensitized rats were confirmed by a significant increment, in plasma histamine levels and, in anaphylactic symptom scores compared with negative controls[(p=0.008) and (p=0.000), respectively], as well as by positive id- and ip-challenges outcomes.Conclusions: Because of/adverting to, possessing the necessary qualifications including the availability of an impressive armoury of essential facilities/reagents also, economic advantages, etc., the majority of animal-modeling studies have been concentrated on rodent mammals. Taking to account the homeostatic similarities between rat and man, previous studies have suggested that the Brown Norway(BN)-Rats are the most pertinent model for human allergic diseases. Here, based on our findings, we dare report, for the first time that, the wistar model may even be more predictive of human food-allergic responses.

Keywords: Adjuvant, Anaphylactic Parameters, Peanut Allergy, Wistar Model.

1553P

Quercetin-therapy of Peanut Allergy; a nutraceutical-means to an end

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Background: Assuming of, the huge figure of population suffering from potentially deathful peanut allergy, the possibility of unavoidable occurrence of unexpected exposures and, the paucity of promising acceptable immunotherapy approaches, more applicable and at the same

time, secure preventative and/or remedial strategies are necessitated.Regarding the allergies, a compound of supreme interest is the flavonoid quercetin, which is present in daily often-consumed, plant-origin food ingredients in fairly great levels. Owing to its anti-inflammatory and anti-allergic characteristics (have mainly been achieved in test-tube researches), quercetin has been propounded for the prevention or therapy of humankind allergic diseases and might be helpful for the treatment of food allergies.**Objective:** The goal of our study was to validate the anti-allergic properties of quercetin in an In-vivo system of a peanut allergy model. **Methods**: One week post-sensitization period, the wistar rats in treatment-group received daily, quercetin powder (50mg/kg.BW) dissolved in 5% dimethyl sulfoxide (DMSO) aqueous solution by interagastric gavaging, over a period of four weeks.In the like manner, the animals in sham-group and in both of the control groups, were gavaged with 1 ml/rat of 5% DMSO aqueous solution and tap water, respectively.**Results:** Based on our results, after daily-repeated gavaging during the intervention period, quercetin efficiently suppressed the peanut-induced anaphylactic reactions subsequent to ig challenges.**Conclusions:** To our knowledge, we are the first to report a significant appeasement of some well-known anaphylactic reaction parameters by quercetin intervention in an In-vivo system in a rodent model of IgE-mediated food allergy.

Keywords: Dimethyl sulfoxide, Flavonoids, Peanut Allergy Model, Quercetin.

1970P

Confirmation of a sensitization operation based on in-vitro analyzing-data

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Background: Evident intensification in the incidence of food allergies notifies the necessity of urgent/additional assessments to enhance any requisite encountering strengths including preventative/therapeutic strategies. Extensive investigations of humans are restricted moralistically also, considering the risk of possible life-threatening events. This prompts the researchers for employing ofpertinent animal models in order to depict any appropriate action plan for food allergy. Animal models, as a reliable means, possess the large quantities of qualifications to ease manage many of the problematic complications encompassing the food allergies, as yet.**Objectives**: To confirm the induction of peanut allergy in a wistar-rat model and eventually, pave the way of more improving of our understanding concerning the temperament of the IgE-mediated food allergies, in depth.**Methods**:Orbital-plexus blood samples were collected into microtubes (1.5 mL in size, 0.75 mL in each one/rat). Subsequently, the levels of total serum IgE immunoglobulins and plasma histamine were determined by using an enzyme immunoassay kit, as described by the manufacturer. All analyses were accomplished in duplicate.**Results**: On week post sesitization-period and following to challenge, total serum IgE and plasma histamine levels had significantly been elevated in all the sensitized rats. **Conclusions**: Despite the fact that the larger animals are of prominent precedence/preference as models for food allergy; reflecting more closely the mankind allergic associative entity

owing to their physiology and outbred characteristics, routinely/laboratorially small animal models are often being utilized to characterise the underlying immunological pathways, rendering a foundation for the evolution of various food allergy models. Now here, according to our findings, we state strongly that, the wistar model may even be better predicting of human food-allergic reactions.

Keywords:Sensitization operation ,In-vitro

1711P

In Vivo Diagnostic-procedures of the IgE-mediated Food Allergies

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Background: The minute that a food-allergen was nominated as a possible culprit of symptoms, the verification of principal associations and eventually, identifying the incriminated food(s) are warranted.As to confirming of an IgE-mediated food allergy, some idiosyncratic assays can be considered in order to include or exclude the susceptible food(s). One technique of precise deducing regarding the production of specific IgE immunoglobulins, is the well-known, prick-puncture skin testing. Skin Prick Tests(SPTs) are most predicting in cases where they are negative(over 95 percent).**Methods**: Ventral aspects of wistar rats were used for intra-dermal skin testing with Crude Peanut Extract(CPE). 5-min before the test, 100 µL of Evan's blue dye was injected into the tail vein to ease visualize the wheal reaction. Subsequently, 66 µL of the filter-sterilized CPE was administered intra-dermally into the firstly shaved abdominal skin. (Negative controls were put as well). A positive test response was determined as a wheal reaction appearing as a blue area measuring greater than 5-mm in diameter.**Results**: As expected, abdominal surfaces of the test animals only in positive group, showed-up a wheal reaction as a blue circle close to one cm in diameter, at read time (20-min after id peanut-challenge). **Conclusions**: Collectively, animal models for food allergy are useful to study of the due pathomechanisms, and so forth. But, a lot of validating- studies remain still to be completed.In this respect, any requisite and sufficient skillfully-modified protocols and acceptable conducts should be finely familiarized for masquerading the pertinent complications in contemplation, and ultimately, exploiting of the newly explored data in oncoming humankind researches.

Keywords: Food Allergy, IgE, In Vivo Diagnostic-procedures.

1831P

Role of excretory-secretory products of *Marshallagia marshali* on TLR4 expression in spleenocytes of asthmatic mice

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Background: Epidemiological studies suggest an inverse relationship between helminth infections and allergic diseases. Innate immunity especially antigen presenting cells (APC) have key role for initiation of allergic responses. Numerous studies revealed the role of pattern recognition receptors (PRRs) on APCs to balance or imbalance the immune responses. It seems Toll-like receptor 4 (TLR4) is the most important PRRs that plays important role during asthma development. On the other hand several studies have shown helminth parasites are masterful modulators of TLRs. We are interested in studying the effects of excretory-secretory (ES) product of *Marshallagia marshalli* on expression of TLR4 in spleenocytes of asthmatic mice. **Methods:**After allergic airway inflammation is induced in BALB/c mice by sensitization and challenging with ovalbumin. ES together with ovalbumin will be injected intraperitoneally during sensitization. The effect of ES components on the development of asthma will be evaluated by ovalbumin specific serum IgE titers, lung histology and cell counts.**Results:**This study is ongoing and preliminary results on diffrential expression of TLR4 will be analyzed and presented accordingly.**Conclusion:**This study could provide new insights into immune modulation through TLR4 by *M.marshali* ES products.
Keywords:*Marshallagia marshali*, TLR4, Asthmatic mice

1484P

In vitro cytokine responses of peripheral blood mononuclear cells from Asthmatic human to excretory–secretory products of *Marshallagia marshalli*

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Background: Helminths and their products are considered to possess therapeutic capability to control or even prevent immune-mediated diseases. Studies suggest that helminths induce a systemic immuno-modulatory network, including regulatory T cells and anti-inflammatory IL-10, which might play a key role in the protection against the allergic phenotype. Thus, helminthic therapy is becoming of major interest, and many researchers are enthusiastic to explore its role in allergic diseases. **Methods**:For obtain of ES antigen of *Marshallagia marshalli*, *M.marshalli* was obtained from abomasums of sheep and was cultured. Peripheral Blood Mononuclear Cells (PBMCs) from asthmatic and healthy human were collected. After isolation of PBMCs, they were stimulated with ES antigen of *Marshallagia marshalli*, in incubator with 5%CO2, 37 °c. Total RNA was isolated from all cells from antigen stimulated and unstimulated control PBMC in asthmatic and healthy human after 24, 48 and 72 h and the concentration of Th1 type cytokines (IFN-γ) and Th2 type cytokines (IL-4) and Treg cytokines (TGF-β, IL-10) was determined using Real Time PCR.**Results:**Result of our study is now evaluating and we hope present result of this study in time of congress. **Conclusion:**We hope that Helminths products induce immunomodulation and were becoming significant candidates for anti-asthmatic agents in this context.
Key word:Asthma, PBMC, *Marshallagia marshalli*, cytokine responses

1500P

Assessing the effect of high school students' training program on peers performance suffering from asthma after 6 months of study in Esfahan high schools 2010

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Background: Asthma is the most common disease in childhood which is considered as the forerunner of the acute diseases and simply can cause disability among the children. Since childhood and adolescence are the most important periods of growth and perfection and incidence of asthma can bring about distortion in this process, the present study done aimed to assess the effect of conducting high school training program on peers' performance with asthma.**Methods**: This was a quasi-experimental study in which the knowledge and performance rate of the students at the time of artificial attack of asthma was directly assessed and compared through demographic data questionnaire and performance assessment check list and knowledge questionnaire. Eighty individuals from the second grade of high school students in 2010 in Isfahan City were randomly selected to participate in the present study, among which, 40 individuals were entered in the test group and 40 of them also were placed in the control group. After conducting the training program for the test group, which had been designed both by direct method (in person and face to face by asking and answering and group discussion) and by indirect method (using pamphlet and other educational materials), the level of the training effectiveness was assessed on the students 'performance and knowledge. **Results:**The findings of the present study indicated that the performance of students at the test group 2.2±0.8 before and 80.9±18.4 six month after the intervention and there was a significant difference between them ($p<0.001$). The knowledge of peers of asthmatic student in test group1.3±0.03 before the intervention and80.5±21.5 six month after the intervention and difference was significant ($p<0.001$). **Conclusion:**Considering the results of the present study and regarding to the importance and role of students as the future makers of the country, and also the cost-effectiveness of the training programs and the positive effect of peers on increasing the level of health among the students with asthma and consequently decreasing the school absence, it obviously seems necessary to generalize and expand these training programs.

Keywords: Health promotion, Knowledge, Performance, Asthma

1501P

An examination the effect of health promotion plan in high school students on knowledge and performance of their peers suffering from asthma in high schools of district 3 in Esfahan, 1389

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Background: Asthma is the most prevalent disease during childhood, known as the most important reason for children's disability adolescences truancies thanks to their hospitalization and as a result intensification of disease's symptom.**Methods**: The present study is quasi-experimental kind, implemented with two groups. Sampling method was straight forward.80 student's second-grade high school student constitute participants. Both groups were

homogenized considering age, gender, education and parent's vocation. First of all a letter agreement was received from students.In the next step during 4 session educational content specified beforehand was presented to students via interview, lecture, group discussion and display methods .one month after the last session attended by test group students. The knowledge and accomplishment of both groups' students was measured through questionnaire and checklist. Finally the data was analyzed by spss 16 and statistical test of t-paired, independent-t, man-Whitney and ANOVA. **Results:** The finding demonstrated that group's student accomplishment registered 91.8±1.3 while being 2.2±0.6 before holding session. In addition their knowledge increased significantly, registering 99.6±1.2 while it had measured as 1.3±0.3 before sessions. The results of paired t test indicated that the average of difference between knowledge and accomplishment grades of two groups was significant. ($p<0.05$)**.** **Conclusion:** Regarding the finding of the present study and the fact that asthma is on the rise resulting in an increase in truancies as well as stressing the efficacy of training peers suffering from the disease, the implementation of the curriculum could be necessary.

Keywords: Asthma, Knowledge, performance, health promotion

2672P

Effect of food allergen exculsion on symptoms and quality of life in irritable bowel syndrome

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Background: Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders characterized by abdominal pain or discomfort and altered bowel habits. Although IBS etiology is poorly understood, some psychological disturbances and food allergies have been proposed. This study was done to determine the effect of food allergen exculsion on symptoms and quality of life in IBS.**Methods:** In this descriptive and anolytical study was done on 100 IBS patients in Tabriz, Iran during 2011. Food allergies of specific origin and effects of non allergic regimens on quality of life, pain severity and disease symptoms were evaluted during one month period. Data were analyzed using SPSS-16, student's t-test, Chi-Square and Fisher's exact tests.**Results:** Patients age were 31.5±7.2 between 15-43 years. Specific antibodies against allergic foods were detectable in 31 (31%) cases. Penaut showed to induce the highest food allergy in 5 (16.1%) patients. The median disease duration was 4.5±2.6 years in allergic patients while 3.5±2.9 in non allergic group with no significant difference. The changes is quality of life was significant in non allergic regimens after one month in allergic group ($P<0.05$) while disease symptoms such as mucus defecation and flatulence had no significant changes.**Conclusion:** This study showed that one month non allergic regimens reduce abdominal pain andimprove quality of life in IBS patients.

Keywords: Food allergy, Irritable bowel syndrome, Quality of life

1820P

Evaluation of the immunoregulatory effects of somatic antigens of *Marshallagia marshalli* in a mouse model of experimental allergic asthma

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Backgrand: Epidemiological studies suggest an inverse relationship between helminth infections and allergic disease. Recently several helminth-derived products have been shown to suppress allergic responses in animal models. In this study we are investigating whether somatic of *Marshallagia marshalli* suppress the development of allergic asthma in a mouse model.**Methods**: Allergic airway inflammation isinduced in BALB/c mice by sensitization with ovalbumin. The effect of the *M.marshalli* somatic Ags on the development of asthma is evaluated by analyzing serum antibody titers, lung histology,cell counts and cytokine levels in the bronchoalveolar lavage fluid.**Results:** This study is ongoing and preliminary results of a pilot study, when mice is sensitized with ovalbumin together with the somatic Ags of *M.marshalli*, cellular infiltration into the lung, ovalbumin specific serum IgE and Th2/ Treg cytokines level will be analyzed and presented accordingly.**Conclusion**: This study could provides new insights into immune modulation by the *M.marshalli*, with suppressive potentiative airway inflammation in mice during the development of asthma. The identification and characterization of parasite-derived immune-modulating molecules might have potential for designing novel prophylactic/therapeutic strategies for immune-mediated diseases.

Keywords:immunoregulatory, somatic antigens ,*Marshallagia marshalli* , allergic asthma

1639P

The effect of the Phytolacca's fruit extract on activities of the peripheral blood's PMN

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Background: The Phytolacca plant can be found in huge amounts at the northern district of Iran (Caspian sea zone). Despite being toxic, this plant specially its foliage has herbal usage. The Phytolacca's seeds, are full of extract when they are riped that make them black in color. This palnt's seeds are one of the nighthingales's favorite meals.In this research, we study the effect of Phytolacca's fruit on the avtivities of the peripheral blood's PMN.**Method:**We extract Phytolacca's seeds and neutrophils from whole blood with dextran 3% method and adjoining them in 37 degree bain marie for 1 hour and then adding them prepared NBT/PMA and incubate them in 37 degree bain marie for an hour, then we mount neutrophils on a slide, fixed them with Ethanol and then stain them with giemsa.**Results:**The results of the experiment, show that even 1/64 dilutioned Phytolacca's extract can force more than 50% of the neutrophils to the self- destract themselves. (Apoptosis process)This means that Phytolacca's extract not only dosen't stimulate the neutrophils but also inhibits their activities.**Conclusion:**This extract in high dilution can induce apoptosis in neutrophils.

Keywords: Neutrophil, Phytolacca, PMN

2123P

Comparison of allergenicity of petals in two ontogenical stages of *Cytisusscoparius*L. in guinea pig

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Background: *Cytisusscoparius* is an ornamental and highly aromatic plant. It is the aim of this study the comparison of allergenicity of very aromatic petals in two ontogenical stages of middle-aged and older in *Cytisusscoparius* .**Methods:** In this experimental study, 18 guinea pigs from hartlystirps were randomly selected and divided into three equal groups. Petals buffered extracts were prepared with 16% concentration and injected intraperitoneally to guinea pigs. In the first group phosphate buffered saline ,inthe second group middle-aged petal extract and in the third group extract of older petal was used. Afterintraperitoneal injections within 4 weeks, once per week, and subcutaneous injection in the fifth week, was taken blood sampling directly from the heart of animals, and the number of eosinophils, immunoglobulin E and blood sugar levels were measured in experimental groups.**Results:**Created wheals diameter in Skin test in both treatment groups significantly increased compared to controls ($p<0.001$). serological tests (levels of IgE, eosinophils, and blood glucose) in group treated with middle-aged petal extract significantly increased compared to controls. Atelectrophoretic profiles was observed highest and the lowest protein bands about 4 bands(2 light bands and 2 pale bands) in the range of 27 to 85 kD in the middle-aged petal and 3 bands in the range of 46 to 85 kD in older petal ,respectively.**Conclusion:** The results showedthat the highest and lowest potency of extreme stimulation of the immune system belongs to middle-aged petal and old petal, respectively.

Keywords: Allergenicity, *Cytisusscoparius*L.,IgE, Petal.

1636P

IgE-mediated food allergy in children

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Background: Food allergy is a serious health issue affecting roughly 4% of children, with a substantial effect on quality of life. Food allergy is caused by an immunological reaction that results in nontolerance to a specific food or foods. In most children,this disorder is mainly mediated by IgE. **Methods**: Diagnosis of IgE-mediated food allergy is indicated through medical history and physical examination and is confirmed by the presence of antigen-specific IgE and positive food challenge when necessary. **Results:**In this study, the reported resolution rates for cow's milk allergy were roughly 45–50% at age 1 year, 60–75% at age 2 years, and 85–90% at age 3 years.Also, a study reported 21% non-tolerance in children aged 16 years; these children had increased concentrations of cow'smilk IgE at all ages. As a result, the prognosis for cow's milk allergy in this population is thought to be worse than previously reported, and the increased potential for persistent allergy to cow's milk and the

effect of specific IgE concentrations on prognosis should be considered in the counselling of families on expected clinical course. **Conclusion:**Because sensitisation to foods can change over time, children should be monitored for evidence of tolerance to previously allergenic foods or development of novel food allergies. In children who test positive for food-specific IgE (with or without atopic dermatitis), avoidance of foods regularly eaten without allergic symptoms can be detrimental because this practice disrupts oral tolerance and increases the risk of anaphylaxis upon re-exposure.

Keywords: Food allergy, IgE, Children

1641P

The evalution of Glycyrrhiza Glabra extract on neutrophil function

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Background: Licorice is the root of Glycyrrhiza Glabra from which is some what sweet flower can be extracted.**Methods:**In this research, we obtain extract of Glycyrrhiza Glabra powder by alchohol and water, and so PMNs from health donor by obtaining heparinised blood with dextran. We incubate of PMNs with different dilution of licorice of Glycyrrhiza Glabra extract. **Result:** We showed that, this extract in some dilution conduce increase neutrophil function and in some dilution is not much effect in neutrophil function. **Conclusion:** In this research, we showed that, extract of Glycyrrhiza Glabra, in some dilution can induce neutrophil function and can conduce a inducer for PMNs in innate immunity. But, in some dilution it can decrease PMNs function. Because of, this plant is consumped for treatment of stomach disease, this effect of plant can harmuful for stomach in high consumped.

Keyword: Neutophil, Glycyrrhiza Glabra

1710P

Effects of body mass index on spirometry parameters in persistent allergic rhinitis

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Background: Close association exists between allergic rhinitis and asthma as it considers a strong risk factor for the onset of asthma in adults. Moreover several studies have outlined a possible relationship between increased body mass index (BMI) and allergic rhinitis. The aim of this study was to investigate the relation between BMI and respiratory function tests in persistent allergic rhinitis patients.**Methods**: This cross sectional study carried out on 126 patients with persistent allergic rhinitis who referred to Allergy Clinic of Mashhad University of Medical Sciences, Mashhad, Iran. Skin prick test andspirometry were performed in all patients. We excluded all patients with asthma symptoms, acute or chronic upper respiratory infections, anatomical nasal disorders (i.e. nasal polyps, septum deviation, etc.), smokingimmunotherapy, nasal or oral corticosteroids,vasoconstrictors,antileukotrienes for 4 weeks. All patients were

treated with drugs (e.g. antihistamines) alone only on demand.**Results**: We studied 126 individuals (72 female and 54 male) with duration of allergic rhinitis 6.94±0.65 month. They were ranged between 9 to 72 years old (28.74±11.36 yrs). Skin prick test showed that outdoor allergens (60%), mixed allergens (26%) and indoor allergens (14%) had the most frequencies respectively. Besides skin prick test of all aeroallergens showed that female (72%) had more frequencies than male (54%). There was no significant difference among FEF25-75, FVC and FEV1 for BMI values (P>0.05). **Conclusion**: This study provides the first evidence about evaluation of BMI on spirometric parameters in allergic rhinitis patients in Iran.

Keywords: Body mass index, allergic rhinitis; spirometry; Allergens

1717P

Prevalence of allergens in persistent allergic rhinitis patients who referred to allergy clinic, north east of Iran

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BackgroundAllergic rhinitis is one of the most common allergic disorder and health problem worldwide including our country. Indoors and out door allergens could be caused and triggers of allergic rhinitis symptoms. Our Allergy Clinic is the referral center in Northeast of Iran. In this study we aimed to evaluate the prevalence of allergens in persistent allergic rhinitis patients.**Methods:** This cross sectional study carried out on 126 patients with persistent allergic rhinitis who referred to Allergy Clinic of Mashhad University of Medical Sciences, Mashhad, Iran. Skin prick test and was performed in all patients. We excluded all patients with asthma symptoms, acute or chronic upper respiratory infections, anatomical nasal disorders (i.e. nasal polyps, septum deviation, etc.), smoking immunotherapy, nasal or oral corticoster oids,vasoconstrictors,antileukotrienes for 4 weeks. All patients were treated with drugs (e.g. antihistamines) alone only on demand.**Results**: Our analysis showed that the house dust mites (16 cases) (D.Farine, D.Pt) were the most common indoor allergens (28 people). And the least indoor allergens were cat dander (2) and molds (clodosporium and Penicilium, 2 cases respectively). About the outdoor allergens, Ash (70), weeds (60) and salsola (50) had the most frequencies respectively. Grass and birch had the lowest frequencies. The most common sever symptoms were runny nose (79) and sneezing (76), while smelling disorder (9) had the least one. There was no significant difference among age (childhood and adults) and type of allergens (indoors and outdoors allergens) (P>0.05). The most common sever symptoms in male and female wasrhinorhea (35) and sneezing (45) respectively.And the least sever clinical symptoms in male and female was smelling disorder in both groups (4).**Conclusion**: In present study we concluded that house dust mite and ash were the most common indoor and outdoor allergens respectively. About the severe symptoms, runny nose was the most common symptom and rhinorhea in males and sneezing in females had the most frequencies.

Keywords: Persistent allergic rhinitis; Allergens

1853P

Investigation the prevalence of airborne antigens in patients with allergic rhinitis in Kerman

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Background:Allergic rhinitis is an inflammatory disease of mucous membrane of the nose. the irritating symptoms are associated whit asthma and conjunctivitis. Mostly symptoms are troublesome .after sensitization IgE cross link and cause inflammatory mediators release.for diagnosis giving history and using examination can be helpful.the purpose of this study was to investigate of prick skin test in AR patients in Kerman.**Method**:Prick skin test was done by 20 airborne antigens for 70 patients with AR and at last result was analyzed by SPSS16 and chi-square test.**Conclusion:**There was no relationship between gender and sensitivity to allergens such as animal hair antigen and plants,by using chi-square test.in addition the highest prevalence was belong to chenopodiaceae and after that compositae and salicaceae were the most common allergen in AR patients.

Keywords: Airborne antigen,Allergicrehinitis,Prick skin test

1725P

Effects of perennial allergic rhinitis on spirometric parameters

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Background: There isintimate association exists between asthma andallergic rhinitis.However allergic rhinitis is considered as the strong risk factor for the onset of asthma inadults. The aim of this study was to evaluate group of patients withmoderate-to-severe and persistent allergic rhinitis alone for investigating thepresence of spirometric abnormalities and role of this skin prick test results on this abnormalities.**Methods**: This cross sectional study carried out on 126 patients with persistent allergic rhinitis were evaluated. After complete follow-up, the other rhinitis causes were rule out. Skin prick test andspirometry were performed in all patients. We excluded all patients with asthma symptoms(cough, wheezing, dyspnea and shortness of breathing), acute or chronic upper respiratory infections, anatomical nasal disorders (i.e. nasal polyps, septum deviation, etc.), smokingimmunotherapy, nasal or oral cor ticosteroids,vasoconstrictors,antileukotrienes for 4 weeks. All patients were treated with drugs (e.g. antihistamines) alone only on demand. **Results**: We studied 26 individuals (72female and 54 male) with duration of allergic rhinitis 6.94±0.65 month. They were ranged between 9 to 72 years old (28.74±11.36 yrs).There was no significant difference among FVC and FEV1 for allergens ($P>0.05$). Whilefor indoor allergens, FEF25-75<80 was significantly more in female than male ($P=0.003$). There was negative correlation between FEF25-75 and duration of disease ($r=-0.13$). **Conclusion**: This study highlights the close link between upper and lower airwaysand the role of some risk factors such as duration and mites' sensitization, and FEF25-75 as a marker of early bronchial involvement in patients with moderate-tosevereand persistent allergic rhinitis alone.

Keywords: allergic rhinitis; asthma; spirometry; Allergens

2054P

Role of Nutritional knowledge in allergic reactions

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Backgrounds: A food allergy is an adverse immune response to a food protein. These kinds of allergies occur when the body's immune system mistakenly identifies a protein as harmful. Research done in respect to nutrition knowledge on people dealing at the same time with allergy is seldom the subject of dissertation in Iran as well as worldwide social medical literature. **Methods:**To examine the influence of food allergy, we studied 210 person15 to 75 years. These allergic reactions have an acute onset (from seconds to one hour) and may include: Hives, Itching of mouth- lips- tongue- throat- eyes- skin or other areas, Swelling of lips- tongue- eyelids or the whole face, Difficulty swallowing, Runny or congested nose, Hoarse voice, Wheezing and/or shortness of breath, Nausea, Vomiting, Abdominal pain and/or stomach cramps, Lightheadedness, Fainting.**Results:**On the basis of allergic questionnaire form, in 31.4% food allergy was confirmed. Although sensitivity levels vary from person to person, the most common food allergies are allergies to milk, eggs, peanuts, tree nuts, seafood, shellfish, seeds, soy, wheat, rice, fruits, vegetables, maize, spices, synthetic and natural colors, and chemical additives.**Conclusions:** In response to the risk that certain foods pose to those with food allergies, countries have responded by instituting labeling laws that require food products to clearly inform consumers if their products contain major allergens. Therefore, nutritional knowledge and early prevention of exposure to them may help reduce the allergic reactions.

Keywords: Nutritional knowledge,Food allergic, Immune response, Social medical literature

2088P

Determination of the correlation between serum levels of total IgE antibody and the percentage of eosinophil cells in patients with asthma referred to the Yasuj Mofatteh clinicin 2013

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Background: According to the role of eosinophil cellsandIgE antibodyin the pathogenesisof asthma, the aim of this study was to determinethe correlation betweenserum levelsoftotalIgEantibody andthe percentage of eosinophil cells in patients with asthma.**Methods**: This case-control studywasperformedon30patientswithasthmareferredtoYasujMofattehClinic in 2013. The disease had been confirmed byrelevant specialistsasthe case group and30healthy subjectsas controls. After completing thequestionnaireincludeddemographicandclinical status, 2 ml of oxalated blood and2ml of clotted bloodwere taken. Eosinophil cells percentage and totalIgEantibodylevelswere measuredby counter and ELISA respectively.**Results**: This study indicated that the total IgE levels 34 % of patients with asthma and 20% of control group were

higher than normal ($p<0.05$). The 36% of cases showed eosinophil above 4%, while the control group no positive cases were found ($p<0.05$). There was a significant correlation between the percentage of eosinophil cells and extent of symptoms of asthma with its severity ($p<0.05$). A significant correlation was seen between total IgE levels and the percentage of eosinophil in asthma patients ($p<0.05$).**Conclusion:** The amountoftotalIgEantibody and the percentage of eosinophil cellsin patients with asthmaare high. Due tothesetwo factorsassociatedwithsymptoms andseverity it can be used of these two factors for controlling, prognosis andtreatmentof the disease.

Keywords: Asthma, IgE antibody, Eosinophi

1987P

Substitution of boiled cow's milk in feeding of infants with cow's milk allergy

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Background:Cow's milk allergy is the most common food allergies in the first year of life and virtually all milk allergies develop by 12 mo of age. Appropriate identification and elimination of foods responsible for food hypersensitivity reactions are the only validated treatments for food allergies.It has attempted to survey the efficacy of boiled Cow's milk in children with cow's milk allergy. **Methods**:Fifty-six cow's milk allergy children selected for the study by clinical manifestations and positive skin prick test and then questionnaire completed for each of them. Cow milk was prepared as boiled form. Consequently, follow up studies on the prescribed milk as well as clinical examinations of the patients conducted.**Results**:In this study, the mean age of children was 30 months; 38% were boys and 62%were girls respectively.Children challenged with boiled cow milk, respectively. Following the challenge, symptoms of 24 (42.85%) child resolved.**Discussion and Conclusion**:Cow's milk proteins include casein and whey. It well known that structure of casein by heat does not change and allergenicity potential of casein remains, whereas heating destroys several of the whey proteins and decrease allergenicity of whey.So many children with milk allergy tolerate extensively heated milk in baked products and it can be a sutitable solution for children with Cow's milk allergy.

Keywords: Cow's milk allergy, Boiled cow milk .

2065P

Correlation of IFN-λ3 (IL-28B) gene polymorphism with total serum IgE levels in asthmatic patients

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Background: Interferon lambda (IFN-λ), the newly discovered type III family of IFNs, comprises three structurally related cytokines, IFN-λ1 (IL-29), IFN-λ2 (IL-28A) and IFNλ-3

(IL-28B). IFNλ-3that last one functions in several diseases such as asthma. The purpose of this study was evaluation of rs8099917 allele polymorphism in the IFN-λ3 in Iranian asthmatic patients and its relation by total Serum IgE level. **Methods**: DNA was extracted from whole blood 99 doctor diagnosed asthmatics patients. Evaluation of rs8099917 allele polymorphisms were done by Nested-PCR and RFLP method using BsrDI and Tsp451 restriction enzymes. The total serum IgE levels were determined by ELISA method. **Results**: Analysis of the results showed that there was not a significant correlation between the polymorphisms of rs8099917 allele of IFN-λ3 and the total serum IgE in examined asthmatic patients (P>0.05). **Conclusion**: Although there are some evidences of IFN-3 role in asthma and asthma features, our study didn't show a positive correlation between rs8099917 allele genetic variants and evaluated levels of IgE in Iranian asthmatic patients. Further studies on different features of asthma may show clearer role of IFNλ-3 (IL-28B) in asthma disorder.
Keywords: IFN-λ3, Polymorphism, rs8099917 allele, Iranian Asthmatics, IgE

2369P

Evaluation of the ratio of T helper 17 and T regulatory cells in patients with chronic idiopathic urticaria

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Background: Chronic idiopathic urticaria (CIU) is a common skin disorder characterized by hives for at least 6 weeks without any known etiologic agents. T helper 17 and regulatory T cells (Treg) ratio plays a critical role in suppressing immune response or maintaining of immunological's homeostasis. The aim of this study was to examine the potential role of Th17 and Treg by measuring of RORγt and FOXp3, master regulator transcriptional factors, in patients with CIU. **Methods**: We studied 20 CIU patients (mean age: 28±6, mean duration of disease: 27±4) and 20 healthy individuals (mean age: 28±9). Proliferation assay of peripheral mononuclear cells was carried out by PHA stimulation and real-time PCR was applied to quantities RORγt and FOXp3 gene expression.**Results**: There was a significant difference between FOXP3 gene expression in cases (1260±400) and control groups (14.35±2.92) ($p<0.001$), while no significant differences in RORγt gene expression was observed between patients (128.46±38.11) and healthy controls (90.91±32.30). Furthermore, the ratio of RORγt /FOXP3 was significantly lower in patients than controls ($p<0.001$; $Z=-4.6$).**Conclusion**: The high expression of Foxp3 in patients with CIU might suggest that this factor has not a pivotal role in disease's pathogeneses. Other factors such as "neurogenic inflammation" which mediates the inflammation by degranulation of mast cells and induces Treg cells might involve in disease. Further studies are needed to clarify the roles of neurogenic inflammation in CIU.
Keywords: chronic idiopathic urticaria (CIU), T helper 17 (Th17), T regulatory (Treg), RORγt, FOXP3

2158P

Bully victimization and social anxiety in adolescents with allergies

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Background: An allergy is a hypersensitivity disorder of theimmune system. Although an allergy can have serious health consequences, little is currently known about the psychological experiences of people with allergy. **Method**: The present study examined the differences in experiences of bullying and social anxiety between adolescents with and without an allergy. The study sample comprised 86 adolescents (37 girls and 49 boys) aged 14 to 17 diagnosed with a specific allergy.**Result:** All the adolescents with allergies were recruited from among patients visiting medical clinics for treatment, while adolescents without allergies were matched for age and recruited through convenient sampling. All adolescents completed the Bullying Prevalence Questionnaire, and the Liebowitz Social Anxiety Scale along with a socio-demographic sheet. Data were analyzed using descriptive statistics and multivariate analysis of variance. Of the 86 participants, 63 (73.25%) reported they were allergic to at least one food. Seafood, nuts, bananas and broad beans were reported as allergens. Six (6.97%) of the adolescents reported multiple allergies and 17 (19.77%) had non-food allergies. The prevalence of bully victimization was found to be 72 % and 27.9% among adolescents with allergies and their normal counterparts, respectively. Much of the bullying reported was in the form of teasing and taunting. The prevalence of social anxiety was also greater among adolescents with allergies (76.74% vs. 22.1%). Significant gender differences emerged with both bully victimization (77.55% boys vs. 64.86% girls) and social anxiety (81.6% boys vs. 70.27% girls).**Conclusion:**Findings indicate that people need to be made aware how serious allergies can be. Findings are discussed in terms of health education implications and possibilities.

Keywords: Allergy, Bullying, Victimization, Social anxiety, Adolescent

1938P

The effects of pilates training on pulmonary indices in femaleasthmatic patients with asthma

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Background: Asthma is one of the most common disorders of respiratory system. Sport exercises are main part of the pulmonary rehabilitation programs. This study designed to investigate the effects of the Pilates training on pulmonary indices(FEV1,FVC and FEV1%) in female patients with Asthma.**Methods:**In this semi-experimental study a total of 24 femaleasthmatic patients, among all patients referred to Amin hospital in Isfahan, with mean age and standard deviation (31.3±3.6) , height (162.5±5.2) and weight (65.4±8.6) , were purposefully and availably selected as the sample and were randomly divided into two groups: control(n = 12) and experimental (n = 12). During 8 weeks of workouts, the experimental group performed the Pilates exercises, three sessions per week, each session lasted 60 minutes; while the control group didn't participate in any special training. Pulmonary indices(FEV1,FVC and FEV1%) were measured bySpirometer device. At the end of this period both groups were post-tested. Repeated measure ANOVA was used to statistical analysis with $\alpha = 0.05$. **Results:** The group within changes(posttest than pretest) and also the process of change (slope

change)in all variables(FEV1,FVC and FEV1%) was significant. Although they did not differ in FEV1% and FVC(P = 0/06 and P = 0/6),But in FEV1 difference was significant (P = 0/04).
Conclusion: The Pilates exercise can be used as a complementary and useful method to reduce some problems in patients with asthma.
Keywords: Pilates, Asthma, Pulmonary indices.

3154P

Food allergy as a predictor of asthma; a 4-year follow-up

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Background: Associations have been observed between asthma and food allergy, through shared mechanisms, similar genetic factors, and co-existance. We aim to assess food allergic children, documented in the Iranian Food Allergy Registry. **Methods**: 45 children with clinical complaints of food hypersensitivity (23 positive specific IgE (sIgE) patients and 22 negative sIgE controls) were evaluated after a 4-year follow-up. Asthma and food allergy status was assessed by telephone interview, using a modified version of the International Study of Asthma and Allergies in Childhood, ISAAC, questionnaire. **Results**: At the follow-up, 48% of patients with positive sIgE reported persistent food allergy, while 14% in the group with negative sIgE reported the same outcome (p=0.013). Out of the patients without positive sIgE, none reported ever having asthma and 45% reported wheezing ever, while 13% of patients with positive sIgE stated that they've been diagnosed with asthma and 57% in this group reported wheezing ever. Out of 23 patients with positive sIgE, 39% had milk allergy, 44% wheat allergy, 39% egg allergy and 13% had allergy to nuts. Wheezing ever was reported among 66% of patients with egg allergy, 66% with nut allergy, 50% with milk allergy and 50% with wheat allergy. **Conclusion**: There is a need to include more patients and follow them for a longer period, but the results so far show a relationship between positive sIgE and diagnosis of asthma. As most of food allergic patients with positive sIgE report wheezing ever, further evaluation of this group regarding asthma is needed.
Keywords: Food allergy, asthma, children, milk

2811P

An efficient and economical method for purification of β-Lactoglobulin from bovine milk

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Background: Cow's milk allergy (CMA) affects about 2-7.5% of infants. β-Lg is a major protein of bovine whey (2-3 g/l) is one of the main milk allergens. This protein produces the Immunoglobulin E antibodies and causes the most clinical problems. To use the β-Lg in allergy diagnosis and preclinical allergy studies, the preparation of high pure β-Lg in biologically active form is required. Because production of β-Lg using recombination or subsequent chromatographic steps is onerous, we have developed a simple and efficient method for the purification of bovine milk β-Lg. **Method:** Dry citric acid was added to bovine milk whey (80

mM) and pH was adjusted to 3.9 by *tri*-sodium citrate. Then the solution was heated at 55 °C for 150 min. The precipitated proteins were removed by centrifuge at 12000 g for 30 min at 4 °C. The supernatant was lyophilized and stored at -70 °C. The purity of protein was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).**Results:** The clear supernatant contains pure β-Lg. SDS-PAGE analysis showed over 97% purity. Also, 1.4g β-Lg was obtained from 2g initial amount. In comparison, the β-Lg produced by Sigma Co. is very expensive and have just ≥90% purity. **Conclusion:** The recovery of this method is 70%. Hence, this simple, efficient and economical method can be applied to purification of large amount of β-Lg.

Keywords: β-Lactoglobulin, Bovine milk, Allergen, Citric acid, Heat

3079P

Prevalence of *broussonetia papyrifera* pollen and its impact on human health in the atmosphere of islamabad city: a two year study

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Background: An aerobiological study aiming towards sampling of pollen diversity and intensity was carried out in the atmosphere of Islamabad city for a period of two years using Roto Rod Sampler Model 40. **Method:** The prime source of allergy in the area was observed to be *Broussonetia papyrifera*-an exotic species releasing large amount of pollen in to the air, posing serious implications on pollinosis. Variations in the daily pollen count over the study years have proven that peak pollen count release was recorded during the months of March till start of April. This reveals a positive correlation with temperature. **Result:** The results of the study were compared with the allergy patients of adjoining areas to determine the general trend regarding close liaison between the upward trends of the number of allergy patients in accordance with the number of pollen count release. **Conclusion:** This analysis confirmed the findings that Islamabad city is considered as a high pollen risk locality for the residents and the visitors.

Keywords: Daily Pollen Count , Allergy Patients ,Broussonetia papyrifera ,Temperature Humidity

1567P

Modification and Evaluation of Avidity IgG Testing for differentiating of Toxoplasma gondii Infection in Early Stage of Pregnancy

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Background: Toxoplasma gondii infection, an intracellular parasite, is often asymptomatic or is caused by different clinical diseases without being detected. Evaluation of IgG, IgA, and IgM in order to diagnose the pending Toxoplasmosis may confront some problems. Several researches has showned that Toxo IgG avidity can be useful in the recent active Toxoplasmosis. In current study, modification and importance of improved Toxoplasma Avidity IgG testing has been evlauated for differentiating Toxoplasma gondii infection in early stage of pregnancy. **Methods:** This experimental study included 300 pregnant women with risk of Toxoplasmosis

in their initial months of pregnancy. We randomly divided 300 serum samples into A group (n=60) with high avidity and B group (n=40) with borderline avidity. The samples with Toxo IgG levels were classified to four subgroups. IgG avidity was evaluated by enzyme-linked immunosorbent assay (ELISA) method. **Results:** The mean absorbance of 100 samples in two groups was calculated, and then, two dilutaion curves with plotted absorbance against dilution were drawn for each serum sample. The results of this study showed that in groups with different concentrations of toxo IgG, appropriate dilution of serum is suitable for testing of Avidity. Our findings revealed the subgroups of 1, 2, 3, and 4 with serum dilutios of 1/3 , 1/6, 1/9, and 1/18 respectively, had real and good avidity. **Conclusion:** One of the issues affectig the results of avidity is high concenteration of Toxo IgG in serum sample. As shown in this study, the best points of dilution for well avidity in both high and borderline avidities are marked with arrows in figures 1-8. This study confirmed that improved methods of measuring Toxo Avidity IgG are very important.

Keywords: Toxoplasmosis, Pregnancy, ELISA, IgG

1442P

A Case Control Study of Asthma Symptoms and Sensitization among Workers Exposed to Toluene Diisocyanate, TDI

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Background: Toluene diisocyanate (TDI) is a necessary chemical substance in the production of paints, elastomers, polyurethane foams that cause a variety of health problems in workers who are exposed in work places. The aim of this study was to determine asthma symptoms and IgE levels in TDI exposed workers and comparing the results with healthy control group. **Methods:** In this case control study we include all paint and glue factories that use TDI in the west of Tehran province, 550 workers completed modified initial questionnaire of the NIOSH, the questions were consist of asthma symptoms. For each symptomatic exposed worker one healthy, sex and age matched control selected. Total IgE and Specific TDI IgE tests were done for each case and control groups by RAST method. **Results: A**mong 550 exposed workers, 26(4.7%) workers had asthma symptoms. Nine (34.6%) of symptomatic workers who were exposed to TDI were active cigarette consumer versus 3(11.5%) unexposed workers, P=0.049(CI= 0.953-17.29)OR=4.059. Nine(34.6%) workers had positive family history of atopy versus 1(3.8%) unexposed workers, P=0.0138(CI= 1.45-305.41)OR=13.24. TDI specific IgE was found in 2 TDI exposed workers and 1 unexposed worker (P=0.5). Mean of total IgE was 339.05 in exposed (P=0.201).**Conclusion:** Clinical and paraclinical data of workers who are exposed to TDI show a relation between atopy and smoking habit with asthma symptoms that offer preventing recommendations for TDI exposed workers and their heath administrators

Keywords: Toluene diisocyanate (TDI), Asthma symptoms, IgE

3342P

Zizyphus spina-christi(L.) Extract is a New Amazing Traditional Supplement Drug for Asthmatic Patients Treatment

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Background: Asthma is the result of chronic inflammation of the airways via internal and external stimulator which subsequently results in increased contractibility of the surrounding smooth muscles. On the other hands, many study obviously revealed that medication management procedures in the some asthmatic case are very difficult. Therefore, Our research team in this regard investigated the actual anti inflammatory properties of Zizyphus spina –christi leaf extract in adult asthmatic patients via pulmonary function assessments.**Methods**: Eighty patients with asthma and stability with their current therapy were selected in the investigation. All patients are randomly divided in two arms. Group A (30 patients) were those who had their regular repeat medications with added Z.spina –christi extract, twice a day every other day as a steam inhalation for 2 months. Group B (30 patients) with their regular repeat medications only, without any added Z.spina –christi extract. Two arms were compared together in regard to spirometry parameters before and after this study. And also SGRQ score was assessed for all subjects and comprised between two groups.**Results**: Spirometry analysis revealed that group A has a obvious increase and improve in FEV1 with the $P< 0.01$ and consequently its ratio with FVC in comparison to group B. Intra group analysis in Z.spina –christi extract arm, group A, has indicated that all spirometry parameters in this group after investigation notably improved in comparison to before study starting. **Conclusion**: Accordingly, the quality of life parameters, pulmonary function test and physical symptoms in group A patients when compared against group B has improved. Our finding revealed and robustly confirmed that the extract of Ziziphus Spina-Christi can be used a traditional supplement drug for the management of asthma. Other supplementary data about our study would be released in the future papers.

Keyword: Zizyphus spina-christi, asthma, Pulmonary Functions

3139P

Study of relationship between asthma and parasitic infections

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Background: It is estimated that 130 million person all over the world suffer from asthma. Allergic asthma is a disorder of respiratory system in air filtration that is caused by inflammatory cells.Some parasites are caused asthma symptoms due to migration and establish in the lung. Asthma symptoms are cough, shortness of breath, wheezing, bloody sputum, inflammation of

the lungs and pneumonia.But some parasites prevent asthma via interfere with immune factors. However there isn't a comprehensive report of these parasites and their effects.This study was done whit the aim of understanding a relationship between asthma and parasites.**Methods:** In this review article several reports about different parasites and their effects on lung all over the world have been reviewed.They are divided according to two criteria:parasites that cause asthma and parasites that prevent asthma. **Results:** Asthma symptoms are caused by parasites, especially worms for example *Ascaris lumbricoides*, *Trichinella spiralis*, *Strongyloides stercuralis*, hook worms, *Schistosomas*, alveolar *Echinococcus*,*Toxocara*, *Paragonimus westermani* and some arthropods such as bugs and mites.Of course for pathogenesis and causing asthma parasite burden must be optimum. Chance of asthma is greatly enhanced in developed countries that don't have health poverty. Epidemiological data suggest that respiratory allergies in those facing intestinal parasites have been significantly reduced.Intestinal Parasite suppresses the immune system of type 1 and 2.*Toxoplasma gondii*, *Plasmodium falciparum*, intestinal parasites and *Schistosomas* are such parasites to prevent asthma. An example is the researcher who infected himself with hook worms and hay fever that had suffered him for a long time, didn't came to him forever.**Conclusion:** Data of this review article show that some parasites are directly injurious for lung and cause asthma. Recently, one of the reasons for the prevalence of asthma is listed, control of parasitic diseases and immune reactions to other allergens.

Keywords: Parasites.Asthma,Disease

2335P

Proteomic response of fenugreek (Trigonella foenum-graecum) as an antiallergic plant to vermicompost amounts

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Background: Fenugreek (Trigonella foenum-graecum L.) has a broad variety of therapeutic properties for allergic and inflammatory diseases and is applied as a traditional functional food, but its antiallergenic mechanism in these diseases is yet to be clearly explained. Fenugreek proteins are a main role in antiallergic properties. Allergenic fenugreek proteins have been identified by immunoblotting methods. In this paper the effects of vermicompost on protein pattern of fenugreek has been investigated.**Methods:** In order to determine effects of vermicompost amounts and planting date on protein patten of Fenugreek (Trigonella foenum-graecum L.) this experiment was laid out. The experiment was laid out as experimental design in three planting date (20 May, 20 July and 20 September) and five different fertilizers (0, 5, 10 and 15 ton/ha vermicompost and a chemical fertilizer based on seed requirement) in three replications. The leaves were used for protein pattern analysis. For this purpose leaves protein were purified by extraction buffer. SDS-PAGE method was applied for extraction and resolving of this medicinal plant.**Results:** statistical analysis and SDS-PAGE electrophoresis results showed that planting date and different amounts of vermicompost affect on protein pattern in Fenugreek. More investigation in necessary for antiallergic proteins changes due to vermicompost application. **Conclusion:** Fenugreek proteins are affected by vermicompost application.

Keywords: Antiallergic, Fenugreek, proteins, vermicompost

2501P

Study of Osteomalacia prevalence and its related factors in patients with chronic backache in Torbat-Heydarieh

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Background: While backache is one of the common complaints in clinical medicine, specific cause of it is unclear. Enough studies have not been conducted to investigate the role of Vitamin C as a cause of backache, however, the present study was conducted to investigate the prevalence of Osteomalacia and its related factors in patients with idiopathic backache in 87-88 in Torbat-H. **Method:** This is a sectional descriptive study, conducted on 85 patients complaining of idiopathic chronic backache, and its cause was not found in the clinical reports and scanning. Biochemical tests related to serum level of calcium, phosphor, phosphatase alkaline, Parathyroid hormone, and 25 hyroxy vitamin D have been measured and analyzed. Clinical and para-clinical information were analyzed by using statistical tests.**Resulte:** We selected 85 patients, 25 male and 60 female, out of which 45 people had Osteomalacia. The highest rate of the patients having such disease were 40-49 years old, and the lowest rate were under 20 years old (6%), 27 patients with lack of vitamin D haven't taken advantages of the sun. The most common sign in the patients with Osteomalacia was found in their lower limb(56%). 22 patients, not being exposed to the sun sufficiently, depite to reciving Vitamin D had Osteomalacia. The average of vitamin D in subjects with and without Osteomalacia and was 10.52 mg/ml and 58/54 mg/ mg, respectively. 50% of the patients with Osteomalacia had a low deficiency, 24% medium deficiency, and 27% had a high deficiency. **Conclusion :**Because of relatively high prevalence of vitamin D deficiency in patients with idiopathic backache, applying methods such as food fortification in order to provide vitamin D needed to the body and self-protection instructions in order to take appropriate advantages of the sun light is required.

Keywords: Osteomalacia, Idiopathic backache, vitamin D

2812P

Assessing Obesity and Overweight as Risk Factor for Developing Asthma Later on among Children in Primary Girl School in 18th Region in Islamshar.

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Background: Over the last two decades asthma and overweight or obesity prevalence are both increasing worldwide! It's well known that obesity can cause diabetes and heart disease, but studies show that it also leads to higher rates of asthma. Many studies in this area results showed that overweight and obesity has been suggested as a risk factor for developing asthma. Also some of the cohort studies results showed that there is more strong relationship of obesity/ overweight and asthma among women than men. Body mass index (BMI) is a measure used to determine childhood overweight and obesity. The aim of this study is to determine obesity and overweight as risk factor for developing asthma among primary girl school (Ghods) in 18th region of Islamshar. **Methods:** This cross sectional study was performed in 18th region of Islamshar. There was only one primary school (Ghods) in this region. After getting the permission from the head of the school and the students' parents, all students whom attending

to this school (n=155) were participated to this study. A questionnaire was designed to collect the demographic data which completed by face to face interview. Also some of the data were extracted from the students' health records. The weights and heights of the students without shoes and with the appropriate methods of anthropometry were taken by using a standard instrument for measuring weight and height which was related to the region's health house. Trained field nursing students took both the measurements of weight and height. In this study, BMI for age (Body Mass Index) was used as the anthropometric indicator for determining obesity and overweight as risk assessment for asthma among the students. The value of BMI for age used, were based on the reference data of the national BMI percentile for this age groups of girls which the charts were on their health records. A child was considered underweight or having low BMI for age when her BMI for age was $<5^{th}$ percentile and overweight when her BMI for age was $> 95^{th}$ percentile. Appropriate descriptive statistical analysis was performed. **Results:** Majority (6/25%) of the students were 11 years old. Data from the students' health records and the research questionnaire showed that 94% of the samples did not have any health problem. In general 81/93% of the students were underweight. Among this group lowest rate of median score of BMI was 14 related to grade one and highest median score of BMI belong to grade four which was 17/39. It showed that students in higher grade in primary school had and highest median score of BMI. % 17/41 of the students had normal range of BMI and only 0/64% of the study samples were overweight. **Discussion:** Therefore it is concluded that obesity and overweight is not a major problem, as risk factor of developing asthma later on among the study samples. Anyway according to results which showed that majority of the students were under weight so probably they are at risk for other health problem related to their undesirable range of BMI. Hence it is usually recommend further studies on other risk factors affecting onset of asthma for this group.

Keywords: Obesity, Overweight, Risk factors and Asthma, Primary School.

3013P

Circulating Level of $CD4^+CD25^+$ $Foxp3^+$ T Cells in Patients with Chronic Urticaria

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Background: CD4+CD25+ T regulatory (Treg) cells play critical roles in maintaining peripheral tolerance and preventing autoimmunity. As characteristics of Treg cells have not been precisely investigated in chronic urticaria (CU) yet, this study was performed. To determine the frequencies of circulating CD4+CD25+ FOXP3+ T cells, and serum level of IL-10, TGF-β, and IL-17 in chronic autoimmune urticaria (CAU) and chronic idiopathic urticaria

(CIU) patients compared to healthy controls. **Methods:** Peripheral blood mononuclear cells (PBMCs) were obtained from patients with CU and healthy controls. The frequency of CD4+CD25+ T cells in PBMCs and expression levels of FOXP3 were detected by flow cytometery. The serum level of IL-10, TGF-β and IL-17 were measured by ELISA. **Results:** A significant decreased in the percentage of circulating CD4+CD25+FOXP3+T cells was detected in CU patients, compared to control subjects. However, no significant difference was detected on the serum levels of IL-10, TGF-β, and IL-17 between CU patients and control subjects. **Conclusions:** This study demonstrated that the frequency of Treg cells in PBMCs was decreased in CU patients. Further studies are needed to clarify the exact role of Treg cells in the pathogenesis of CU and factors regulating their function.

Keywords*:* Treg; chronic urticaria; IL-10; IL-17; TGF-β

2862P

The effect of symptoms based written action plan on asthma control according to Asthma Control Questioner score in children

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Background: Little understanding of the severity of symptoms in patients with asthma led to the misuse of drugs frequently and increases the risk of asthma attacks an visit to the emergency room. The symptoms-based written action plan (WAP) is recommended for most patients with asthma to reduce the incidence of chronic disease. Unfortunately, despite these recommendations, the use of these tools to control asthma is still not common. The aim of this study was to assess the effect of WAP on asthma control in children aged 7-18 years.**Method:** This study was randomized control trial that has been done on children and adolescents with asthma. Sixty patients who referred to the asthma clinic were included in this study. Patients were randomly divided in to two groups to receive or not receive the WAP in addition to standard treatment of asthma. Patients in both groups were followed weekly for 3 months. Data was analysed using SPSS version 16.0 (Chicago, IL, USA).**Result:** At the end of the study period, there was significant difference between the level of asthma control (ACQ) in case compared to control group (p=0.0001). **Conclusion**: The use of written asthma action plan with effective control, can led to decrease in asthma attacks, hence reduced the additional costs of asthma complications.

Keywords: Asthma, Action plan, Asthma control questionnaire

2379P

The investigation of MMP-2 protein expression in lung fibrosis induced by bleomycin in mice

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Background:pulmonary fibrosis (PF) is a chronic and progressive disease with a median

survival of 3–5 years following diagnosis. It can be conceptualized as resulting from an imbalance between normal processes of synthesis and degradation of extracellular matrix (ECM) components. Matrix Metalloproteinases (MMPs) in particular MMP-2, a member of proteolytic enzyme family, degrade the extra cellular matrix and basement membrane and have an important role in the development of pulmonary fibrosis. In this research, the expression of MMP-2 protein in the pulmonary fibrosis induced by bleomycin was investigated in the form of qualitative and quantitative in mice. **Methods**: In this experimental study, sixteen C57BL/6 mice were divided into 2 groups. Mice received in group1(Experiment group), bleomycin sulfate and in Group2(control group) CMC intraperitoneally. The mice were sacrificed at the end of experiment and Lung samples were collected from two groups and were prepared for histological and immunohistochemical studies. Then investigated the histological changes of lung tissues and MMP-2 protein expression and Results were analyzed using Student t-test. **Results**: Histological studies in the experimental group showed inflammatory response, collagen deposition and increase of connective tissues amount in the lung alveolar septa in comparison to control group. Immunohistochemical studies showed that the number of MMP-2 protein expressing cells significantly increased in the experimental group compared to the control group(P<.001).**Conclusion**: The results of this study showed that the number of cells expressing MMP-2 protein increases in pulmonary fibrosis induced by bleomycin.

Keywords: Pulmonary fibrosis, MMP-2 expression, bleomycin

3083P
Neurological effects in patients with Rheuma toidrathritis

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Background: Rheumatoid arthritis (RA) is a chronic sickness in which system organs conflicted by 1-3 percent. Outer joint involvement including Lungs, Eye, Vascular, Neurological manifestations. neurological effects of the disease are divided by tow, 1_involving central nerve system, mainly Cervical spinal cord . 2_Involving peripheral nervous system includes involvement of peripheral nerves, Poly neuropathy, Mononorit multiplex (sensory & motor) assessment and a better understanding of neurological effects in RA patients provides the possibility of early diagnosis and appropriate treatment.to achieve this goal, from 88_87 years ,for 18 months a search has been done in Torbal-heydariyeh.**Method :** This search is of the cross-sectional that due to it, the patient were 60 and base on ACR criteria the diagnostic actions was under staging of disease improving. all patients were performed under Nasvltral neck radiography & upper extremity Electrodiagnostic studies. The result of research in this perusal 72/2% female and 72/2% male were between 50/21±14/9.Te most common clinical symptom was morning stiffness(84/2%) and the most common involving joint was the wrist (75/6%).ERS was increased in 53/7% of patients and CPR was positive in 61/7% and also RF in 51/2%.0/4 of patients were in Stage I, 48/9% in Stage II, 25/2% in Stage III and 21/9% in Stage IV. **Result:** In 57/3% of patients observed nervous system involvement, in 23/4% involvement of central nervous and in 33/8% peripheral nervous system involvement. The most common effects of central nervous system involving the lower cervical spine in11/3%, 8/2% involving cervical spine, 3/8% involving the Atlantoaxial. The most common effects of

peripheral nervous system was obstruction of nerve 19/3 percent and sensory Polyneuropathy 12/2 percent. The most common type of nerve involvement was Carpal tunnel syndrome that reported In 9 patients.**Conclusion:** One of the serious and threatening symptoms of Rheumatoid arthritis was nervous system involvement ,rate of neurological effects increases with disease progression .performing periodic neck radiography and studies in patients with disease duration of more than 2years, Deformity or Erosions is one of necessary doing in order to control the neurological effects.

Keywords: Neurology, Rheuma toidrathritis.

3320P

Comparison of the prevalence of allergies between two ISAAC studies in Birjand city

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Background: The prevalence of asthma and other allergic diseases has been increasing significantly during the past decades. Many studies have confirmed this finding not only in modern countries but also in developing and under developed countries. Many factors contribute to this increase but the main factors are different in different societies and areas. Data about the change in prevalence and risk factors in each society is primary step for prevention and management of allergic diseases. The aim of this study was to evaluate the changes in prevalence of asthma and other allergic diseases among 13-14 years old children in a Birjand city after 16 years. **Methods:** In a cross-sectional study, validated Persian version of ISAAC written questionnaire was used to evaluate prevalence of allergic symptoms among 13-14 years old students in March 2011. The same questionnaire and protocol has been used in 1994 ISAAC survey. 3320 and 3100 questionnaires were returned in 2011 and 1994 respectively. **Results:** Out of the 3320 students, 3000 were participated in this study. 56% were female and 44% were male. In comparison to 1994 survey, prevalence of asthma has not been changed significantly (3.8% vs. 3.7% in 1994 and 2011 respectively); but prevalence of rhinitis (9.6% in the year 1994 versus 19.7% in 2011) and eczema (11.3% in 1994 versus 26.8 in 2011) has been increased during the 16 years period significantly. **Conclusion:** The result of this study confirmed the low prevalence of asthma bus also showed that wheeze, nasal and ocular symptoms, as well as eczema have been increased in a large extent. Further studies need to reveal the underlying factors for this increase.

Keywords: Allergy, ISAAC, Questionnaire

2844P

Temporal expression profile of CXC chemokines in serum of patients with spinal cord injury

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Background: Chemokines, a subclass of cytokine superfamily have both pro-inflammatory and migratory role and serve as chemoattractant of immune cells during the inflammatory

responses ensuing spinal cord injury (SCI). The chemokines, especially CXCL-1, CXCL-9, CXCL-10 and CXCL-12 contribute significant part in the inflammatory secondary damage of SCI. Inhibiting chemokine's activity and thereby the secondary damage cascades has been suggested as a chemokine-targeted therapeutic approach to SCI. **Methods:** To optimize the inhibition of secondary injury through targeted chemokine therapy, accurate knowledge about the temporal profile of these cytokines following SCI is required. Hence, the present study was planned to determine the serum levels of CXCL-1, CXCL-9, CXCL-10 and CXCL-12 at 3-6h, 7 and 28days and 3m after SCI in male and female SCI patients (n=78) and compare with age- and sex-matched patients with non-spinal cord injuries (NSCI, n=70) and healthy volunteers (n=100). ANOVA with Tukey post hoc analysis was used to determine the differences between the groups. **Results:** The data from the present study show that the serum level of CXCL-1, CXCL-9 and CXCL-10 peaked on day 7 post-SCI and then declined to the control level. In contrast, significantly elevated level of CXCL-12 persisted for 28 days post SCI. In addition, post-SCI expression of CXCL-12 was found to be sex-dependent. Male SCI patients expressed significantly higher CXCL-12 when compared to control and SCI female. We did not observe any change in chemokines level of NSCI. **Conclusion:** Further, the age of the patients did not influence chemokines expression after SCI. These observations along with SCI-induced CSF-chemokine level should contribute to the identification of selective and temporal chemokine targeted therapy after SCI.

Keywords: Chemokines, Neuroinflammation, Secondary injury

Cancer immunology & Immunotherapy

Oral Presentations:

2897O

CD4$^+$CD25$^+$ lymphocytes are involved in initial tumor development

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Background: CD4$^+$CD25$^+$ regulatory T cells (Tregs) are the most studied regulatory T cell subset that mediates immunological tolerance to self and non-self antigens. These cells suppress effector cells of immune system by various mechanisms. Increased levels of Tregs have been reported in cancer patients. We investigated the frequency of CD4$^+$CD25$^+$ T cells in tumor model and their role in tumor development. **Methods:** Murine fibrosarcoma tumor was inoculated subcutaneously in flank. Percent of CD25 expressing cells was determined by flow cytometry in tumor draining lymph nodes. Gene expression analysis was performed by real time RT-PCR. In order to investigate the role of CD4$^+$CD25$^+$ Tregs in tumor growth, these cells were depleted using anti-CD25 monoclonal antibody. **Results:** CD4$^+$CD25$^+$ T cells were increased in tumor draining lymph nodes in comparison with healthy hosts. Gene-expression analysis showed expression of genes related with Tregs such as the transcription factor Foxp3 and interleukin-10 in the lymph node cells before tumor inoculation. Depletion of Tregs before tumor inoculation clearly led to delay in tumor growth even though depletion after tumor inoculation had not notable effect on tumor growth. **Conclusion:** These results demonstrated that CD25$^+$ Tregs are associated with tumor growth especially in initial phase of tumor development.

Keywords: Regulatory T cells, Lymph node, Gene expression, Depletion, Tumor development

2896O

Foxp3-Fc (IgG) fusion construct efficiently decreases regulatory T cells in mice

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Background: Apparently, regulatory T cells (Treg)play a critical role in the attenuating immune responses against tumor cells in tumor microenvironment. Therefore, several methods have been introducedfor eliminatingTreg. Among them, instructing immune response against Treg expressing Foxp3transcription factor is a hopeful approach to decrease Treg frequency. In current study, we design a fusion construct expressing Fc portion of IgG and Foxp3 to effectively instruct immune response against Treg. **Methods:** DNA construct containing Fc portion of IgGfused to Foxp3 was designed. The expression of DNA construct was investigated by transfection into HEK cells. This DNA construct was used as DNA vaccine. For protein vaccine, FOXP3-Fc fusion construct was inserted into pET21a vector and consequently, E. coli strain BL21 was selected as host cells. The expression of recombinant fusion protein was assayed bywestern-blot analysis. Afterward, fusion protein was purified by SDS PAGEreverse staining. This purified fusion protein was considered as protein vaccine. DNA construct and respective protein were injected into *C57BL/6*mice. After 2 weeks, the frequency of Treg in spleen was investigated by FACS analysis. **Results:** The expression analysis of DNA construct by flow cytometry and fluorescent microscopy showed that this construct successfully expressed in eukaryotic cells. Moreover, the Foxp3-Fc expression was confirmed by western blot analysis. Additionally, the presence of fusion protein was shown by specific antibody after purification. FACS analysis of Treg in spleen revealed that the frequency of Treg significantly decreased in mice vaccinated with fusion protein compared with groups vaccinated with Foxp3 construct and recombinant protein. **Conclusion:** We conclude that targeting Treg expressing FOXP3 by our novel fusion constructcause more efficient decrease in Treg. This novel approach can be considered for efficient elimination of Treg in tumor bearing mice.

Keywords: T regulatory, FOXP3, Fragment c,IgG, Tumor

1968O

Ligation of human Fc receptor like 4 (FCRL4) by specific monoclonal antibodies down-regulates B cell receptor mediated signaling

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Background: FCRL4 are exclusively expressed on memory B-cells and has inhibitory roles due to the presence of ITIM motif in its cytoplasmic tail. In the present study we aimed to investigate the FCRL4 signaling on B-cells by specific anti-FCRL4 monoclonal antibodies (mAbs). **Methods:** FCRL4 gene was cloned in eukaryotic and prokaryotic expression vectors to produce stable transfected cell lines and recombinant FCRL4 protein. Different strategies were applied to immunize mice for mAb production. Hybridoma cells producing FCRL4-specific mAb were generated, selected, cloned and screened by FACS and ELISA. FCRL4 signaling was studied in DG-FCRL4 transfected cells by immunoblotting and FACS using

specific mAbs. **Results:** We successfully established stable cell lines (CHO and DG75) expressing FCRL4 molecule which were used for mAbs screening and also signaling studies. Investigation of the signaling potential of mAbs showed that signaling with anti-FCRL4 mAbs (1A5-C10 and 1A2-C6) inhibited the calcium influx, but not phosphorylation of the total protein tyrosine induced by anti-IgM Ab in FCRL4-transfected DG75 cell line. Moreover, FCRL4 signaling substantially reduces phosphorylation of Erk1/2 and p38 in the stimulated target cells. **Conclusion:** Our findings indicate that the anti-FCRL4 mAbs potently inhibit BCR signaling mainly through MAP kinase (Erk1/2 and p38).

Keywords: FCRL, B-cell receptor, Monoclonal antibody, Signaling, MAP kinase

1811O

Antagonistic effects of IL-21 and vitamin A on B cell proliferation from different subsets of chronic lymphocytic leukemia

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Background: Interleukin-21 (IL-21) is a potent regulator of B cell proliferation and differentiation. While IL-21 induces apoptosis in effector B cells activated through toll like receptor (TLR), it enhances B cell proliferation, isotype switching and differentiation in B cells stimulated through CD40L. Vitamin A, on the other hand, enhances B cell proliferation in the presence of TLR9 agonist, CpG-ODN. In the present study we report the combination effect of IL-21 and CpG on leukemic B cells from patients with chronic lymphocytic leukemia (CLL). **Methods:** Patients were broadly classified into either progressive (n=6) or indolent (n=18), and immunoglobulin heavy chain variable region (IGHV) genes mutated (n=16) or unmutated (n=8) subsets. Peripheral B cells were isolated and immunophenotyped using magnetic beads and flow cytometry, respectively. The proliferative effects of different combinations of IL-21, vitamin A and CpG-ODN on CLL and normal B cells were analyzed using H3-thymidine incorporation assay. **Results:** Our results showed that while vitamin A enhances CpG-induced proliferation in normal B cells it inhibits leukemic B cell proliferation. Addition of IL-21 to vitamin A significantly inhibits proliferation of normal B cells as well as leukemic B cells from progressive, but not indolent patients. **Conclusion:** Our results indicate antagonistic effects for IL-21 and vitamin A in regulation of B cell proliferation in different subsets of CLL patients and normal subjects.

Keywords: IL-21, vitamin A, CpG, Chronic lymphocytic leukemia, B cell proliferation

1515O

Evaluation of TCD3 Foxp3 expression, NK cytotoxicity, IFN-γ, IL-4 levels and Her2 expression in samples from breast Cancer

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Background: In order to evaluation immunological parameters status and their relationships in breast cancer progression the present study was design. **Methods**: The diagnosed breast cancer samples (n=29) were prepared and the following tests were applied. Tissues were process for immunohistochemistry (IHC) to determine Her2 expression. The obtained blood serums were kept for cytokines measurements. The PBMC were used for NK cell cytotoxicity against sensitive K562 cells. And TCD3Cells were isolated and the Foxp3 expression cells were assayed by flowcytometry. **Results**: The 84% of samples were diagnosed as invasive ductal carcinoma, the means ± SD of NK cells cytotoxicity percentage at both E:T ratio 50:1 and 25:1 were significantly lower as compared with control group. The CD3 T cells expressing Foxp3 were 1.23%± 0.46% in breast cancer samples and 0.94%± 0.9% in control group. The serum IFNγ level was 4.3 ±2.3 ng/ml in patient serums and 7.4±2.7 ng/ml in control group, and IL-4 was 24.5 ±3.9 ng/ml in patients and 12.5±4.4 ng/ml in control group. 38% of the patients were HER2 positive with more than one fold overexpression. The Pearson correlation for the parameters assayed does not reach statistically significant (P> 0.5). **Conclusion;** the data obtained indicate independent role for the parameters assayed and weak immune response status overall in the host and skew to escaping mechanisms for tumors.

Keywords: Breast Cancer, IFN-γ, IL-4, Foxp3

1902O

Deciphering cellular characteristics of tumorogenic subpopulations in human colorectal cancer reveals cellular plasticity

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Background: A great deal of study has been revealed that human colorectal cancer consists of a phenotypically distinct minority population of tumorigenic cancer cells known as Cancer Stem Cells (CSCs) which play a pivotal role in cancer progression, maintenance, metastasis and the relapse. The aim of this effort was to investigate and compare cellular characterizations of CD133+ with CD133- cell subsets isolated from both primary and metastatic human colorectal tumors. **Methods:** Using our optimized protocols, unfixed colorectal tumors were enzymatically and mechanically dissociated into single cells followed by evaluation

of post-digestion viability. The obtained single cell suspensions were then subjected to cell sorting using magnetic beads according CD133 marker. The resultant CD133+ and CD133- cell subsets were cultured in specific cell culture medium. Finally, flow cytometric analyses were performed to compare cellular characterizations of CD133+ and CD133- cell subsets. **Results:** The results demonstrate that CD133+ cells have smaller size and lower complexity of intracellular structure, sphere formation ability and ALDH enzyme activity. CD133- cells isolated from primary colon cancer samples were not able to form sphere and did not show ALDH enzyme activity. Intriguingly, CD133- cells isolated from metastatic colorectal cancer specimen were able to form sphere and shown ALDH enzyme activity. **Conclusion:** The present study indicates that our results are in agreement with stem cell theory and portrays the possibility of the existence of cellular plasticity among cancer subpopulations, similar to their normal compartment. Therefore, clinical cancer immunotherapeutic strategies directed solely against CD133 antigen should be reconsidered.

Keywords: Colorectal cancer, CD133 antigen, Cellular plasticity, Immunotherapy

2041O

Induction of HER2 specific anti tumor antibody response by DNA immunization

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Background: *HER2* protooncogene is overexpressed in approximately 20-30% of breast cancer patients. Anti-HER2 monoclonal antibodies (mAbs) are currently employed for immunotherapy of breast cancer. An alternative to passive immunotherapy by mAb is active immunization using an immunogenic preparation of *HER2*. In the current study, we immunized Balb/C mice with a plasmid construct containing the human *HER2* gene. **Methods:** The extracellular domain (ECD) of human *HER2* was amplified and cloned in a eukaryotic expression vector. This construct was then used to immunize Balb/C mice. Serum antibody levels in hyperimmunized mice was titrated by ELISA and functional activity of anti-HER2 Ab was assessed on HER2 overexpressing breast cancer cell line (BT-474) by flow cytometry and XTT assays. **Results:** Serum titration of immunized mice demonstrated induction of a high titer of HER2-ECD specific antibody. Flow cytometric analysis showed efficient binding of the antibody to BT474 cells. Proliferation of BT474 cells was inhibited by treatment with HER2-immunized serum. **Conclusion:** Induction of an efficient antibody response to HER2 by DNA immunization in an animal model indicates immunogenicity of the construct and suggests that this approach might be applicable in breast cancer patients.

Keywords: HER2, DNA vaccination, Breast cancer, Monoclonal antibody, Immunotherapy

1956O

Modulation of Wnt5A expression by Stat-3 and NF-ĸB signaling pathways in ovarian cancer

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Background: Wnt5A is a member of the non-transforming Wnt protein family implicated in inflammatory processes and is highly expressed by ovarian cancer cells. This study sought to determine the possible modulation of Wnt5A expression by inflammatory pathways in ovarian cancer cell line, SKOV-3. **Methods:** SKOV-3 cells were treated with 25 µM Stat-3 inhibitor (S1155S31-201) or 10 µM NF-ƘB inhibitor (BAY11-7082) for 8, 12, 24 and 48 hours. To assess the modulatory role of proinflammatory cytokines on Wnt5A expression, cells were treated with 10 ng/ml recombinant human IL-6, TNF-α (rhIL-6, rhTNFƘ) or with combinations of cytokines and the aforesaid inhibitors for different periods of time. At the end of incubation times, Wnt5A expression levels were determined by western blot analysis. **Results:** There was a strong down regulation of Wnt5A expression in the presence of Stat-3 or NF-ƘB inhibitors. Wnt5A expression was increased 4-fold after 8 hours stimulation with rhIL-6, while rhTNF-α exerted no detectable effect. Increased Wnt5A expression by rhIL-6 was abrogated in cells treated with Stat-3 or NF-ƘB inhibitors. **Conclusion:** This study revealed for the first time the critical role of Stat-3 and NF-ƘB transcription factors on Wnt5A expression by ovarian cancer cells. Our data suggest that IL-6 exert its modulatory role on Wnt5A expression through Stat-3 and NF-ƘB signaling pathways.

Keywords: Wnt5A, Ovarian cancer, Inflammation, Stat-3, NF-ƘB

1427O

The effect of IL-12 gene therapy in the regression of tumor masses in mouse model

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Background: Immunotherapy is one of cancer treatment strategies. Recently, interluekin 12(IL-12) has been used as immunotherapeutic agents in cancer gene therapy. IL-12 is one of prominent cytokine in activating DCs and boosting anti-tumor immune responses. Here, we investigated the antitumor effects of gene therapy with IL-12 in the regression of tumor masses in mouse model. The aim of this study is to determination the effect of gene therapy with IL-12 in the regression of tumor masses in mouse model. **Methods:** To study the therapeutic efficacy of this cytokine, we used tumor cells transfected with mIL-12 plasmids. Then ELISA test was used to check the cytokine production by transfected cells. Tumoral transfected cells were injected subcutaneously to inoculate tumor in BALB/c mice. We measured tumor volumes by caliper. Then the mice were sacrificed and tumors extracted. The expression of IL-12 and IFN-γ were studied with Real-time PCR and immunoblotting. The expression of Ki-67 (tumor proliferation marker) in tumor masses was studied by immunohistochemistery staining. **Results**: Our results demonstrated that, gene therapy with IL-12 displayed therapeutic effects on the regression of tumor masses in mouse model. IL-12 and INF-γ expression enhanced in mice treated with IL-12 in comparison to control group and Ki67 expression declined in

group treated with IL-12. **Conclusion:** Gene therapy with IL-12 has therapeutic effects on the regression of tumor masses in fibrosarcoma mouse model.
Keywords: Gene therapy, IL-12, Tumor

1594O
5-Flurouracil (5-FU) modulates the function of myeloid derived suppressor cells (MDSC) in melanoma bearing mice

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Background: Myeloid-derived suppressor cells (MDSC) are one of the major cells with immunosuppressive role that accumulate in the spleen and tumor microenvironment in tumor bearing subjects. Inflammatory mediators such as reactive oxygen species (ROS) and nitric oxide (NO) are the main products of MDSCs that mediate chronic inflammation in cancer. Previous reports have shown that suboptimal doses of anti-cancer agent 5-Flurouracil (5-FU), not only selectively decrease the number of MDSC in tumor bearing mice, but also improve tumor specific immune responses. In this research, we evaluated the effect of suboptimal doses of 5-FU on the frequency of MDSCs and expression of ROS and NO in spleen and tumor infiltrated cells in experimental model of melanoma. **Methods:** B16-F10 cell line was injected subcutaneously to induce melanoma in C57/BL6 mice. 50 mg/kg of 5-FU injected intra-peritoneally in tumor bearing mice. Spleen and tumor infiltrated leukocytes separated and the frequency of MDSCs expressed ROS analyzed by flow cytometry using Gr1, CD11b and DCFDA. Moreover, the nitrite derived from NO catabolism evaluated by Griess reagent. **Results:** we showed that not only 5-FU does decrease ROS production in MDSC population in spleen and tumor but also it can reduces nitrite derived from NO which was produced by these cells. **Conclusion:** According to the finding of current study, 5-FU reduces the production of inflammatory mediators in MDSCs and this specificity in combination with other therapies may improve the immune responses against tumors.
Keywords: Myeloid derived suppressor cells, 5-Flurouracil, Reactive oxygen species, Nitric oxide

1738O
Chimerization and humanization of an inhibitory anti-HER2 mouse monoclonal antibody

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Background: Humanized monoclonal antibodies (mAbs) against HER2 including Trastuzumab and Pertuzumab have been approved for treatment of HER2 overexpressing metastatic breast cancers. Pertuzumab and Trastuzumab recognize different epitopes of HER2

and their combination was found to enhance blockade of HER2 signaling than Trastuzumab alone. Recently, we have reported production of a new inhibitory anti-HER2 mouse mAb (m-1T0), which binds to an epitope different from that recognized by Trastuzumab. Here, we report chimerization and humanization of this mAb. **Methods:** Chimerization was performed by amplification of the immunoglobulin variable region heavy (VH) and light (VL) chain genes from cDNA of 1T0 hybridoma cells and their ligation to human gamma-1 (γ1) and kappa (κ) constant region genes using Splice Overlap Extension (SOE) PCR. The humanized form of 1T0 was designed by grafting all six complementarity determining regions (CDRs) onto the most homologous human variable germline genes while preserving the donor framework residues which seem to contribute to HER2 binding. Humanized VH and VL genes were synthesized and ligated to human γ1 and κ constant region genes as described earlier. Subsequently, the chimeric (c-1T0) and humanized (h-1T0) mAbs were expressed in CHO cells and characterized by ELISA, Western blot and flow cytometry. **Results:** The purified chimeric and humanized antibodies specifically bind to recombinant HER2 and HER2 overexpressing tumor cells and inhibited proliferation of these cells. The binding affinities of the chimeric and humanized mAb were comparable to the parental mouse mAb. **Conclusion:** This is the first report on chimerization and humanization of a mouse mAb in Iran. This new humanized mAb is potentially a valuable tool for breast cancer immunotherapy.

Keywords: Chimerization, Humanization, Monoclonal antibody, HER2, Breast cancer

1603O

Improvement of immune response against Her2-derived p5 and p435 peptide by PADRE peptide in breast cancer vaccine

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Background: It is now clear that $CD4^+$ T cells play a crucial role in the generation of $CD8^+$ T cells effector and memory T-cell immune responses. Furthermore, it is desirable to generate antigen-specific CD4+ T cells in addition to antigen-specific CD8+ T cells in vaccination strategies. The pan HLA DR-binding epitope (PADRE) has been proposed as a simple carrier epitope suitable for use in the development of peptide vaccines. Here in line with our previous study, we investigate whether administration of the PADRE peptide enhances antigen-specific $CD8^+$ T-cell immune responses against rHER2/neu-specific CTLs epitopes (p5 and p435). **Methods**: BALB/c female mice were vaccinated 3 times with p5 or p435 peptide, alone or co-administered with PADRE peptide.14 days after last vaccination 3 mice per group were euthanized and immune responses were studied in their spleens for assessment TCD4 and TCD8 subpupulation by flow cytometry and IFN-g by Elispot assay. Six mice per group were challenged by live TUBO cell line and followed for tumor size and survival. **Results:** Our result show that mice vaccinated with p5 and p435 peptide in combination with PADRE induced higher antigen-specific T-cell responses compared with groups without and PBS. Also tumor in these mice grew slowly compared with group without PADRE and survival rate

was significantly improved. **Conclusion**: The combination of PADRE peptide with antigenic peptides (p5 and p435) is capable of generating potent antigen-specific CD8+ T cell immune responses and antitumor effects in vaccinated mice.
Keywords: PADRE, Her2/neu, MHC class II

2149O
The effect of mir-143 on decrease expression levels of pro-apoptotic TNFRSF10a molecule in Jurkat cells

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Background: miRNAs act as a potential regulator of physiological and genetical process like apoptosis. The previous studies express the intervention of miRNA in apoptosis but its molecular mechanism is unknown. The aim of this study was to determine the effect of miRNA-143 on the expression of TNFRSF10a as pro-apoptotic molecule. **Methods:** MiR-143, PBS and a scramble sequence were introduced, separately, to the Jurkat cell lines (T cell leukemia) and the mRNA levels of TNFRSF10a were examined in parallel with beta-actin and GAPDH (as housekeeping genes) using Real-Time PCR technique. Apoptotic changes also were identified using annexin-FITC staining method. **Results:** Our results indicated that 30% of miR-143 transfected Jurkat cells had apoptotic features. The results also demonstrated that mRNA levels of TNFRSF10a were significantly increased in the miR-143 transfected Jurkat cell line in compare to the scramble sequence or PBS transfected Jurkat cells as controls. **Conclusion:** According to our results, it may be concluded that miR-143 can lead to increase expression of pro-apoptotic molecule, TNFRSF10a, in Jurkat cell. Thus, it seems that miR-143 can consider as a therapeutic agent to treatment of cancers to induce apoptosis.
Keywords: MiR-143, TNFRSF10a, Jurkat cell

2068O
Potential application of 3'-noncoding region of poliovirus RNA genome for stabilization of RNA-based vaccines in cancer immunotherapy

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Background: Application of RNA-based vaccines, due to their higher safety measures, has recently gained importance in cancer immunotherapy. However, labile nature of RNA is a major drawback for *in-vivo* applications. The 3'non coding regions (3'NCRs) of RNA virus genomes which contain cis-acting elements that contribute in RNA stabilization might be employed to increase the stability of heterologous RNAs. **Methods:** Two reporter plasmids encoding for IRES-GFP and IRES-GFP-Poliovirus (PV)-3'NCR in tandem were constructed in pCDNA3.1 as negative and positive vectors, respectively. The effect of Poliovirus (PV)-3'NCR on mRNA stability and protein (GFP) expression was assessed by qRT-PCR, flowcytometry and fluorescence microscopy respectively in three different eukaryotic cell lines (BHK, Hela and HEK 239). RNA secondary structure prediction and motif searching was performed by

web-based softwares. **Results:** Application of PV-3'NCR affected the GFP-expression via regulation of mRNA stability in a cell dependent manner (enhancing and declining effects for BHL and Hela cells, respectively), although prediction of secondary structures indicated a generally more thermodynamically stable mRNA in the presence of PV-3'NCR. Analyses of the PV-3'NCR sequence for the presence of cis-acting motifs indicated the occurrence of hexamer; "AUUUUU" known for its destabilizing effects in eukaryotic mRNA molecules, that might be countered in part for lower GFP-mRNA stability in Hela cells. **Conclusion:** Results implied the presence of still unidentified cellular factors that might alter mRNA stability through interaction to PV-3'NCR in BHK or HEK-293 cell lines and suggested the potential application forPV-3'NCR to improve the stability of RNA molecules in cancer immunotherapy via a cell-dependent/targeted manner.

Keywords: Poliovirus, 3'NCR, RNA stability, R.NA vaccination,

2402O

Identification of a novel cancer-testis antigen, PLAC1, as a target for immunotherapy of prostate adenocarcinoma: Correlations with Gleason score and PSA expression

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Background: The scarcity of effective therapeutic approaches for prostate cancer has encouraged steadily growing interest for identification of novel antigenic targets. **Methods**: Here, we investigated for the first time the differential expression of placenta-specific antigen 1 (PLAC1) in Prostate adenocarcinoma (PCa), High-grade prostatic intraepithelial neoplasia (HPIN), benign prostatic hyperplasia (BPH) and normal prostate tissues by immunohistochemistry on tissue microarrays (n =227). The correlation of PLAC1 expression with certain clinicopathological parameters and expression of prostate specific antigen (PSA), as a prostate epithelial cell differentiation marker, was investigated. **Results**: PLAC1 expression was increased in a stepwise manner from BPH to PCa which expressed highest levels of this molecule, while in a majority of normal tissues, PLAC1 expression was not detected. Moreover, PLAC1 expression was positively associated with Gleason score ($p \leq 0.001$). Interestingly, there was a negative correlation between PLAC1 and PSA expression in patients with PCa and HPIN ($p \leq 0.01$). Increment of PLAC1expression increased the odds of PCa and HPIN diagnosis (OR: 49.45, 95% CI for OR: 16.17-151.25). **Conclusion**: Our findings on differential expression of PLAC1 in prostate cancer plus its positive association with Gleason score and negative correlation with PSA expression highlight the potential

usefulness of PLAC1 for targeted prostate cancer immunotherapy especially for patients with advanced disease.

Keywords: PLAC1, Prostate cancer, Gleason, PSA, Immunohistochemistry

3349O

Natural Killer T Cells and γd T cells in Patients with Peptic Ulcer and Gastric cancer

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Background: To clarify the effect of Natural Killer T (NKT) cells and γd T cells in pathophysiology of dyspeptic disorders and gastric cancer, the number of these two cells in patients with non-ulcer dyspepsia (NUD), peptic ulcer disease (PUD), and gastric cancer (GC) were compared. **Methods:** Patients with dyspepsia who were positive for *Helicobacter pylori* infection were selected and divided into three groups of NUD, PUD, and GC according to their endoscopic and histopathological examinations. *H. pylori* infection was diagnosed by rapid urease test and histopathology. The number of peripheral blood $CD3^+V\alpha24J\alpha18^+$ NKT cells and $CD3^+TCR\gamma d^+$ cells were measured in all patients, by flow cytometry. The number of $TCR\gamma d^+$ T cells was also determined by immunohistochemistry (IHC). **Results:** Forty six patients with NUD (32.2%), 46 with PUD (32.2%), and 51 with GC (35.7%) were enrolled in this study. The results showed that the percentage of $CD3^+TCR\gamma d^+$ cells in peripheral blood from patients with GC (2.71±0.25) were significantly lower than that in NUD (3.97±0.32, $p<0.05$) and PUD groups (3.87±0.32, $p<0.05$). However, there was no significant difference in $CD3^+TCR\gamma d^+$ cells cell percentage between the NUD and GC groups ($p>0.05$). Furthermore, the percentage of NKT cells was not significantly different among the three groups. **Conclusions:** Increased number of NKT and γd^+ T cells may contribute in the pathogenesis of PUD and GC.

Keywords: NKT cell, γd^+ T cells, Gastric cancer, Peptic ulcer

1694O

Expressions of NKG2D and NKG2A on NK cells in the presence of adipose derived stem cells isolated from breast cancer tissues

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Background: Adipose derived stem cells (ASCs) are important for their presence in tumor microenvironment due to their immunomodulatory effects on both innate and adaptive immune responses. It has been reported that ASCs can affect on the maturation, proliferation and phenotype of NK cells. The aim of this study was to assess the change in activatory receptor (NKG2D) and inhibitory receptor (NKG2A) expression on NK cells in the presence and absence of breast cancer ASCs. **Methods:** ASCs were isolated from 5 breast cancer patients

using collagenase digestion. Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors by centrifugation over Ficoll-Paque. PBMC were activated with PHA and then co-cultured with ASCs isolated from breast cancer patients. Data were compared to those from PBMCs which have not been co-cultured with ASCs. Expressions of CD3, CD16, CD56, NKG2D and NKG2A were then evaluated on PBMCs by flow cytometry method using appropriate antibodies. **Result:** The presence of ASCs caused downregulation of both NKG2D and NKG2A on NK cells. The expressions of NKG2D and NKG2A on co-cultured NK cells were 2.8-fold and 2-fold lower than that of non co-cultured NK cells, respectively. These differences were statistically significant (*p* value ‹ 0.05). **Conclusion:** Our data conclude that ASCs have the ability to induce phenotypic changes in NK cells in favor of tumor progression. Considering ASCs as important cells in tumor microenvironment, investigating their crosstalk with other cellular components of tumor microenvironment is recommended.

Keywords: Adipose derived stem cell, NKG2D, NKG2A

2223O

Comparison of functional and regulatory T cells in the mice model of melanoma microenvironment

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Background: It is believed that the tumor development is a result of an aberrant chronic inflammation in tumor microenvironment which may be due to a lack of natural balance between $CD4^{+}CD25^{high}FoxP3^{+}$ regulatory T cells (Tregs) and pro-inflammatory Th17 cells. The aim of this study was to investigate if there is a significant correlation between Treg and Th17 cells in the tumor microenvironment and lymph node in a mice model of melanoma. **Methods:** A melanoma model was developed in C57 mice. Tumor tissue and draining lymph nodes were dissected from 15 mice. The expression level of STAT3, a Th17 cell specific transcription factor, and FoxP3, a Treg marker were analyzed using reverse transcriptase real-time polymerase chain reaction. **Results:** Tumor tissue and draining lymph nodes showed increased levels of STAT3 and Foxp3 expression during tumor progression. Furthermore, there was a significant correlation between the expression level of STAT3 and Foxp3 ($r2 = 0.87$, $p<0.01$). **Conclusion:** Our results indicate a simultaneous increase in STAT3 and Foxp3 gene expression in tumor tissue and draining lymph nodes that can lead to increased inflammation and tumor growth.

Keywords: Chronic Inflammation, Gene Expression, Tumor Microenvironment, Lymph Node, Regulatory T Cells

1776O

Nuclear pattern of CXCR4 expression is associated with a better overall survival in patients with gastric cancer

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Background: Previous studies have shown that stromal derived factor-1 (CXCL12) and its receptor, CXCR4 play a crucial role in metastasis of various tumors. Similarly, it has been cleared that CXCR4 is expressed on the cell surface of gastric cancers. However, nuclear expression of CXCR4 and its clinical importance has not been yet studied. **Methods:** Herein, we studied the expression of CXCR4 in gastric samples from patients with gastric adenocarcinoma as well as human gastric carcinoma cell line, AGS, by employing RT-PCR, immunohistochemistry and flow cytometry techniques. **Results:** RT-PCR data showed that CXCR4 is highly expressed on AGS cells. This was confirmed by IHC and FACS as CXCR4 was detected on cell membrane, in cytoplasm and nucleus of AGS cells. Moreover, we found that both cytoplasmic and nuclear CXCR4 are strongly expressed in primary gastric cancer and the cytoplasmic pattern of CXCR4 tends to be associated with a shorter overall survival than nuclear staining. **Conclusion:** we present evidence for the first time that both cytoplasmic and nuclear expression of CXCR4 is detectable in gastric cancer tissues. However, the role of both cytoplasmic and nuclear CXCR4 needs to be further elucidated.

Keywords: Nuclear pattern, CXCR4, Overall survival, Gastric cancer

Poster Presentations:

2627P

1,3-Di amino propan vanadium acetil acetate 3- metoxy mediated cell cycle arrest in k562 cell line

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Background: Previous studies revealed that Vanadium complex exhibit interesting biological properties**.** The goal of this study is to examine the antiproliferative and apoptosis induction virtue of newly synthesized shift-base of vanadium (VOLComplex: $C_{19}H_{20}N_2O_5V$) on K562 leukemia cells. **Methods:** The MTT results showed that the K562 cells viability were sensitive to Vol complex in a concentration dependent manner The results of apoptosis and flow cytometry examination showed that exposure of K562 cells to non-cytotoxic dose of VOL complex led to induction of apoptosis in a dose and time dependent manner. **Results:** The most apoptosis (37.96%) occurred after 48 h treatment in response to 350 µg/ml Vol complex. Also, The cell cycle analysis showed that, VOL complex induced G0/G1 arrest. **Conclution:** This finding indicated that Vol complex, even in noncytotoxic dose, have just a apoptotic inducing effect and capable of arresting the affected cell in Go/G1 phase of cell cycle.

Keywords: Apoptosis, MTT, Cell cycle, Flowcytometry, Leukemia

2472P

Cytokine profile in peripheral blood and tissue samples from patients with breast cancer

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Background: CD4+ T cells are the central part of the adaptive immunity that can be divided into different subtypes based on their cytokine profile. Despite the effector responses formed in patients with cancer, such responses often fail to eliminate tumors. In this study, we investigated the frequency of IL-4 (Th2-), IL-17 (Th17-), IFN-γ (Th1-) and IL-10-producing CD4+ T cells in blood of patients with breast cancer and compared the results with those of healthy volunteers. We also evaluated the mRNA expression of these cytokines in tumoral and normal breast tissues. **Methods:** The frequencies of the cytokine-producing CD4+ T cells in peripheral blood samples were analyzed by flow cytometry. The expression of IL-4, IL-10, IL-17 and IFN-γ genes were analyzed in tumoral and normal breast tissues by real-time PCR. **Results:** The flow cytometry analyses revealed increases in IL-4 (p=0.001) and IFN-γ (p<0.0001) -producing CD4+ T cells in the patients' peripheral blood and the IFN-γ/IL-4 ratio was higher in the patients (12.21), compared to the normal individuals (6.6). The real-time PCR data showed increased gene expression for IL-4 (2.51±0.59), IL-10 (4.78±2.85), IL-17 (2.3±0.7) and IFN-γ (8.25±1.87) in the tumor tissues. **Conclusion:** Our results suggest that patients with the breast cancer exhibit strong Th1 and Th2 effector responses in their peripheral blood and a significant increase in IFN-γ gene expression in the tumor tissues. Therefore, it appears that patients with the breast cancer do not fail to produce the effector responses; however due to the tumor suppressive microenvironment (influenced by IL-10), such responses are not sufficiently effective.

Keywords: Breast Cancer, Th1 and Th2 responses, IFN-γ/IL-4 ratio

2840P

The cytotoxicity of sodium butyrate (NaB) on human colorectal adenocarcinoma cell line

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Background: Colorectal carcinoma (CRC) is the fourth the most frequent causative agent of cancer worldwide with high mortality. Histone deacetylase (HDAC) inhibitors induce growth arrest and apoptosis in a variety of human cancer cells. Sodium butyrate (NaB), a short chain fatty acid, is belong to HDAC inhibitors which is released in the colonic lumen as a consequence of fiber fermentation. **Methods:** The cell viability was determined by MTT assay. The Adherent SW480 (the human colorectal adenocarcinoma cell line) cells were seeded in 96-well plates, NaB (0,1.0,2.5,5.0,10,20 μmol/L) was added and incubated for 12, 24, 48, and 72 hours at 37◦C with 5% CO2. MTT solution was added to each well. dimethylsulfoxide was added After 4h. The absorbance was measured at 550 nm in a spectrophotometer. **Results:** After 24 hours, a dose dependent cytotoxicity and morphological changes were observed which were

accentuated after 48 and 72 hours. MTT assay results confirmed the morphologic observations statistically. IC50 after 48 hours was 10 μmol/L. Results confirmed the anti-proliferative effect of NaB on SW480 cells and also revealed that at concentrations less than 10 μmol/L, it has no significant cytotoxicity effect on SW480 cells. **Conclusion:** Our results may suggest that the gene expression which were contributed in cell proliferation and apoptosis has been changed under pressure of HDAC inhibition. Our results suggesting that the naturally colonic NaB secondary to the microbial degradation of dietary fibers, may play as a natural preventive factor for CRC.

Keywords: Sodium butyrate, Colorectal carcinoma, Cytotoxicity, MTT

2454P

Lack of prognostic value of VEGF-A and VEGF-C expression in peripheral blood mononuclear cells of adult AML

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Background: The crucial role of angiogenesis in the pathophysiology of acute myeloid leukemia (AML) has been proposed. One of the key regulators of angiogenesis is the vascular endothelial growth factor (VEGF). Among the VEGF family, it has been observed that VEGF-A and VEGF-C are expressed by AML cells and mediate leukemic cell proliferation, survival, and resistance to chemotherapy. The aim of this study is to evaluate gene expression of these angiogenesis promoters and its possible prognostic value in peripheral blood mononuclear cells of patients with AML. **Methods:** We investigated the mRNA expression of VEGF-A and VEGF-C in peripheral blood mononuclear cells of 27 patients with newly diagnosed AML by quantitative real-time PCR. Also, we performed a univariate Cox regression analysis of the impact of VEGF-A and VEGF-C gene expression on overall survival. **Results:** Data analysis showed that VEGF-A and VEGF-C gene expression were not significantly related to prognosis in the study population (p=0.793, and p=0.548; respectively). **Conclusion:** Our data showed that expression levels of VEGF-A and VEGF-C did not influence the clinical outcome. It seems that angiogenesis is affected by different cytokines other than VEGF-C or VEGF-A and VEGF is also affected by different cytokines. Taken together, these findings help to provide new insights into the investigation of other angiogenic factors and cytokines that may play roles in the pathogenesis of AML.

Keywords: Acute myeloid leukemia, VEGF-A, VEGF-C, Gene expression, Prognostic value

2620P

SDF-1/CXCL12 and CXCR4 expression levels in the tumoral and normal tissues of basal cell carcinoma

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Background: Basal cell carcinoma (BCC) is regarded as one of the non-metastatic malignant tumors of skin. However, progression of BCC occurs by invasion of cancer cells into surrounded tissues. Delineating invasion mechanisms of BCC will be helpful in finding new therapeutic methods. **Methods:** In this study, the levels of SDF-1 and CXCR4 expression were determined by quantitative Real Time PCR in the tumoral and normal tissues of 25 BCC patients which BCC of them was confirmed by pathological finding. **Results:** Our data showed that the expressions of both SDF-1 and CXCR4 mRNA about 3 fold increased in tumoral tissues of BCC patients in comparison with normal tissues (P>0.05). Our result also showed that history of radiotherapy leads to the increase 100 fold SDF-1 expression and about 7 log increase in level of CXCR4 transcripts in normal tissues of BCC patients (p=0.043 and p=0.0154, respectively). **Conclusion:** It is tempting to conclude that the increased level of SDF-1 in the normal tissues of patients with radiotherapy provide a migratory signal for tumor cells and accelerate their invasion into normal tissues. This is especially important in the light of latest studies showing increase CXCR4 expression in the BCC tumor cells. Slightly increased of SDF-1 and CXCR4 in tumoral tissues of non-invasive BCCs may play a role in keeping the cells in place and localize the disease. Hence, application of CXCR4 antagonists may prevent the invasion and progression of BCC.

Keywords: Basal cell carcinoma, Chemokine, SDF-1, CXCR4, Real time PCR

2485P

Cytotoxic CD8+ subsets in tumor draining lymph nodes of breast cancer patients

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Background: CD8+ cytotoxic T lymphocytes have been recently divided based on their cytokine expression profile. The aim of the present study was to evaluate the percentages of CD8+ lymphocytes and their effector subsets including Tc1, Tc2 and Tc17 in the tumor draining lymph nodes (TDLNs) of patients with breast cancer. The comparison was made between LN+ versus LN- patients, as well as patients in different clinico-pathological status. **Methods:** Single cell suspensions were obtained from TDLNs of 42 patients with breast cancer. Staining of the cell surface markers and intracellular cytokines was performed using appropriate fluorochrome-conjugated antibodies. The data was acquired on a four-color flow cytometer and was analyzed by CellQuestPro software package. The percentages of different CD8+ cell subtypes (Tc1, Tc2 and Tc17) were quantified in CD8+ lymphocytes gate. **Results:** Despite no difference in the percentage of Tc1 cells in LN+ patients with infiltrative ductal carcinoma (IDC) type of breast cancer, the mean expression of IFNγ by Tc1 cells decreased significantly in comparison to LN- patients. On the other hand, the percentages of Tc2 and Tc17 effector subsets were increased in advanced stages. **Conclusion:** As the first study to investigate various effector subtypes of CD8+ lymphocytes in TDLNs of patients with breast cancer, our data collectively suggests a positive association between Tc2 and Tc17 effector subsets in TDLNs with breast cancer progression. Conversely, down-regulation of IFNγ by

Tc1 cells seems to be associated with tumor metastasis to TDLNs. These findings may have implications in cancer immunotherapy based on CD8+ effector subsets.
Keywords: Breast cancer, Tumor draining lymph nodes, Tc1, Tc2, Tc17

2536P
Helper and regulatory subsets of CD4+ lymphocytes in breast cancer tumor draining lymph nodes

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Background: Lymphocyte subpopulations activated during anti-tumor response determine the outcome of host-tumor interaction. We investigated different subtypes of CD4+ lymphocytes, including regulatory cells (CD25-, and CD25+ Treg cells), helper subsets (Th1, Th2, Th17 cells), and the expression level of their cognate cytokines (IFNγ, IL4, and IL17) in tumor draining lymph nodes of patients with breast cancer. **Methods:** Forty-seven lymph nodes with or without tumor involvement were collected from untreated breast cancer patients undergoing surgical resection. Mononuclear cells obtained from fresh homogenized lymph nodes were subjected to surface and intracellular staining by flow cytometry. **Results:** Our results demonstrated that CD25+ Treg subset showed higher frequency among LN+ patients (P=0.007), although the frequency of Th1, Th2, Th17 and CD25- Treg cells observed not to be different between LN+ and LN- patients. Whereas, geometric mean expression of IL17 in Th17 cells showed a significant reduction in LN+ patients (P=0.015). The frequency of Th2 (P=0.005) and CD25+ Treg cells (P=0.007) were also increased with tumor progression. Conversely, the expression intensity of IL17, and IFNγ, in CD4+ lymphocytes were decreased in stage III compared with stage II (P=0.002 and P=0.030, respectively). **Conclusion:** Along with metastasis of tumor cells to lymph nodes and the progression of the disease stage, the immune responses changed from an inflammatory to an inhibitory state, as evidenced by a reduction in pro-inflammatory and anti-tumor cytokines, IL17 and IFNγ, as well as an increase in pro-tumorigenic phenotypes, Th2 and Treg cells. This situation may provide a favorable condition for tumor growth and spread.
Keywords: Breast cancer, Tumor draining lymph nodes, Th1, Th2, Th17, Treg

2531P
Helper and regulatory follicular T cells in breast cancer tumor draining lymph nodes

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Background: Follicular helper T cell (Tfh) is a new subset of helper cells, which have the responsibility for helping B cells during the development of a T cell dependent antibody response. Recent studies have also demonstrated the presence of a new subgroup of Foxp3+ T cells in

lymph nodes designated as follicular regulatory T cell (TFR), which is vital for controlling Tfh cells, germinal center responses and the prevention of auto-antibody production. **Methods:** To investigate the presence of Tfh and TFR cells, 47 auxiliary lymph nodes with or without tumor involvement were collected from untreated breast cancer patients. Mononuclear cells obtained from fresh homogenized lymph nodes were subjected to surface and intracellular staining using CD4, CXCR5, Bcl6 and Foxp3 antibodies by flow cytometry. The data was acquired on a four-color flow cytometer and was analyzed by CellQuestPro software package. **Results:** The results indicated the presence of a subgroup of CD4+Bcl6+CXCR5$^{int/hi}$, which was positive for Foxp3 in the TDLNs of patients with BC. This subgroup, known as TFR, comprised from 0.1 to 8 percent of CD4+ lymphocytes. However, no significant difference was found in the frequency of Tfh and TFR cells between LN+ and LN- patients as well as patients in different stages of the disease. **Conclusion:** This is the first study that showed the presence of newly identified subtypes of CD4+ T cells, Tfh and TFR, in TDLNs of BC patients. However, it seems that the percentages of these subsets were not affected along with tumor cell metastasis to LN and tumor progression.

Keywords: Breast cancer, Tumor draining lymph nodes, Tfh, TFR

2397P

Olive leaf extract affects frequency and function of myeloid-derived suppressor cells in experimental model of melanoma

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Background: Myeloid derived suppressor cells are heterogeneous populations of immature myeloid cells which are strong inhibitors of antitumor responses. MDSCs are mainly induced by pro-inflammatory mediators. Olive leaf extract (OLE) as an herbal product has been indicated to possess anti-inflammatory, anticancer and antioxidant properties. We sought to determine olive leaf extract's effects on the frequency and function of MDSCs population on experimental model of melanoma. **Methods:** Different doses of OLE were administered orally to C57BL/6 mice for 3 days. The frequency of MDSCs isolated from Splenocytes were evaluated using flow Cytometry. Moreover, for assessing MDSC's function, generation of ROS and NO were respectively analyzed by DCFDA and Griess techniques and compared with control group. **Results:** Our findings support the hypothesis that Olive leaf extract can be considered as a therapeutic agent by regulatory effect on MDSCs. Based on our results 500 and 1000 mg/kg of OLE, significantly inhibited increases in the frequency and function of MDSCs. **Conclusion:** Since MDSCs function as a barrier for antitumor immunity, OLE appears to be one of the agents that serve as a modulator of the function of MDSCs. These effects may suggest a new method to treat some types of cancers that are resistant to different kinds of therapy.

Keywords: Olive leaf extract, Myeloid derived suppressor cells, Melanoma

2740P

Expression of interleukin-25 (IL-17E), interleukin-17B and interleukin-25 receptor in breast cancer

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Background: Breast cancer is malignant proliferation of stromal or acinar cells in breast ducts. There are indications that normal breast epithelial cells inhibit tumor growth and metastasis by producing IL-25. IL-25 which is produced by normal epithelial cells, binds to IL-17B receptor and triggers apoptosis of tumor cells. Inconsistently IL-17B also binds to the same receptor leading to tumor progression. Dominance of one of the cytokines in the tumor microenvironment would determine the fate of tumor towardtumor progression or regression. **Methods:** Total RNA extracted from 30 formalin fixed paraffin embedded breast cancer specimens as well as normal tissue around the tumor mass from the same patients and 10 nonmalignant breast specimens by means of Roche high pure RNA extraction kit. The integrity of RNA was checked by 1.5%agarose gel electrophoresis. cDNA was synthesized by Fermentase cDNA synthesis kit according to guidelines of the provider. Expression of IL-25, IL-17B, IL-25R and GAPDH as housekeeping gene were measured by ABI 7900HT Real Time PCR using sequence specific Taq Man Probe. The results were analyzed by SPSS software using relevant statistical tests. **Results:** The results showed that the expression levels of IL-25 mRNA in normal tissue around the tumor mass are higher than tumor tissue itself in the same patient. Expression levels of IL-17B and IL-25R in tumor tissue were higher than normal tissue around the tumor mass as indicated by Real Time PCR relative comparison. **Conclusion:** IL-25 produced by normal tissue surrounding the tumor cells can bind to IL-25R on tumor cells. It could be part of defense mechanisms of preventing tumor progression in competition toIL-17B which leads to tumor progression.

Keywords: Breast cancer, IL-25, IL-25R, IL-17B, Tumor progression

3367P

Diverse activation of prostate tumor cells by TLR ligation

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Background: TLRs trigger inflammatory responses upon microbial sensing. Inflammation has long been associated with cancer development. **Methods:** In the present study, the impact

of lipopolysaccharide (LPS) and lipoteichoic acid (LTA) stimulation on functional cancerous behavior of prostate tumor cells were investigated. **Results:** We showed that prostate cancer cell lines differentially express TLR1-10, MyD88 and CD14 transcripts. LNCaP and PC3 cells expressed functionally active TLR2 and TLR4. Regardless of lacking TLR4, DU145 responded to LPS stimulation. Positively identified TLRs expression by Western blotting was not accompanied by cell surface expression, even after LPS, LTA or IL-1 stimulation, as judged by flow cytometry. Immunofluorescent staining clearly demonstrated predominantly perinuclear localization of TLR2 and TLR4. LPS and LTA treatment of DU145 and LTA activation of LNCaP and PC3 cells significantly increased cell proliferation. While LPS caused increased invasiveness of LNCaP, invasive capacity of PC3 was significantly reduced after LPS or LTA stimulation. Stimulation of all prostate tumor cells with LTA was associated with increased cell adhesion. All prostate tumor cells increased IL-8 production in response to LTA activation. IL-6 production, however, was differentially regulated by LPS stimulation in prostate tumor cells. **Conclusion:** Overall, we showed for the first time differential consequences of TLR2 and TLR4 activation in prostate tumor cells. The data shows that cancer cells originated from the same histologically origin exhibit heterogonous response to the same TLR ligand. Therefore, a thorough and comprehensive judgment on how and to what extent a particular cancer is affected by TLR agonist could not be inferred by studying an individual cell line.

Keywords: Toll-like Receptor, Prostate cancer, Proliferation, Invasion, Adhesion

2377P

MiR-155 induces apoptosis via down-regulation of BAG1

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Background: One of crucial roles of micro-RNAs (miRNAs) in regulation of cell functions is apoptosis. Previous studies reaveled that miRNA-155 (miR-155) can induce apoptosis but its molecular mechanisms is yet to be clarified. Therefore, the aim of this study was to determine the effect of this miRNA on the mRNA level of BAG1, BAG3 and BAG4 as anti-apoptotic molecules. **Methods:** MiR-155 and a scramble sequence as well as PBS were defined, separately, to the Jurkat cell lines (T cell leukemia) and the mRNA levels of BAG1, BAG3 and BAG4 were examined in parallel with beta-actin and GAPDH (as housekeeping genes) using Real-Time PCR technique. Apoptotic changes also were identified using annexin-FITC staining method. **Results:** Our results indicated that 28% of miR-155 transfected Jurkat cells had apoptotic features. The results also demonstrated that mRNA levels of BAG1, were significantly decreased in miR-155 transfected Jurkat cell line in comparison to the scramble sequence or PBS transfected Jurkat cells as controls, while the mRNA levels of BAG3 and BAG4 were not changed in miR-155 transfected Jurkat cell line when compared to the scramble sequence or PBS transfected Jurkat cells. **Conclusion:** According to our results, it may be concluded that miR-155 is able to induce apoptosis via decrease mRNA expression or half-time of BAG1 in Jurkat cells. Therefore, it appears that, miR-155 can be considered as a novel target for molecular therapy of cancers.

Keywords: MiR-155, BAG1, BAG3, BAG4, Jurkat cell

P2506

Expression of the markers ERp57 and VEGF in patients with gastric cancer

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Background: Studies of gastric metaplasia and neoplasia in mouse models have informed us about the pathway to gastric carcinogenesis. VEGF and ERp57 were identified as markers for gastric metaplasia. To investigate the expression of VEGF and ERp57 in gastric metaplasia and adenocarcinoma in humans. **Methods:** 224 paraffin-embedded tissues from 112 patients with gastric cancer were stained for ERp57 and VEGF by horseradish peroxidase immunohistochemical technique, in which VEGF or ERp57 quantification score is obtained. **Results:** The level of VEGF expression was significantly higher in metaplasia and adenocarcinoma than that in normal gastric mucosa ($p \leq 0.01$).VEGF score revealed significant differences in differentiation degree, and histological type of the gastric carcinoma and depth of invasion ($p \leq 0.05$).ERp57 staining significantly increased in normal gastric mucosa, compared with that in cancer and metastases ($p \leq 0.05$).ERp57 expression correlated with greater depth of tumor and advanced stages ($p \leq 0.01$). **Conclusion:** High VEGF expression and low ERp57 expression are correlated with the progression of gastric cancer. Therefore, VEGF and ERp57 are prognostic markers for such patients.

Keywords: VEGF, ERp57, Gastric Cancer

2775P

The CXC chemokine IP-10 is differentialy expressed before and following bone marrow transplantation in acute myeloblastic and lymphoblastic leukemia patients

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Background: Immune system-related factors are important in development of leukemia. IP-10 as CXC chemokine are involved in the immune responses. Hence, this study was designed to assess the serum levels of IP-10 in acute lymphoblastic and myeloblastic leukemia. **Methods:** In this experimental study, blood samples were collected from 49 Leukemia patients including 37 AML and 12 ALL patients and 50 healthy controls. Sample were collected from patients (before and after transplantation) and controls and subjected to ELISA. Demographic data were also collected by a questionnaire which was designed specifically for this study. **Results:** Our results showed that serum level of IP-10 increased in both AML and ALL patients in compared to healthy controls significantly. **Conclusion:** Based on the results of this study, it can probably be concluded that serum level of IP-10 have an important role in pathogenesis of acute leukemia. It is also worth noting that this factor could probably use as pivotal biological marker in diagnosis and possible treatment factor.

Keywords: Acute lymphoblastic leukemia , Acute myeloblastic leukemia , IP-10

2513P

IL-17 serum level in patients with non small cell lung cancer

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Background: Lung cancer is considered to have the highest mortality rate among all malignant cancers. Several studies have revealed the roles of cytokines in lung cancer to facilitate screening, early diagnosis and treatment of lung cancer. This study was aimed to investigate Interleukin-17 (IL-17) serum concentration in blood samples of lung cancer patients and healthy controls. **Methods:** Serum concentration of IL-17 was measured in blood samples of 130 subjects including 65 patients with Non-Small Cell Lung Cancer (NSCLC) and 65 healthy controls using ELISA. Association of IL-17 serum level with clinicpathological characteristics of the disease was then investigated in the patients. **Results:** There was no statistically significant difference between IL-17 concentration in NSCLC patients and control group (0.511±2.438 and 0±0, respectively, P value > 0.05). Despite of this, IL-17 serum concentration was observed to be higher in NSCLC patients with stage IV compared to stage III (P value= 0.008). No statistically significant correlation was observed between IL-17 serum concentration and age, sex, tumor subgroup and histo-pathological grade of patients (P value > 0.05). **Conclusion:** Although no difference was found in the concentration of IL-17 between NSCLC patients and healthy controls, higher IL-17 concentration in patients with stage IV compared to stage III suggests the likely impact of this cytokine on cancer progression in NSCLC patients.

Keywords: Non Small Cell Lung Cancer, IL-17, ELISA

2322P

B cell subsets in breast and colon cancer draining lymph nodes

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Background: Tumor draining lymph nodes (TDLNs) have a pivotal role in the formation of immune responses against tumors. As little is known about different subpopulations of B cells in TDLNs in human, this study was designed to assess the frequency of some subpopulations of B cell in TDLNs of breast (axillary lymph nodes) or colon (mesenteric lymph nodes) cancer patients. **Methods:** Mononuclear cells were isolated from lymph nodes using Ficoll-Hypaque gradient centrifugation. Cells were stained with anti-CD19, CD25, CD5, CD27, CD1d and CD24 or isotype matched antibodies and subjected to flowcytometry. **Results:** Our data revealed that 27.7-44% of B cells in mesenteric lymph nodes and 31.7-76% of B cells in axillary lymph nodes expressed CD27. $CD19^+$ $CD5^+$ cells comprised 2.2 – 13.8% of B cells in mesenteric lymph nodes and 1.7–5% of B cells in axillary lymph nodes. The frequency of

CD25^{+} cells among B cells were 3.4-6.8% and 1.3-10.9% in mesenteric and axillary lymph nodes respectively. Of CD19^{+} cells, 22- 36.9% in mesenteric lymph nodes and 27.2-58% in axillary lymph nodes were CD24^{hi} CD27^{+}. 0.8-3.5% of B cells in mesenteric lymph nodes and 0.8-1.6% of B cells in axillary lymph nodes had the unique phenotype of CD19^{+} CD1d^{hi} CD5^{+}. **Conclusion:** In this study the frequency of active/memory B cells (CD19^{+} CD27^{+} ,CD19^{+} CD25^{+}) ,those similar to B1 cells (CD19^{+} CD5^{+}), and subsets which are called "enriched for regulatory B cells"(CD19^{+} CD24^{hi} CD27^{+} and CD19^{+} CD1d^{hi} CD5^{+}) were assessed in TDLNs of breast and colon cancer patients.

Keywords: B cell, Subset, Lymph node, Colon, Breast cancer

2321P

IgM, IgD expressing B cells in breast and colon cancer draining lymph nodes

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Background: B cells are important cellular components of tumor draining lymph nodes (TDLNs).The aim of this study was to assess the frequency of different populations of B cells with regard to their surface IgM and IgD expression in TDLNs of patients with breast or colon cancer. **Methods:** Mononuclear cells were isolated from lymph nodes using Ficoll-Hypaque gradient centrifugation. Cells were stained with anti-CD19, IgM and IgD or isotype matched antibodies and subjected to flowcytometry. **Results:** B cells of mesenteric lymph nodes obtained from colon cancer patients consisted of 41-61% IgM^{+}IgD^{+} and 12.5-29.2% IgM^{-}IgD^{-} cells. In these lymph nodes there was another population with CD19^{+}IgM^{+}IgD$^{dim/-}$ phenotype. An unexpected finding in one of mesenteric lymph nodes was an IgM^{-}IgD$^{dim/+}$ population that comprised about 9% of B cells in that lymph node. In axillary lymph nodes obtained from breast cancer patients 24.8-87% of CD19^{+} cells were IgM^{-}IgD^{-} and 3.5-65% were IgM^{+}IgD^{+}. The CD19^{+}IgM^{+}IgD$^{dim/-}$ subset were also seen in these lymph nodes. **Conclusion:** Regarding IgM and IgD expression, we could detect different subpopulations of B cells in breast and colon TDLNs. Two subpopulations need further assessment. The first one is CD19^{+}IgM^{+}IgD$^{dim/-}$, as they can be either IgM-only memory B cells or B1 like cells; moreover half of regulatory B cells have such a phenotype. The second one is CD19^{+}IgM^{-}IgD$^{dim/+}$ cells. To know whether they are so-called IgD-only plasmablasts requires further phenotypical and functional analysis in larger sample size.

Keywords: IgM, IgD, B cell, Breast, Colon cancer

3457P

The effects of plant flavonoid, fisetin on proliferation and apoptosis of a gastric cancer cell line (AGS)

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Background: Cancer results from structural and quantitative alterations in molecules that control different aspects of cell behavior. Fisetin, a natural flavonol present in edible vegetable and fruits were reported to exertanticarcinogenic effects. The objective of the current study was to examine the effect of fisetin on proliferation and apoptosis of a gastric cancer cell line (AGS). **Methods:** Cells were cultured in DMEM medium and treated with different concentrations of fisetin for three consecutive days. Cell viability was quantitated by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) assay. The percentage of apoptotic cells was determined by flow cytometry using Annexin V fluorescein isothiocyanate. **Results:** The MTS assay revealed that fisetin had an antiproliferative effect on AGS cells in a dose- and time-dependent manner.fisetin induced apoptosis in AGS cells, as determined by flow cytometry. **Conclusion:** Our results suggest that fisetin has anti-proliferative effects on gastric cancer cells. Therefore, fisetin may be a potential compound for both cancer prevention and treatment.

Keywords: Fisetin, Apoptosis, Gastric cancer

2442P

Cytotoxic/Proliferative effects of umbelliprenin on colon cancer cell lines

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Background: Colon carcinoma growth depends on many factors like different organisms, as well as immune cells, which induce and produce inflammatory cytokines. Umbelliprenin is a naturally prenylatedcoumarin with anti-inflammatory activities. **Methods:** In the present study, invasive SW48 and noninvasive SW1116 were treated with umbelliprenin (6.25, 12.5, 25, 50, 100, and 200 μM), and the cytotoxicity was determined using methyl thiazolelydiphenyl-tetrazolium bromide (MTT) assay. **Results:** Umbelliprenin had significant cytotoxic activity against SW48 cells at the all study concentrations (except for 6.25 μM). However, it was cytotoxic against SW1116 only at higher concentrations. At lower concentrations, umbelliprenin showed a significant proliferative effect on this noninvasive cancer cell line. Our data were validated by eye and microscopic images. **Conclusion:** Using umbelliprenin as an anti-inflammatory or cytotoxic compound for patients with colon cancer should be with caution and care.

Keywords: Colorectal Neoplasms, MTT assay, Umbelliprenin

3222P

Induced Apoptosis with TGF-B1 associated with Mitochondrial Bcl-2 Expression

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Background: Transforming growth factor (TGF-b1) can elicit various cellular responses, including inhibition of cell growth, migration, differentiation, and apoptosis. In addition, TGF-b1 is able to induce apoptosis in certain lymphomas. In the present study, the role of SMADs, Bax, Bcl-xl, and Bcl2 was characterized in 2 B-lymphoma cell lines, Burkitt and pre–B cell. **Methods:** Apoptosis was detected after exposure of TGF-b1 on Raji and Nalm 6 cell lines and was evaluated by flow cytometry by using annexin V, reverse transcriptase–polymerase chain reaction, and Western blot analysis. **Results:** Flow Cytometry with Cell Sorting analysis showed that apoptosis could be observed after 24 hours of TGF-b1 treatment and was continued after 48 hours. TGF-b1 downregulated the Bcl-xl and Bcl-2, whereas the Bax was upregulated. Furthermore, messenger RNA of SMAD6 and SMAD7 showed the significant upregulation. **Conclusion:** The results indicated that alteration in gene expression and protein level may determine the induction of apoptosis pathway in these lymphoma cell lines.

3166P

Preparation of polyclonal antibodies against NGEP protein; as a potential biomarker in prostate cancer immunotherapy

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Background: NGEP (New Gene Expressed in Prostate), is a prostate-specific gene is only expressed in normal prostate and prostate cancer. Because of its selective expression in prostate cancer cell surface, NGEP makes an excellent immunotherapeutic target. **Methods:** In this study, polyclonal antibodies against epitopes of NGEP extracellular domains were prepared as immunogen. Specification of these antibodies determines by ELISA, Western blotting and immunohistochemistry. The intensity of NGEP expression were analyzed in 152 cases of adenocarcinomas, 28 cases of hyperplasias and 10 cases of normal prostate tissues and also distribution of NGEP expression investigated with PSA level serum in prostate cancer. **Results:** The Western blot and immunohistochemistry results showed that anti-NGEP-p2 antibody had the advantages of high specificity and sensitivity. Utilizing anti-NGEP-P2 antibody, showed that NGEP protein was significantly reduced in high grade of malignancy ($p<0.001$). Also, there were inverse relative association of serum PSA with NGEP expression ($p= 0.05$). **Conclusion:** The expression of NGEP protein in prostate tissues by anti NGEP-p2 antibody was clarified. The anti NGEP-p2 antibody can be served as a good tool for prognostic prostate cancer, NGEP protein function research and targeting therapy in prostate cancer.

Keywords: Biomarker, NGEP

1984P

Novel thiazole thione derivatives as apoptosis inducer

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Background: Apoptosis is defined as programmed cell death that occurs during development, differentiation, in tumor cell deletion, and in response to a variety of insults, such as cytotoxic molecules or compounds. Since the induction of apoptosis by chemotherapeutic agents reserves a physiological advantage in the cancer treatment, we evaluated the ability of new synthesized thiazole thione derivatives to induce apoptosis in human cancer cell line. **Methods:** Apoptosis was investigated morphologically using acridine orange/ ethidium bromide staining by fluorescence microscopy. The percentage of cells within a population that are undergoing apoptosis was determined by flow cytometric analyses of annexin V-PE/ 7-AAD. **Results:** The analysis of the acridine orange/ethidium bromide staining showed that the thiazole thione derivatives induced apoptosis in MCF-7 cell line. The apoptotic cells had orange particles in their nuclei, whereas the viable cells were observed green. Apoptosis induction for synthetic compounds was further confirmed by Flow cytometry analysis. Annexin V/7-AAD double staining followed by flow cytometric analysis revealed that MCF-7 cells undergo apoptosis after treatment with IC_{50} concentrations of compounds. **Conclusion:** The results of apoptosis evaluation suggested that the cytotoxic activity of these compounds in MCF-7 cells occurs via apoptosis.

Keywords: Apoptosis, Cancer, Cell line

3165P

Prevalence of cancer in patients referred to pathologic center Amir al- Mu'minin hospital.

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Background: Cancer is the imbalance between cell growth and cell death that leads to the accumulation of excessive cells . Accumulated cells is called a tumor. There are two forms of tumors . Benign tumors that could be removed surgically and Malignant that can be dangerous. **Methods:** Among statistical population of the Gerash city in five consecutive years (2009 -2013) .The people suspected to cancer (1500) referred to pathologic centers for precise assessment. **Results:** Among this population (1500 case), 225 patients were positive (Benign – Malignant) equal to 15% person referred . Prevalence of Breast and lung cancer were both in the first place (13 – 30 % cases). Brain tumor was placed second (10-24% cases). **Discussion:** Breast cancer incidence in Iran is in the age between 40-50 , while it was 54 in the region of study . Lung cancer incidence was very high in addition to Brain tumor incidence (0.01 %) was very high While the national rate in Iran is 0.0002 % . Can be justified high Brain tumor incidence because of exposure to intense sunlight , reduced cultivation and consumption of vegetables and high lung cancer due to excessive use of tobacco.

1944P

A Comparative survey on the effects of Extracellular fractions from *Lactobacillus casei* and *Lactobacillus paracasei* on k562 cell line

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Background: The goal of this study was to compare the effects of different extracellular fractions of *Lactobacillus casei* and *L. paracasei* on the growth of K562 cell line. **Methods:** *Lactobacillus casei* and *L. paracasei* isolated from the intestine of common carp were obtained from microbiology lab of Artemia and Aquatic Research Institute, Urmia University and cultured in MRS broth medium. Then extra cellular secretions were collected and protein contents were measured with Bradford technique. Proteins were precipitated and fractionated using G100 Gel Filtration Chromatography. SDS-PAGE electrophoresis was used for the evaluation of the fractions. Then the anti turmeric properties was evaluated by MTT technique on K562 cell line. **Results:** Results showed extracellular fractions of *Lactobacillus casei* and *L. paracasei* had anti turmeric properties ant their molecular weights were different from each other and their antitumoric effects were different depending on the concentration and incubation time. **Conclusion:** We conclude that after more study, the extracellular fractions from Lactobacillus casei and paracasei could use for the treatment or prevention of cancer.

Keywords: K562, *Lactobacillus casei*, *Lactobacillus paracasei*, Extracellular fraction, Gel Filtration Chromatography

3276P

Blockage of hypoxia inducible factor: The impact on tumor growth in mouse model of melanoma

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Backgrounds: Considerable evidence shows that the tumor microenvironment (TME) is an active participant in preventing immunosurveillance and limiting the efficacy of anticancer therapies. Hypoxia within the TME has been proven to play a critical role in the development of immune escape mechanisms by tumor cells.The heterodimeric hypoxia-inducible factor-1 (HIF-1), is an important mediator of tumor response to hypoxia. Over expression of HIF-1α, the regulatory subunit of HIF-1, under hypoxic conditions, is associated with increased tumor growth via multiple mechanisms.PX-478 is an experimental anti-cancer drug known to inhibit constitutive and hypoxia-induced HIF-1αlevels and thus HIF-1 activity. Moreover, there are evidences for Celecoxib, a selective COX-2 inhibitor, to exert its antitumor activity in part via down-regulation of HIF-1α. Here, we designed a study to evaluate HIF-1α inhibition by PX-478 and Celecoxib and their effect on tumor growth in experimental model of melanoma. **Methods:** PX-478 and Celecoxib were administered i.p. to C57/BL6 mice with established subcutaneous B16-F10 tumors at 20, 40, and 60 mg/kg for PX-478 and 75 mg/kg for Celecoxib, once daily for 3 days/week for 1 week. HIF-1α expression by Real-Time PCR analysis and Tumor growth was measured as an indicator of HIF-1a inhibition effect. **Results:** The results showed that both agents can reduce tumor growth via HIF-1α down-regulation.

PX-478 exerts its effect in a dose dependent manner. **Conclusion:** As shown by the results, HIF-1α suppression had significant activity on subcutaneous model of melanoma suggesting it to be considered as a therapeutic strategy to enhance the efficacy of cancer immunotherapies.
Keywords: PX-478, Hypoxia inducible factor-1α, Tumor

1945P

Novel chalcone and flavanone analogues as apoptosis inducer

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Background: Defects in apoptosis pathways are implicated in the development of cancer and have been extensively linked to the resistance of tumors to chemotherapy. The goal of cancer therapy is to kill cancer cells. Many anticancer drugs are designed to kill cells by inducing apoptosis. Flanvonoids and chalcones were recognized as anti cancer agent. The apoptotic effects of a series of chalcone and flavonone with various substituents was examined. **Methods:** Apoptosis was determined morphologically using staining with acridine orange/ethidium bromide by fluorescence microscopy. The percent of cells under apoptosis was assessed by Annexin-V-PE/7-AAD double staining using an Annexin-V-PE kit. **Results:** The analysis of the acridine orange/ethidium bromide staining showed that the synthetic compounds induced apoptosis in MCF-7 cell line. The viable cells were observed green and the apoptotic cells had orange particles in their nuclei. Staining with Annexin V-PE is used in combination with 7-AAD, which is a vital dye to identify early apoptotic cells before membrane integrity has been lost. According to the results, the cells undergo apoptosis after treatment with IC_{50} concentrations of synthetic compounds. **Conclusion:** According to acridine orange/ethidium bromide staining and flow-cytometric data, synthetic compounds induce apoptosis in MCF-7 cell line.
Keywords: Cancer, Apoptosis, Chemotherapy

3289P

Th1 (IFN-γ) and Th2 (IL4) cytokines in salivary gland tumors

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Background: Salivary gland tumors are among the cancers with high recurrence rate. Immune responses in salivary gland tumors have not been well elucidated. Given the role of Th1 and Th2 cytokines in the outcome of tumors and the high recurrence rate of salivary gland tumors, we aimed in this study to evaluate the serum level of IFN-γ and IL4 cytokines in the patients with benign and malignant salivary gland tumors in comparison with healthy controls. Methods: 50 patients with benign and 15 patients with malignant salivary gland tumor, as well as 23 healthy individuals were participated in this study. IFN-γ and IL4 serum levels were measured by using sandwich ELISA method. The data are presented as mean ±standard error

of mean (SEM). Nonparametric tests were used for data analysis. **Results:** Serum level of IL4 and IFN-γ was observed not to be significantly different between patients compared with the control group (4.5±1.5 versus 4.4 ±1.3 pg/ml, respectively, P=0.26 for IL4, and 0.7±0.3 versus 1.0±0.6 pg/ml, respectively, P=0.569, for IFN-γ). IL4 and IFN-γ serum level was also not significantly different between patients with benign and malignant salivary gland tumors (4.9±1.9 versus 2.9±1.6 pg/ml, respectively, P=0.9 for IL4, and 0.7±0.3 versus 0.4±0.4 pg/ml, respectively, P=0.75 for IFN-γ). **Conclusion:** The serum level of IL4 and IFN-γ seems not to be associated with salivary gland tumors.

Keywords: Salivary gland tumors, IL4, IFN-γ, ELISA

2153P

Study of apoptotic pathway induced by vincristine in mouse proliferating and resting normal lymphocytes and BCL 1 cell line

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Background: Vincristine is the effective anticancer drug, widely used as a potent chemotherapeutic agent in the treatment of various cancers such as lymphoma. Most anti-cancer chemotherapy drugs such as vincristine target mitosis and induce apoptosis in cancerous cells. On the other, proliferation and apoptosis of lymphocytes are essential parts of the immune system. So, cells undergo more apoptosis when they are more dividing, which may also include the normal proliferating lymphocytes responsive to malignancies as well. **Methods:** Resting and proliferating spleen lymphocytes and BCL1 (mouse lymphoma cell line) were cultured with different concentrations of vincristine for 48 h. IC50 was calculated for cells. Then a cell lysate was prepared from them. In order to determination of apoptotic pathway induced by vincristine, the activity of caspases-3, -8 and -9 in aboved-mentioned cells were measured using specific chromogenic substrates. **Results:** The activities of caspase-3 and -9 were increased in a dose-dependent manner in resting cells, proliferating cells and BCL1 cell line exposed to different concentrations of vincristine. On the other, activity of caspase-8 was increased only in BCL1 cells. The results were proportional to two methods that had already been done (fluorescence microscope and MTT assay). **Conclusion:** The results suggesting that vincristine induce internal pathway of apoptosis through activation of caspase-9 in all of the cells while it induce extrinsic pathway through activation caspase-8 only in BCL1. **It seems that**, this effect is highly dependent on concentration and activation of the cells.

Keywords: Apoptosis, BCL1, Vincristine, Spleen Lymphocytes, Caspases.

2971P

Evaluation the correlation between clinical symptoms and cytogenetic features in chronic myelogeneous leukemia patients exposed to sulfur mustard

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Background: Chronic Myeloid Leukemia (CML) is the one of the malignancy of hematopoietic system. In more than 90% patients, a translocation is happened between chromosome 9 and 22 and is formed bcr/abl oncogene on Philadelphia (Ph) Chromosome. In our study we found the incidence of Ph chromosome in CML patients exposed to SM, is 1.778 times more than CML patients not exposed to SM. Considering to effect of Ph chromosome on expression of other gene including clinical symptoms such as splenomegaly, we should evaluate genetic mutation to control leukemia disorders. **Methods:** In our research, we collected peripheral blood of 5 and 10 CML patients exposed and not exposed to SM, respectively. For determining of clinical signs, we followed the disease process of the patients from diagnosis to our research. By using SPSS ver. 16 software, we analyzed the correlation between cytogenesis and clinicians by running Generalysed Estimation Equation (GEE) program. **Results:** Our results show CML patients exposed to SM significantly suffer from splenomegaly more than the other group (PV=0.011). So we can emphasize on the effect of exposing to SM by genetic changes. **Conclusion:** By the way, the rate of in Ph^+ exposed CML patients is 5.204 times more than Ph^- exposed to CML patients.

Keywords: CML, SM, Ph chromosome, Clinical symptoms

2969P

Studies on bcr-abl oncogene effects on laboratory assigns in Chronic Myelogenous Leukemia Patients exposed to Sulfur Mustard

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Background: Chronic Myelogenous Leukemia (CML) is a malignancy of hematopoietic system which is remarked by Philadelphia chromosome (Ph). Formation of an abnormal tyrosine kinas is the Ph oncogene production. A point mutation has been diagnosed in JAK2 (JAK2 V617F) myeloproliferative disorders patients which result in polycytemia Vera like erythrocytosis and Leukocytosis. It seems that significant mutations are responsible for clinical and laboratory disorders in leukemia hematopoietic proliferation. **Methods:** In our research, we collected peripheral blood of 5 and 10 CML patients exposed and not exposed to SM, respectively. For determining of clinical signs, we followed the disease process of the patients from diagnosis to our research. By using SPSS ver. 16 software, we analyzed the correlation between cytogenesis and clinicians by running Generalysed Estimation Equation (GEE) program. **Results:** We found that Wight Blood Cell (WBC) numbers in Ph^+ cases, totally, 18740.35 cells in micro liter more than in Ph^- cases (PV=0.020). On the other hand, the incidence of Ph chromosome in SM exposed CML patients is 1.778 times more than the others (PV=0.047). **Conclusion:** So, it is expected to diagnose more laboratory disorders in SM/ CML patients in comparison to non SM/ CML patients.

Keywords: SM, CML, Laboratory assigns

2733P

Apoptotic effect of all-trans retinoic acid (ATRA) on gastric cancer cells through down-regulation of Bcl 2 and increased Caspase-3 activity

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Background: Gastric cancer is one of the most common and lethal malignancies with high mortality rate in the world. Retinoic acid and its derivatives (Retinoids) have been utilized as potential chemopreventive and chemotherapeutic agents due to their anti-proliferative,anti-oxidant, pro-apoptotic and differentiation effects. Currently, all-trans retinoic acid (ATRA) is an approved drug for the treatment of acute promyeloid leukemia (APL) patients. One underlying mechanism by which ATRA carries out its anti-cancer effectis induction of apoptosis. Despite extensive studies, the main mechanism of apoptosis induction by ATRA has not been well elucidated. Caspases are central components of cell death machinery. Caspase-3, the executioner Caspase, is a key effector molecule of apoptosis and its activation is critical to induce programmed cell death in variety of cancer cells. Bcl2, a member of anti-apoptotic proteins, appears to protect cells from apoptosis by sequestering pro-apoptotic proteins or interfering with their activities. **Methods:** In this study, AGS cells were treated by different concentrations of ATRA dependent. Caspase-3 activation was measured by luminescent kit and was confirmed by RT-PCR. Moreover, down-regulation of Bcl2 expression was also evaluated by RT-PCR. **Results:** In this study we showed that ATRA treatment of gastric cell line (AGS)induced apoptosis through Caspase-3 activation. Furthermore, ATRAled to decrease in expression of Bcl2, an anti-apoptotic protein. **Conclusion:** ATRA is a potent agent to induce apoptosis in gastric cancer cells. These data suggests that induction of apoptosis through Caspase-3 activation is a novel mechanism of action for ATRA during therapeutic intervention in gastric cancer.

Keywords: Gastric cancer, Apoptosis, Caspase-3, Bcl2

1598P

Expression level of miR-34a, miR-126, miR-128 , miR-210 and their target genes in Non-small cell lung Cancer (NSCLC) patients

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Background: Non-small cell Lung cancer is one the most common types of lung cancer (80%). MicroRNAs are non-coding small RNAs that have key roles in cell proliferation, apoptosis,

invasion and differentiation. The human miR-34a precursor is transcribed from chromosome 1 and it acts as a tumor suppressor gene that its validated target gene is bcl2, an antiapoptotic gene. miR-126 is located within the 7th intron of the EGFL7 gene which resides on human chromosome 9.Its target IRS-1 inhibits the cell cycle from progressing from G_0/G_1intoS phase. miR-128 target is EGFR that is usually mutated ,so miR-128 is not able to downregulate this gene, therefore these patients are resistant to tyrosin kinase Inhibitors.mir-210 has been strongly linked with the hypoxia pathway, and is upregulated in response to Hypoxia-inducible factors. **Methods:** microRNA were isolated from tumor samples and adjacent normal tissues by miRNaeasyKit(Qiagen). Then cDNA was synthesized in two methods by using specific stem-loop primers for Taqman real time RT PCR and oligodT for Syber Green real time RT PCR. **Results:** miR-34a , miR-210 are downregulated in our patients with p< 000 . miR-126 and miR-210 are upregulated in tumor samples in comparison with pooled normal tissues with p<003 and p<0.008 ,respectively. However, BCL2, IRS-1 and HIF-1a were all downregulated significantly. **Conclusion:** In Our samples miR-34 acts as a tumor suppressor gene, however, the expression level of its target BCL2 is downregulated either. It showsthat there must me another pathway which regulate BCL2's expression level. On the other hand, HIF-1a that is a transcription factor is downregulated so the expression level of miR-210 is influenced transcriptionally as HIF-1a binds to miR-210 promoter. miR-126 is upregulated so its validated target IRS-1 is negatively regulated and in this way cell will trends toward proliferation.

Keywords: MicroRNA, BCL2, IRS-1, HIF-1a, NSCLC

3300P

MiR-143 induces apoptosis via alteration in expression of BCL2 family molecules in Jurkat cell line

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Background: BCL2 family members play important roles in apoptosis regulation of human cells. Additionally, micro-RNAs (miRNAs) are important candidates for regulation of apoptotic molecule expression. It has been documented that miRNA-143 (miR-143) induces apoptosis in cancer cell lines but its molecular mechanisms has yet to be clarified. Therefore, the aim of this study was to determine the mRNA levels of BCL2 family members in Jurkat cell line following treatment with miR-143. **Methods:** MiR-143, a scramble sequence and PBS were introduced, separately, to the Jurkat cell lines and the mRNA levels of BCL2 family members were investigated in parallel with beta-actin and GAPDH (as housekeeping genes) using Real-Time PCR technique. **Results:** Our results indicated that the mRNA levels of AIM2 and ASC were significantly increased in the miR-143 transfected Jurkat cell line in compare to the scramble sequence or PBS transfected Jurkat cells as controls. **Conclusion:** According to our results, it may be concluded that miR-143 can lead to increase half-time of AIM2 and ASC mRNAs. Thus, it seems that miR-143 can consider as a therapeutic agent to treatment of chronic infectious diseases, especially viral infections.

Keywords: MiR-143, BCL2 family, Jurkat cell.

1905P

Evaluation of relationship between station at onset of active labor innulliparous patients and risk of cesarean delivery in Tonekabon Shahid Rajaeehospital in years 2007-2008

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Background: Station is determined by noting the position of the fetal presenting (bony) part relative tothe ischial spines stations of the fetal head. At the 0 station, the fetal head is at the bony ischial spines and fills the maternal sacrum. Positions above the ischial spines are referred to as -1 through -5. The active phase commences when the slope of cervical dilation reaches its maximum and especially in the nullipara. **Methods:** This is a prospectively cohort cross sectional study which studied on 418Nulliparous pregnant women at 37- 42 weeks gestation. For the purpose of this study, active labor was defined as regular contractions with cervical dilatation rather than 3cm. Thestation at onset of active labor was record .Engagement was considered to be at station 0 or below. Forstatistic analysis used spss14 and Chi-Square test. **Results:** The Findings point to have significant relation between cesarean delivery in upperstation at onset of active labor in nulliparous patients and fetal macrosomia (P =0.019), oxytocin(P =0.001), Cephalopelvic Disproportion (P =0.001), but no significant relation with Pregnancy weightgain (P =0.403). Type of delivery between upper and lower station have not significant relation(P =0.615). **Conclusion:** seventy-four percent of nulliparas with an unengaged vertex at onset of active labordelivered vaginally, Therefore can conclude that upper station at onset of active labor however increaseduration time of labor, but don't increase cesarean delivery rate.

Keywords: Station, Active Labor, Nulliparous, Cesarean Delivery

1733P

Expression and purification of functional human Vascular Endothelial Growth Factor-A_{121}; the most important angiogenesis factor

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Bachground: angiogenesis or formation of new blood vessels is essential for tumor growth and metastasis. Vascular Endothelial Growth Factor (VEGF) and its receptors play an important role in angiogenesis-dependent tumors. hVEGF-A is the most important factor in angiogenesis process. Recent studies have shown that agent that inhibits VEGF also prevent angiogenesis. **Methods:** in this study, gene of VEGF-A_{121} synthesized and cloned into the PET-26b plasmid. The vector was transferred into appropriate expression strain of BL-21. After induction by IPTG (Isopropyl β D-1Thiogalacto pyranoside) recombinant protein was expressed. Expression of rh VEGF-A_{121} was confirmed with SDS-PSGE and Western-Blotting. Recombinant hVEGF-A_{121} was purified by nickel affinity chromatography. was isolated from umbilical vein. The purified recombinant hVEGF-A was used for studding angiogenesis and

endothelial cell tube formation assay in HUVEC (Human Umbilical Vein Endothelia Cell) cell line. **Results:** SDS-PAGE and Western-Blotting results indicating the success of rhVEGF-$_{A121}$ purification. The final yield of recombinant protein was about 400mg per liter. Endothelial cell tube formation assay result showed that rhVEGF-A_{121} leads to tube formation of endothelial cell on matrix and induces angiogenesis in vitro. **Conclusion:** Recombinant hVEGF-A_{121} is important factor in tube formation of endothelial cell, so it can be use in different cancer research and angiogenesis assay. Moreover inhibition of hVEGF-A signaling with different factors could be useful in prevention of angiogenesis process.

Keywords: hVEGF-A_{121} , angiogenesis, endothelial tube formation assay

3107P

Study on suppressive effect of constructing shRNAs on the expression of apollon in HPV positive cancer cell lines

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Background: Cervical cancer is the second major common cancer among women in across the world. HeLa cells carrying naturally integrated HPV oncogenes. The inhibitor of apoptosis proteins (IAPs) has been implicated in the regulation of apoptosis. Apollon, a membrane of IAPs, protects cells against apoptosis and is upregulated in certain tumor cells. RNA interference (RNAi) inhibits gene expression via the degradation of mRNA. Short hairpin RNA (shRNA) is effective means of harnessing the RNA interference (RNAi) pathway in mammalian cells. The aim of this study is to investigate the effect of small interfering RNA (siRNA) on down-regulating of Apollon as an anti-apoptotic gene. **Methods:** 4 plasmid vectors encoding shRNAs against Apollon were constructed and transfected into hela cells via electroporation. Reduction of Apollon mRNA and protein expression were monitored by real-time PCR and immuno cyto chemistry(ICC), respectively. The viability of hela cells was assessed by LDH-assay. **Results:** mRNA and protein level of Apollon have been decreased via siRNA1. LDH-assay revealed a cell viability decrease in these cells. **Conclusion:** shRNA-expressed from pRNAi suppresses the expression of Apollon in HPV positive cancer cells. Results of this work suggest that RNAi is potentially applicable for treatment of abnormal gene expression and viral contamination.

Keywords: HPV, Apollon, shRNA,Cancer

2541P

Comparison between CYFRA 21-1 and CEA serum levels in Iranian patients with Non-small cell lung cancer (NSCLC)

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Background: Tumor markers play a specific role in diagnosis and monitoring of treatment in different cancers especially lung cancer. Among these tumor markers, a fragment of cytokeratin

19 named, cyfra21-1, encompasses the most diagnostic sensitivity. Another tumor marker which has been studied in NSCLC patients is CEA. The aim of this case-control study was the investigation of Cyfra21-1 and CEA serum levels in patients, detection of correlation between serum marker levels and patients' clinic-pathological factors and finally determination of a proper cut off point for Iranian population. **Methods:** 44 NSCLC patients and 44 healthy individuals were participated in this study. Cyfra21-1 and CEA serum levels were measured with sandwich ELISA method. **Results:** CEA and cyfra21-1 serum levels were significantly higher in patients group (16.3± 4.1 and 24.5±5.5, respectively, $p<0.002$ for CEA and $p<0.001$ for Cyfra21-1).Linear correlation between CEA serum levels and age in controls group was detected. With measured cut off pointof2.26 ng/ml for Cyfra21-1,77.3% sensitivity,97.7% specificity,97% positive predictive value and 81.1% negative predictive value were obtained. Also with cut off point of 1.97 ng/ml for CEA,83.7% sensitivity,72.7% specificity,75.5% positive predictive value and 82% negative predictive value were detected. **Conclusion:** It seems that Cyfra21-1 is a very specific and relatively sensitive discriminative factor in diagnosis of NSCLC patients in suspicious individuals. In addition, CEA is a sensitive tumor marker in diagnosis of lung cancer. These tumor markers can be used as diagnostic factors for early detection of lung cancer in Iranian population.

Keywords: NSCLC, Cyfra21-1,CEA, Sandwich ELISA

2920P

In vitro anti-angiogenic and anti-prolifrative effect of teucrium polium extract on HUVEC cell line

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Background: Angiogenesis is important for growth and invasion of cancer. Development of therapies aimed to inhibit angiogenesis , in combination with classical anti-cancer therapies , is among the most intensively studied approaches to the treatment of cancer. **Methods:** After preparation the extract of teucrium polium with 50%ethanol , the extract was fractioned in to n-hexane, ethyl acetate, n-butanol and aqueous fractions. Anti-angiogenic effect of T.P was assessed on three-dimensional culture of HUVEC(human umbilical vein endothelial cells) in collagen matrix and anti-prolifrative effect of T.P checked with MTT-test .**Results:** Based on our data , angiogenesis perfectly inhibited at 60 µg/ml of ethyl acetate fraction and 300 µg/ml of crude extract. Ethyl acetate fraction and crude extract could inhibit proliferation of HUVEC with IC50 of 68 and 250, respectively, furthermore the results showed that crude extract and ethyl acetate fraction were not cytotoxic at IC50. **Conclusion:** Teucrium polium can be candidate for cancer therapy, however this suggestion needs more investigation for approval.

Keywords: Teucrium polium, Anti-angiogenic, Anti-prolifration.

3303P

Tissue polypeptide antigen (TPA) and neuron specific endolase (NSE) in Iranian patients with non small cell lung cancer (NSCLC)

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Background: Lung cancer is the leading cause of cancer death worldwide. Tumor biomarkers are widely used as the trace of disease occurrence and disease progression in almost all types of cancer. Tissue polypeptide antigen (TPA) and neuron specific endolase (NSE) are two important tumor markers which have been suggested to be useful in detection of lung cancer. The role of these tumor markers have not been well investigated in Iranian population. The aim of this study was to investigate the serum levels of TPA and NSE in patients with non small cell lung cancer (NSCLC) and control population, determine in-home cutoff point and calculate the sensitivity, specificity and predictive values for these tests.**Methods:** 44 patients suffering from non small cell lung cancer and 44 age/sex matched healthy individuals were precipitated in this case-control study.TPA and NSE serum levels were determined by applying sandwich ELISA methods. **Results:** The mean level of TPA in serum of NSCLC patients was significantly higher than normal population (11.20±6.93ng/ml versus 6.20±4.69ng/ml, respectively, $p<0.001$). The cutoff point was calculated to be 6.36ng/ml. With this cut off point, the sensitivity of TPA was determined to be 65.9%, specificity was 70.4%, positive predictive value was 67.3% and negative predictive value was 69%. The mean level of NSE was not statistically different in patients' group in comparison to controls (241.95±313.36ng/ml versus 148.47±148.57, respectively, $p=0.11$). **Conclusion:** Our data confirm the TPA as a significant tumor marker in diagnosis of NSCLC n Iranian population with sensitivity of 65.9% and specificity of 70.4%. Our finding was not able to correlate the NSE with the occurrence of NSCLC in Iranians.

Keywords: Non small cell lung cancer, TPA, NSE, ELISA

2600P

Quantification of HER1 and HER2 expression in CHO and HEK-293 cell lines by real-time PCR to select appropriate cell lines as *in vitro* models in cancer study

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Background: HER family receptor propagates potent signal transduction network regulating normal cell activity and also playing important roles in cancer development. HER1 and HER2 are two main oncogenic components of HER-receptor family considered as targets for cancer therapy especially in breast cancer. Cell lines provide a readily material in molecular and targeted therapy assays. Knowing the precise expression levels of these receptors on cells is a fundamental need in any HER receptor targeted therapy. **Methods:** Absolute and relative expressions of HER1 and HER2 in CHO, HEK-293, MDA-MB-468 (HER1 positive control), and SKBR3 (HER2 positive control) cells were evaluated using quantitative real-time PCR. Absolute expression was carried out by serial dilution plasmids harboring HER1 or HER2 and

relative expression was evaluated by *HGPRT* as reference gene and $2^{-\Delta\Delta CT}$ method. **Results:** CHO and HEK-293 did not express HER1; MDA-MB-468 showed higher expression of HER1 than SKBR3. The expression level of HER2 in CHO and HEK-293 were 9.4075×10^2 and 1.13775×10^5 copy number/µg RNA, respectively. Meanwhile, CHO, HEK-293, MDA-MB-468 had lower HER2 expression compared to SKBR3 (0.007, 0.01, and 0.001 fold expression, respectively. **Conclusion:** Heterologous expression of HER1 and HER2 in cell lines are or (is) the first step in structural and functional studies. Ideally HER1 is not absolutely expressed in CHO and HEK-293 cells. Although HER2 expression is barley detected in CHO and HEK-293 cells, very low endogenous expression level of HER2 marks them as appropriate cell lines in human epidermal growth receptors related studies.

Keywords: HER1/ HER2 expression, CHO and HEK-293, Targeted therapy

1877P

HE4 combined with CA125: favorable screening tool for ovarian cancer

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Background: Ovarian cancer is a common malignancy in women. Diagnostic performance of current biomarkers of disease such as HE4 and CA125 show contradiction in literature. The goal of this study are to evaluate the serumic levels of CA125 and HE4 in patients with ovarian cancer in contrast with healthy women in Iranian people and comparison of specificity and sensitivity of HE4, CA125 and HE4+Ca125 in patients with different stages and diverse histologic subtype to obtain complete view about diagnostic power of above biomarker to distinguish of ovarian cancer at the early stages. **Methods:** To evaluation of CA125 and HE4, 32 patients and 34 healthy women as control was selected. Origin of ovarian cancer and FIGO stage were verified by expert gynecological oncologist. Significance and diagnostic performance were determined by ANOVA and ROC-AUC respectively. **Results:** Serumic CA125 and HE4 was significantly elevated in patients in comparison to control group especially when the origin of the tumor was epithelial cells ($p<0.001$). ROC-AUC for HE4 and CA125 and HE4+CA125 was 0.91, 0.86 and 0.91 respectively. Sensitivity of HE4 was more than CA125 (85% versus 80%). Conversely sensitivity of CA125 was higher in comparison to HE4 (90% versus 80%). It is be noticed that cut-off point of HE4 and CA125 was 150 pmol/ Land 38 U/mL respectively. HE4 is slightly more specific for diagnosis of early stages of disease. But this differences is not remarkable. CA125 and HE4+CA125 have some diagnostic performance to prediction of advanced stages. **Conclusion:** The data of present study suggest that combining of HE4 and CA125 is better screening tool for diagnosis of ovarian cancer generally.

Keywords: Ovarian cancer, HE4, CA125, Tumor marker, Risk of ovarian malignancy

1881P

Evaluation of serum level of MicA / MicB and its relationship with NKG2D gene polymorphisms in women with breast cancer

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Background: NKG2D receptor is one of the activator Immune cells receptors. In human NKG2D gene has two single nucleotide polymorphisms (SNP)(rs1049174G>C). According to other studies GG SNP make a high affinity receptor for NKG2D ligands whereas, CC polymorphism reduce NKG2D affinity for their ligands. The present study investigates NKG2D SNPs and serum levels of soluble MICA /Bin patients with breast cancer in Yazd city for the first time. **Methods:** In this Case-Control study, 108 patients with breast cancer and the same number of healthy women were selected from Yazd Health Care centers. Using specific NKG2D primers the gene amplified and rs1049174G>C polymorphisms were determined by RFLP procedure. MICA/B ELISA kit was used for the detectionof sMICA according to the manufacturer's protocol. **Results:** according to our statistic analysis, Frequency of CC and GG genotypes in the patients were 44.4% and 13% respectively whereas, in the healthy donors the frequency were 71.3% and 6.5%. The difference between the frequency in healthy and patients group was significant. On the other hand, the frequency of GC genotype in patients was considerably higher than the healthy women (PV, 0.0001). With determine of relative risk in the patients it revealed that women with CC genotype have more susceptibility for breast cancer in comparison with Yazd healthy women.(CI=95%, 1.31-2.24, OD=1.7).median levels of sMICA were detected in sera from patient 240± 362.3(Pg/ml) and in healthy women were 127±83.2l(Pg/m).medianlevels of sMICB were detected in patient were162±69.8(ng/ml) and in healthy women were 0.3±0.1 (ng/ml).(Pvalue<0.0001). **Conclusion:** the present study results showed that homozygote CC genotype in Yazd women population will increase chance of breast cancer.The levels of sMICA/B were frequentlyelevated in patients with advanced breast cancer.The elevation of sMICA/B was associated with (SNP)(rs1049174G>C).

Keywords: NKG2D gene, Mic A, MicB

2218P

Metformin attenuates angiogenesis and neutrophil recruitment in air pouch model in rats

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Background: AMP-activated protein kinase (AMPK) activation inhibits mammalian target of rapamycin (mTOR) as a key signaling process in cell proliferation. Recent studies demonstrated that metformin, an AMPK activator, lowers the risk for several types of cancer in diabetic patients. Concerning the critical role of angiogenesis in the incidence and progression

of tumors, we investigated the effect of metformin on angiogenesis. **Methods:** In this study we used air pouch model of rat for assessing the effects of metformin on angiogenesis and inflammation. Carrageenan was used for induction of granulomatous tissue and carmine red dye was processed for the assessment of the dye content. The total leukocyte count was determined in a Neubauer chamber and the differential cell count was determined by microscopic counting of Giemsa stained slides. **Results:** Oral administration of 50 and 100 mg/kg of metformin significantly ($P<0.01$; $P<0.05$) decreased angiogenesis in granulomatous tissue by 34 and 25%, respectively. In agreement with these findings, vascular network formation was also inhibited by metformin. There was a greater growth of new blood vessels in the carrageenan-treated group than in the treated (metformin 25, 50 and 100mg/kg) rats.Oral administration of 25, 50, and 100 mg/kg of metformin produced a marked ($P<0.001$; $P<0.01$) reduction in the neutrophil percentage in the exudates by 13%, 18%, and 11% respectively. **Conclusion:** The present study reported that metformin (as an AMPK activator) inhibited carrageenan induced neutrophil activity and angiogenesis, and the effect may is partially AMPK dependent.

Keywords: Angiogenesis, Metformin, AMPK, Air pouch

2856P

Anti-cancer effect of Ethyl acetate fraction of shallot through modulating the genes involved in cell cycle

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Background: Cancer cells have an uncontrolled and infinitive cell cycle in their growth process. One of the important strategies to control of cancer cell growth is affecting the cell cycle. The present study was performed to examine the potential of the ethyl acetate (EA) fraction of shallot on MDA-MB-231 and MCF-7 breast cancer cells. **Methods:** Human MCF-7 and MDA-MB-231 cell lines were cultured and treated with different concentrations of the EA fraction of shallot. After different time treatments, the expression of three cell cycle-related genes including cyclin-dependent kinase 4 (CDK-4), Cyclin D1 and p27 in treated cells were assayed by real-time PCR technique. **Results:** Gene expression analysis showed that the EA fraction of shallot could alter the cell cycle-related genes including p27, cyclin D1 and CDK4 in the breast cancer cell lines compared to the controls. In other words, the expression of p27 gene was increased by EA fraction while this effect on cyclin D1 and CDK4 genes was decreasing. **Conclusion:** Our study has obviously showed that the flavonoid-rich fraction of shallot may be a potential anti-cancer agent. Further studies including *in vitro* and *in vivo* assays are needed to purify effective compounds from shallot and clarify their chemotherapeutic potency in human breast cancer.

Keywords: Cell cycle, Shallot, Cyclin-dependent kinase, Cyclin D1

2855P

Apoptotic effect of Ethyl acetate fraction (EA) of shallot on two breast cancer cell lines (MCF-7 and MDA)

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Background: Cancer is one of the major threats to public health worldwide. Apoptosis is known to eliminate potentially malignant cells, hence increment of apoptosis can be considered to play a key strategy in combat with cancer cells. In this study, we evaluated the apoptotic potential of ethyl acetate (EA) fraction of shallot on MDA-MB-231 and MCF-7 breast cancer cells. **Methods:** Human MCF-7 and MDA-MB-231 cell lines were treated with different concentrations of the EA fraction isolated from shallot. Then, the expression changes of the apoptosis-related genes including bax, bcl_2 and p53 in the above mentioned cells was determined by real-time PCR technique. **Results:** Our results showed that the EA fraction had a significant effect on expression of the genes involvement in apoptosis in favor of cell death. On the other hand, the EA fraction could down-regulate the anti-apoptotic gene bcl_2 and up-regulate the expression of the apoptotic genes bax and p53 genes on the two breast cancer cells MCF-7 and MDA-MB-231. **Conclusion:** On the basis of our study, the obtained data suggest the EA fraction of shallot causing apoptosis at gene expression level. Future studies focusing on biological significance of EA fraction-induced apoptosis would lead to exploring the mechanisms of chemotherapeutic potency of shallot components in human breast cancer.

Keywords: Apoptosis, Ethyl acetate fraction, Shallot, Breast cancer, Gene expression

3029P

Association of *CTLA-4* gene polymorphisms with sporadic breast cancer in North-West population of Iran

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Background: Breast cancer is the leading cause of female deaths due to malignancies worldwide. The host immunogenetic background plays an important role in the development of breast cancer. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a molecule expressed predominantly on activated T cells and is important during the down-regulation of T-cell activation. To this end, several clinical trials are already underway using anti-CTLA4 antibody for the treatment of various malignancies. **Methods:** This case control study will be conduct in a setting of 100 women with breast cancer and 100 healthy donors. Genomic DNA will be extract from 5 ml frozen whole blood using the DNA Extraction Kit (Qiagen, Germany). A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay will be use to detect dimorphism of -1661 G/A, -658 T/C, -318 T/C, +49 G/A. PCR products will be digest overnight with restriction enzymes (NEB, UK) and will be analyze by 2% agarose gel electrophoresis. **Results:** The aim of this study is to evaluate the potential influences of *CTLA-4* gene polymorphisms on breast cancer risk and the susceptibility to and progression of breast cancer in the north- west population of Iran and comparison of results with similar results in other regions of Iran and world.

Keywords: *CTLA-4* gene, Polymorphisms

2566P

In-vitro effects of hemolymph serum of *Potamon persicum crab (HSPPC)* on MCF-7 and MDA breast cancer cell lines

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Background: Breast cancer is the most common cause of cancer-related death in women worldwide. Therefore, there is an urgent need to identify and develop therapeutic strategies against this deadly disease. This study is the first to investigate the effects of *HSPPC* on MCF-7 and MDA cells. **Methods:** MTT and LDH assays were performed on MCF-7 and MDA cells as well as Human umbilical vein endothelial cells (HUVEC) to determine the cytotoxic activity of the *HSPPC* at different concentrations. Further, the apoptosis inducing action of the hemolymph serum was determined by TUNEL (terminal deoxy transferase (TdT)-mediated dUTP nick- end labeling) and cell death assay. **Results:** IC50 for crude *HSPPC* on MCF-7 and MDA cells was 960 and 850µg/ml, respectively. Growth of both MCF-7 and MDA cell lines were significantly ($P<0.001$) inhibited by *HSPPC* as compared with untreated controls at 48 hours. The results showed that *HSPPC* had no cytotoxic effects but significantly inhibited cell growth in a dose and time dependent manner. In addition, DNA fragmentation analysis (TUNEL) and cell death assay indicated induction of apoptosis by hemolymph serum of *HSPPC* on MCF-7 and MDA cells. **Conclusion:** The results suggest that hemolymph serum of *HSPPC* contains bioactive compound(s) with potentials for treatment of breast cancer.

Keywords: Apoptosis, Hemolymph, *Potamon persicum crab*, Breast cancer

2826P

Effect of Six weeks of Endurance Training on IL-4 Levels of Tumor Tissue in Female Mice with Breast Cancer

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Background: Any type of tumor needs angiogenesis in order to grow. IL-4 inhibits the tumor growth through its anti-angiogenic characteristics. IL-4 is a powerful angiogenesis inhibitor that can act locally and systemic in the inhibition of angiogenesis. The aim of this study was to investigate the effect of a 6-week-long endurance training program on IL-4 tissue levels and tumor mass in breast tumor-bearing female BALB/c mice. **Methods:** Twenty female BALB/c mice (4-5 weeks of age) were randomly divided into identical groups: rest-tumor-rest (RTR) and rest-tumor-exercise (RTE). After induction of tumor, RTE group undertook endurance training at 55-70% VO_2max for 6 weeks. Tumor mass was measured on a weekly manner over the training period. At the end of training period, tumor tissue IL-4 concentrations were measured by ELISA. Data was analyzed using independent t-test and Pearson correlation coefficient test. **Results:** A significant difference (P=0.033) was found in IL-4 tissue levels

between groups. Also, the amount of reduction in tumor mass in RTE group was significantly greater than that of RTR group (P=0.0001). Moreover, a significant reverse correlation (r=-0.501; P=0.048) was found between IL-4 concentration and tumor mass, suggesting an anti-tumor role for IL-4. **Conclusion:** Tumor mass reduction in response to exercise training could to a great extent be dependent on cytokine modulation, which occurs through decreased production of pro-inflammatory and angiogenic cytokines on the one hand, and increased production of anti-angiogenic cytokines, including IL-4, on the other hand. Thus, moderate-intensity endurance exercise could be used as an effective strategy in cancer treatment.
Keywords: Breast cancer; Endurance training, IL-4, Tumor tissue

2801P

Effectiveness of Permanent Implantable Catheter (Polysite) in Children with Cancer

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Background: Totally implantable central venous access devices (ports) have been available for over 10 years, but have not been achieved widespread use in pediatric oncology patients. Ports facilitate the administration of chemotherapy in children with cancer. **Methods:** In this study, early complications of implantable central venous access devices in children with different type of cancer was taken under investigation. All of the complications were recorded by staff nurses by checklist for one week. The study included 68 patients with different cancer (lymphoma-leukemia-sarcoma and wilms' tumor) who were treated between April 2007 and November 2011 in oncology department of Dr Sheikh hospital, Mashhad University of medical science. **Results:** Venous ports were placed in 26 (38.2%) girls and 42 (61.8%) boys aged between 2 and 12 years old (mean: 6 years).We implanted all of the venous ports in patients for chemotherapy, and port implantation procedures were performed by a experienced Pediatric Surgery. 3 cases (4.4%) have needle access site infections which were controlled with antibiotics. Catheter leakage in 3 cases (4.4%), port-catheter disconnection in 4(5.8%) cases and occlusion of the system in 5 cases (7.4%). In this period, there were no major complications. **Conclusion:** With proper placement technique and adequate nursing care, they represent a definite improvement in child cancer therapy. Ports can provide satisfactory for the majority of pediatric oncology patients, with a low risk of line-related complications and a high degree of acceptability to child.
Keywords: Catheterization, Central Venous, Instrumentation, Adverse effects, Child

2784P

Risk Factors and the Most Common Initial Symptoms of Acute Lymphoblastic Leukemia in Children

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Background: The factors involve in Leukemia are not fully understood. However, research has shown the relationship between this disorder and some risk factors. The aim of this study was to determine risk factors involved in acute lymphoblastic leukemia and its most prevalent clinical manifestations in children residing in Khorasan province. **Methods:** It was a case – control study. The adequate sample size was 100 cases and 400 controls. Controls were

matched with cases regarding their sex, age and habitation. Data was collected by face to face interview with patients' mothers and the questionnaires were completed by the investigator. Data was analyzed by conditional logistic regression using SPSS-PC (v.14). **Results:** According to the findings of this study, maternal use of oral contraceptives, living in proximity to high voltage power lines, in-utero ionizing radiation exposure, pesticide exposure in fathers and paternal occupation and parentalsmoking had a significant relation with this type of cancer ($p<0.05$).the most prevalent mitial clinical manifestations were lethargy and weakness, fever, leg pain, cervical lymphadenopathy, bleeding, abdominal pain and cold symptoms respectively. **Conclusion:** According to the results of this study, it seems that environmental factors play an important role in etiology of this kind of cancer. Knowledge abort this fact helps us to find appropriate ways to prevent this disease in children.

Keywords: Acute Lymphoblastic Leukemia, Risk Factors, Initial Symptoms,Children

2867P

Effect of Six weeks of Endurance Training on TGF-β Levels of Tumor Tissue in Female Mice with Breast Cancer

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Background: TGF-β has been shown to possess a tumor suppressor effect in the early phase of cancer, which is of great importance in cancer. Increases in local and systemic levels of TGF-β can contribute greatly to cancer treatment and tumor mass reduction. The aim of this study was to investigate the effect of a 6-week-long endurance training program on TGF-β tissue levels and tumor mass in breast tumor-bearing female BALB/c mice. **Methods:** Twenty female BALB/c mice (4-5 weeks of age) were randomly divided into identical groups: rest-tumor-rest (RTR) and rest-tumor-exercise (RTE). After induction of tumor, RTE group undertook endurance training at 55-70% VO_2maxfor 6 weeks. Tumor mass was measured on a weekly manner over the training period. At the end of training period, tumor tissue TGF-β concentrations were measured by ELISA. Data was analyzed using independent t-test and Pearson correlation coefficient test. **Results:** A significant difference (P=0.035) was found in TGF-β tissue levels between groups. Also, the amount of reduction in tumor mass in RTE group was significantly greater than that of RTR group (P=0.001).Moreover, a significant reverse correlation (r=-0.789; P=0.001) was found between TGF-β concentration and tumor mass, suggesting an anti-tumor role for TGF-β. **Conclusion:** Tumor mass reduction in response to exercise training could to a great extent be dependent on cytokine modulation, which occurs through decreased production of pro-inflammatory and angiogenic cytokines such as IL-17 on the one hand, and increased production of anti-cell proliferation cytokines, including TGF-β, on the other hand. Thus, moderate-intensity endurance exercise could be used as an effective strategy in cancer treatment.

Keywords: Breast cancer; Endurance training,TGF-β, Tumor tissue

2951P

Effects of Chlorella vulgaris supplementation on tumor growth and viability of lymphocytes in spontaneous mouse mammary tumor (SMMT)-bearing BALB/c Mice

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Background: There are many controversial data about Chlorella vulgaris (CV) anti-tumor effects. We examined effects of CV powder supplementation on tumor growth and viability of splenocytes in spontaneous mouse mammary tumor (SMMT)-bearing BALB/c mice. **Methods:** Eighteen 6-8 week-old mice were randomly divided into three groups (n=6); Water+Tumor (WT) group which by gavages received Distilled water (DW), and CV1+Tumor (CV1T) and CV2+Tumor (CV2T) groups which received CV powder (200 and 300 mgCV/kg, respectively)for six weeks. On days 14th and 42thall mice received SMMT and were sacrificed, respectively. Tumor size and body weight (during supplementation) was measured. Splenocytes were cultured either alone or with PHA and/or with SMMT Antigens for 48 hours, and their viability was evaluated by MTT test. Data were compared among and within all groups by ANOVA and Post Hoc Tests. **Results:** There were no significant changes in body weight of mice among all study groups. In CV1T and CV2T groups the tumor sizes on days 27th and 32thwere insignificantly and on day 37th, significantly larger than tumor of WT group(P<0.05). Viability of splenocytes from CV1T and CV2T groups was significantly lower than viability of splenocytes from WT group, after either of three types of culture (P<0.05). **Conclusion:** Our results indicate that CV powder has no effect on body weight and may contribute to the tumor growth in SMMT-bearing mice; possibly by increasing the susceptibility of immune cells to death. However, further studies should be carried out in this respect.

Keywords: Chlorella, Spontaneous mouse mammary tumor, Tumor Growth, Viability

2990P

Production of anti-VEGFR-2 recombinant PE38 immunotoxin and evaluation of its effect on the HUVEC and MCF-7 cell lines in vitro

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Background: Immunotoxins (ITs) have been developed for the treatment of cancer, and are composed of antibodies linked to toxin. Also vascular endothelial growth factor (VEGF) plays a key role in tumor angiogenesis, and the blockade of VEGF receptor-2 (VEGFR2) inhibits angiogenesis and tumor growth. The aim of this study was to produce anti-VEGFR2/rPE (Pseudomonas exotoxin) 38 IT to test its cytotoxiciy activity and mechanism of action. **Methods:** At first, DNA that encodes recombinant PE38 protein inductively expressed in E. coli and was purified by nickel-sepharose chromatography and protein was analyzed by western blot. Then, for production of IT, rPE38 was chemically conjugated to anti-VEGFR2. The cytotoxicity response by IT treatment was evaluated by MTT test in HUVEC and MCF-7 (VEGFR2+) cell lines. The mechanism of IT cytotoxicity was observed by AnnexinV staining

and flow cytometry. **Results:** SDS-PAGE showed 98% purity of rPE38 and IT. In vitro dose-dependent cytotoxicity assay demonstrated that anti-VEGFR2/PE38 is toxic to VEGFR2-positive cells. IT treatment significantly inhibited proliferation of HUVEC and MCF-7 in a VEGFR2-specific manner as compared with the control groups ($P<0.05$). Flowcytometry showed that the mechanism of IT induced cell death is mediated by apoptosis. IT treatment also caused remarkable synergistic cytotoxicity characterized by decreased cell viability, and an increased apoptotic index by both anti-VEGFR2 and PE38. **Conclusion:** These results raise the possibility of using anti-VEGFR2/PE38 IT for cancer therapy because nearly all tumors induce local angiogenesis with high VEGFR expression.

Keywords: VEGFR2, Pseudomonas exotoxin, Immunotoxin

3038P

Single-drug chemotherapy of canine transmissible venereal tumor with vincristine sulphate and doxorubicin: a *hematological,* cytological and *histopathological* study

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Background: In dogs, Canine transmissible venereal tumor (CTVT) grows progressively for a few months and then usually r egresses spontaneously. Several treatment options are available for the treatment of the tumour, with chemotherapy being the most commonly employed. **Methods*:*** In this study, twelve dogs affected by CTVT and testicular seminoma tumour were studied retrospectively. The cytological sample was smeared onto a glass slide and either air-dried for May-Grünwald-**stain** and masses were surgically removed, tumours were grossly examined and sections 4 **µ**m thick were obtained from each sample and H&E stained. For chemotherapy, vincristine sulphate was administered weekly as an infusion over 3 min via cephalic vein at a dose of 0.025 mg/kg after diluting with physiological saline to a total amount of 10 ml. **Results:** Cytological smears from eight tumours in regression after chemotherapy were poorly cellular and many cells were fragmented. In two progressive tumours there was an average of 1406 ±972 CTVT 200 cells/ µl, or 96·71% of total cells counted. Thus, tumour cells represented 96·71% of total cells within biopsy specimens and leucocytes 4·29% (leucocyte: tumour cell ratio =0·062±0·031). Thus, tumour cells represented 97·31% of total cells and leucocytes 2·69% (leucocyte: tumour cell ratio =0·071±0·174). **Conclusion:** Our data suggested that combination treatment with vincristine and doxorubicin could be in future an excellent therapeutic alternative for treatment of TVT for probably reducing the resistance to vincristine and also, treatment success could easily be followed by cytological changes.

Keywords: Chemotherapy, TVT, Seminoma, Cytology, Pathology

3049P

Designing new 4h-chromene compounds with anticancer activity and studying their interaction with tubulin using molecular docking approach

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Background: Microtubules have essential role in cell division, so it appears to be a

preferential target for cancer treatment. Drugs that target tubulin (such as taxol, and vinca alkaloids) are effective anticancer drugs. Among these the 4H-chromene compounds which bind to the colchicine binding site and inhibited microtubules polymerization could be novel lead compounds in the drug design and discovery. **Methods:** In this article, some approaches of rational drug design, including molecular docking and structure based drug design (SBDD) are implemented to achieving new compounds with more effective inhibition of tubulin polymerization; this will result in tumor cells growth inhibition. For this purpose, new analogs of 4H-chromene compounds with anticancer properties were designed based on tubulin structure. **Results:** The most effective compounds were identified by virtual screening docking (VSD) and the lowest binding energy in the first mode was considered as the best conformation docking pose. Then their possible interactions with colchicine binding site were analyzed. **Conclusion:** According to the success of these approaches and considering that the affinity energies of the designed compounds are lower than colchicine, so these new lead compounds could be the subject to *in vitro* evaluations.

Keywords: Tubulin, Cancer, 4H-Chromene, Molecular docking

3136P

Evaluation the effect of recombinant calprotectin on tyrosinase activity

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Background: Calprotectin as a hetrodimeric protein composed of two S100A8(8kDa) and S100A9(14kDa)subunits. This protein has been identified mainly in leukocytes and involves in inflammatory and cancer processes. Alsothe enzyme tyrosinase has been reported to be involved in melanoma skin cancer. In this study, the effect of recombinant calprotectin on tyrosinase activity was investigated. **Methods:** S100A8 and S100A9 were separately expressed in pET15b, *E.coli* BL21 (DE3) system as His-Tagged proteins. Expression and homogeneity of recombinant protein was analyzed using SDS-PAGE electrophoresis. After achievement of hetrodimerical protectin from two subunits and incubation this complex with Tyrosinase for 5min, enzyme activity was measured by **UV-visible spectrophotometry** using L-DOPA as a substrate. **Results:** Protein expression was achieved at 37°C and 3 hour incubation as two distinct band of 8 and 14 kDa.Protein purification was achieved at 150 and 200 mM Imidazole as indicated as homogenous bands. Exposure of tyrosinase with recombinant calprotectin led to increased enzyme activity. **Conclusion:** Increased tyrosinase activity, after interaction with recombinant calprotectin, could be new potential targets to control of malignant melanoma in future.

Keywords: Calprotectin, pET15b,*E.coli* BL21(DE3), Tyrosinase, melanoma

3129P

Association of MTHFR variant C677T with Retinoblastoma

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Background: Folate and methionine metabolisms are involved in DNA synthesis and methylation, and polymorphisms in genes of folate metabolizing enzymes have been associated with some forms of cancer. MTHFR is one of the most important genes of this pathway and the C677T polymorphisms of this gene has been found to reduced the MTHFR enzyme activity and it lead to lower folate levels. Low levels of folate during retinogenesis may have increased uracil misincorporation, hypomethylation and, as a consequence, be more likely to develop postzygotic mutations in RB1. The aim of current study is to evaluate association between MTHFR C677T polymorphism with retinoblastoma. **Methods:** A case- control study of 96 retinoblastoma cases and 204 cancer-free children controls was performed to investigate whether the polymorphism of the methylene tetra hydrofolate reductase (MTHFR C677T) altered the risk for retinoblastoma. C677T polymorphism was evaluated in cases and controls using PCR-RFLP and the products were separated on 12% polyacrylamide gels. The results were analyzed using Chi square test and SPSS software. **Results:** allelic frequencies of C and T alleles were 0.833 and 0.167 in cases and 0.721 and 0.279 in controls, respectively. Genotypes frequencies of CC, CT and TT in cases were 0.688, 0.291 and 0.021 and in controls were 0.51, 0.422 and 0.068, respectively. Chi square test showed significant differences between cases and controls (P= 0.009). **Conclusion:** MTHFR C677T CT plus TT Genotype frequencywere significantly lower in patients than in control and the results show a protective effect for T allele. Further studies in larger cohorts are needed to be performed.

Keywords: Retinoblastoma, polymorphism, MTHFR, Folate

3163P

MiR-155 induces apoptosis via TNFRSF10A but not TNFRSF10B and TNFRSF11B dependent manner

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Background: Micro-RNAs (miRNAs) play important roles in regulation of cell functions including apoptosis. Previous studies demonstrated that miRNA-155 (miR-155) can induces apoptosis but its molecular mechanisms is yet to be clarified, hence, the aim of this study was to determine the effect of this miRNA on the mRNA levels of TNFRSF10A, TNFRSF10B and TNFRSF11B as pro-apoptotic molecules. **Methods:** MiR-155 and a scramble sequence were introduced, separately, to the Jurkat cell lines (T cell leukemia) and the mRNA levels of, TNFRSF10B and TNFRSF11B were examined in parallel with beta-actin and GAPDH (as housekeeping genes) using Real-Time PCR technique. Apoptotic changes also were identified using annexin-FITC staining method. **Results:** Our results show that TNFRSF10A expression increase in miR-155 transfected Jurkat cells 117.78 ± 12 rather than scramble 1± 0.1 and PBS 1± 0.2 transfected cells ($p<0.001$). On the other hand TNFRSF10B and TNFRSF11B did

not show any significant difference in comparison to scramble and PBS groups. **Conclusion:** According to our results, it may be concluded that miR-155 can lead to increase half-time of TNFRSF10A, as pro-apoptotic molecule but not TNFRSF10B and TNFRSF11B mRNAs. Thus, it seems that miR-155 can consider as a therapeutic agent to treatment of cancer cells by inducing apoptosis through up-regulation of TNFRSF10A.

Keywords: MiR-155, TNFRSF10A, TNFRSF10B, TNFRSF11B, Jurkat cell.

3291P

Retinol plasma level and its association with the percentage of regulatory T cells and Th17 lymphocytes in patients with breast cancer

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Background: The use of Retinol (Vitamin-A) and its derivatives in the treatment of breast cancer has shown promising results. In vitro studies, however, indicated the effects of vitamin-A derivatives on the conversion of Th17 cells into regulatory T (Treg) cells. In this study, we aimed to investigate the plasma level of vitamin-A,and the percentage of Treg and Th17 lymphocytes in breast cancer patients. **Methods:** 27 patients in stageII of breast cancer and 26 age-matched healthy women were enrolled. Vitamin-A plasma level was measured by HPLC. The percentage of CD4+CD25+FoxP3+ (Treg) lymphocytes and CD4+IL17+ (Th17) cells was evaluated by flowcytometry. **Results:** Vitamin-A plasma level was not significantly differ between patientsand controls (0.52±0.1 vs 0.53±0.1 ng/ml, P=0.6). The percentage of Treg cells in patients was significantly higher than controls (4.13±1.28 vs. 1.78±0.89, P <0.01). Conversely, Th17 percentage was significantly higher among controls (2.03± 0.7 vs. 1.25± 0.7, P<0.001).A positive correlation was observed between Vitamin-A andTreg cell percentage among patients (P=0.03), but not in control group.No significant correlation was found between vitamin-A plasma level and Th17/Treg ratio neither in patients nor among controls. **Conclusion:** Despite increase in Treg percentage and decrease in Th17 percentage in patients with breast cancer, plasma level of vitamin-A seems not to be associated with the plasticity between these two cell subsets. Plasma vitamin-A might however increase the percentage of Treg cells in a way rather than cell phenotype conversion.

Keywords: Breast cancer, VitaminA, Regulatory T cells, Th17 cells

1408P

Assessment of new gene expressed in prostate (NGEP) in human prostate tissues using tissue microarray analysis

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Background: NGEP is a newly diagnosed prostate specific gene that is expressed only in

normal prostate and prostate cancer cells. Discovery of tissue-specific markers may promote the development of novel targets for immunotherapy of prostate cancer. **Methods:** In the present study, the staining pattern and clinical significance of NGEP was evaluated in a well characterized series of prostate tissues composed of 123 prostate cancer (PCa), 19 High Grade Prostatic Intraepithelial Neoplasia (HPIN) and 44 samples of benign prostate tissue included in tissue microarrays using immunohistochemistry. **Results:** Our study demonstrated that NGEP localized mainly in the apical and lateral membranes and was also partially distributed in the cytoplasm of epithelial cells of normal prostate tissue. All of the examined prostate tissues expressed NGEP with a variety of intensities; the level of expression was significantly more in thebenign prostate tissues compared to malignant prostate samples (p-value <0.001). Among prostate adenocarcinoma samples, a significant and inverse correlation was observed between the intensity of NGEP expression and increased Gleason score (P=0.007).**Conclusion**: Taken together, we found that NGEP protein is widely expressed in low grade to high grade prostate adenocarcinomas as well as benign prostate tissues, and the intensity of expression is inversely proportional to the level of malignancy. NGEP could be an attractive target for immune based therapy of prostate cancer patients as an alternative to the conventional therapies particularly in indolent patients.

Keywords: NGEP, Prostate tissues, Tissue microarray analysis

2077P

Investigation of NGEP expression levels in two standard prostate cancer cell lines

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Background: Prostate cancer is one of the leading causes of cancer deaths among men. NGEP (New Gene Expressed in Prostate), is a prostate-specific gene that expressed only in normal prostate and prostate cancer tissue. Because of its selective expression in prostate cancer cell surface, NGEP makes an excellent immunotherapeutic target. **Methods:** In the present study, we investigated NGEP expression in LNCaP and DU145 cells by RT-PCR, flow cytometic and immunocytochemical analyses. **Results:** RT-PCR analysis of NGEP expression showed that NGEP is expressed in the LNCaP cells but not in DU145 cells. The detection of NGEP protein by flow cytometric and immunocytochemistry analyses indicated that NGEP protein is weakly expressed in LNCaP cell membrane. **Conclusion:** Our results demonstrated that LNCap cell line is more suitable for NGEP expression studies in molecular and protein levels. Furthermore, NGEP expression may be increased by androgen supplementation of culture medium.

Keywords: DU145 cell line, LNCaP cell line, NGEP, Prostate cancer

1983P

The anti cancer activity of novel chalcone and flavanone analogues

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Background: Currently, invasive cancer is the leading cause of death in the developed world and the second leading cause of death in the developing world. Chemotherapy is one of the notable healing techniques. Cytotoxic agents are toxic to all human cells, not just cancer cells, because they act by killing cells that divide rapidly. Therefore, designing effective new anti cancer agents with fewer side effects is a necessary issue in the medicinal chemistry. **Methods:** The *in vitro* cytotoxic activity of synthesized chalcone and flavanone analogues against human cancer cell lines, including MDA-MB231, MCF-7 (Breast cancer), SK-N-MC (Neuroblastoma) and non-tumoral cell line MRC-5 was evaluated by MTT assay. The half maximal growth inhibitory concentration (IC_{50}) values were calculated from the concentration-response curves by nonlinear regression analysis. **Results:** Cytotoxicity evaluation of synthesized compounds against human cancer cells revealed the most compounds were cytotocxic with IC_{50} values in micromolar range. 3′-halogenated chalcones were more potent than etoposide as reference drug and had less cytotoxic activity against MRC-5 cells. **Conclusion:** The anti cancer effects of these analogues were apparently selective for cancer cell lines.

Keywords: Cytotoxic activity, Cancer, Cell line, Anti cancer activity

1982P

The cytotoxic activity of new synthetic dehydroepiandrosterone derivatives against three human cancer cell lines

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Background: The discovery and development of new anticancer agents is required due to problems with currently available medicines, like toxicities and drug resistance. Traditional anti-cancer drug discovery has focused on the identification of cytotoxic chemotherapeutic agents that could be of natural or synthetic origin. Cytotoxic chemotherapy is cancer treatment that kills cells, specifically cancer cells. In this study a series of synthetic dehydroepiandrosterone derivatives have been screened for their cytotoxicity activity against three human cancer cell lines. **Methods:** The cytotoxic activity of synthesized dehydroepiandrosterone derivatives was evaluated against three different human cancer cells including KB (Nasopharyngeal epidermoid carcinoma), T47D (Breast cancer) and SK-N-MC (Neuroblastoma) cell lines by MTT reduction colorimetric assay. Etoposide was used as a standard drug and the half maximal growth inhibitory concentration (IC_{50}) values were calculated from the concentration-response curves by nonlinear regression analysis. **Results:** The results of cytotoxic data indicate that most of synthesized compounds showed moderate to strong cytotoxic potential in all three cell lines. The IC_{50} values of the most potent synthesized derivative were 0.6 and 1.7 μM against KB and T47D cell lines, respectively. **Conclusion:** The cytotoxic activity of these compounds indicated that new derivatives of Dehydroepiandrosterone could be served as a potent anti-cancer agent.

Keywords: Cytotoxic activity, Cancer, Cell line, MTT assay

2002P

Effect of anticancer drug Pomalidomide on vital activity and apoptosis induction of bone marrow mononuclear cells

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Background: Pomalidomide - a combination of drugs Lenalidomide and Thalidomide- is one of the newest anticancer drugs. Pomalidomide induces apoptosis in cancer cells but it has relatively low cytotoxic effect on normal peripheral blood cells. However, it has been a little information about the effect of Pomalidomide on bone marrow cells that contain plasma cells and progenitor cells. In this study the effect of Pomalidomide on bone marrow mononuclear cells was studied. **Methods:** Samples of bone marrow aspirates were obtained from individuals who were pathologically considered normal and mononuclear cells were isolated. Half of the mononuclear cells were cultured with Pomalidomide (final concentration 10μM) and the other half were without drug (control group). After 48 h incubation, vital activity (MTT) and cell apoptosis induction rates (ethidium bromide and acridine orange staining) were investigated. **Results:** The results of culture bone marrow mononuclear cells showed that the vital activity in the presence of Pomalidomide increased significantly compared to cells without drug treatment ($p<0.05$). However, apoptosis induction of normal bone marrow mononuclear cells in the presence of the drug was not significantly different. **Conclusion:** It may be concluded that Pomalidomide stimulates vital activity of bone marrow mononuclear cells (with healthy pathologic conditions) to improve and maintain these cells and on the other hand, it has not cytotoxic effects or apoptosis induction.

Keywords: Pomalidomide, MTT, Apoptosis, Bone marrow

1946P

Cytotoxic activity of thiazole thiones on human breast cancer cell lines

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Background: Cancer is one the global health problem and the most frightening and fatal disease of human. Chemotherapy has still been an important fundament for cancer treatment. Agents that interfere with mitotic progression by perturbing microtubule dynamics are commonly used for cancer chemotherapy. The discovery and development of new anticancer drugs is needed due to problems with currently available medicines, like toxicities and drug resistance. In continuation of our work on new compound derivatives, a new series of thiazole thiones were evaluated for anti cancer activity. **Methods:** The in vitro cytotoxic activity of synthesized compounds against three human cancer cell lines, including MCF-7, MDA-MB-231 and T47D and non-tumoral cell line MRC-5 was determined by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay. **Results:** The cytotoxic activity evaluation of thiazole thiones against human cancer cell lines showed the most compounds were cytotocxic against cancer cells. 4-methyl analog showed the highest activity against all

cell lines. Results of MTT assay showed that the most potent compounds have less cytotoxic activity against non-tumoral cells. **Conclusion:** The cytotoxicity of the most potent compound was apparently selective for tested cancer cell lines and this compound had no significant toxicity towards non-tumoral cell line MRC-5.

Keywords: Anticancer activity, Cytotoxicity, Chemotherapy

1599P

Anticancer activity of piperazin-1-yl- naphthyridin-3- carboxylic acid derivatives against three breast cancer cell lines

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Background: Breast cancer is a heterogeneous disease with different morphologies, molecular profiles, clinical behavior and response to therapy. The development of novel, efficient, and less toxic anticancer agents remains an important and challenging goal in medicinal chemistry. The present work is an extension of our ongoing efforts towards developing effective anticancer agents by evaluation of a new series of Piperazin-1-yl- naphthyridin-3- carboxylic acid derivatives against 3 breast cancer cell lines. **Methods**: New derivatives of the synthetic compounds were evaluated for their cytotoxicity on three breast cancer cell lines including MDA-MB-231, MCF-7 and T-47D by MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay. Apoptosis was determined morphologically after staining MDA-MB-231 cells with acridine orange/ethidium bromide using fluorescence microscopy. **Results**: Results of the MTT assay showed that Piperazin-1-yl- naphthyridin-3- carboxylic acid derivatives with different substituent exhibited differential cytotoxicity in various human breast cancer cells lines. Among the tested compounds derivative with IC_{50}=2.2±1.5 showed the highest cytotoxic activity against T-47D. **Conclusion**: It has been demonstrated that the Piperazin-1-yl- naphthyridin-3- carboxylic acid could be lead compounds in the synthesis of anti tumor drugs.

Keywords: Cancer, MTT assay, Cytotoxic activity

2142 P

SDF-1alpha G801A polymorphism in Southern Iranian patients with meningioma

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Background: Stromal cell derived factor-1 (SDF1) is an angiogenic chemokine with cancer promoting effects. This chemokine has a nucleotide transition from G to A at position 801 in 3`-untranslated region which is indicated to be associated with the susceptibility of distinct types of tumors. The main aim of this study was to investigate the correlation between this genetic variation and susceptibility to meningioma brain tumors in an Iranian population.

Methods: Genotype and allele frequencies of *SDF1* was assessed using Polymerase Chain Reaction-Restriction Length Polymorphism (PCR-RFLP) method in 61 patients diagnosed with meningioma tumor and 262 normal subjects as the control group. **Results:** No statistically significant difference was observed in the frequencies of genotypes and alleles between patients and controls ($p>0.05$). The frequencies of GG, AG and AA genotypes were 57.5, 31.1 and 11.5% in patients and 55.3, 37 and 7.7% in healthy controls, respectively. **Conclusion:** Our data suggest that the *SDF1* gene polymorphism at position 801 G/A is probably not associated with the susceptibility to meningioma in Southern Iranian patients.

Keywords: Chemokine, Meningioma, Gene polymorphism, SDF-1

1942P

Serum levels of VEGF-C correlated inversely with gene expression of VEGF-A in peripheral blood mononuclear cells of acute myeloid leukemia patients

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Background: The pivotal role of angiogenesis has been suggested in the pathophysiology of AML. One of the most key regulators of angiogenesis is the vascular endothelial growth factor (VEGF), which increases permeability and promotes proliferation, migration and differentiation of endothelial cells. Among VEGF family, it has been known that VEGF-A and VEGF-C are expressed by AML cells. The role of VEGF-A as a proangiogenic factor in AML has been well documented. But, the significance of VEGF-C levels in the pathogenesis of AML has not been clarified well; the aim of this study is to evaluate serum levels and gene expression of these growth factors in peripheral blood mononuclear cells of patients with AML. **Methods:** We investigated the serum levels and mRNA expression of VEGF-A and VEGF-C in peripheral blood mononuclear cells of twenty- seven patients with newly diagnosed AML by ELISA and Quantitative Real time PCR. **Results:** Serum levels of VEGF-C correlated inversely with gene expression of VEGF-A in peripheral blood mononuclear cells of AML patients ($rs= - 0.580$, $P=0.023$). Also, patients with low serum levels of VEGF-C (below the mean value) displayed a significantly higher VEGF-A expression ($P=0.005$). However, there was no significant correlation of VEGF-C mRNA expression with serum levels of AML patients ($rs= 0.158$, $P= 0.546$). **Conclusion:** Our data showed that high serum levels of VEGF-C are associated with a decreased expression of VEGF-A mRNA. It seems that VEGF-C had a tumor inhibitory role and high levels of VEGF-C can inhibit the growth and progression of AML leukemic cells.

Keywords: Acute Myeloid Leukemia, Gene expression, VEGF-A, VEGF-C

1647P

The stimulating potential of SW742 allogeneic cell line on PBMCs of Gastrointestinal malignant patients is comparable to autologous tumor cells in vitro

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Background: Natural killer activity is believed to be important contributor of a patient's immune system to fight cancer. However, cancer patients have reportedly defective NK activity and the malignant target frequently has developed mechanisms to escape detection of NK cells. Our research is aimed at overcoming this NK cell deficiency. **Methods**: Malignant autologous epithelial cellsof 10 colorectal carcinoma patients were separated by cell culture procedures. Peripheral blood mononuclear cells (PBMCs) were stimulated with their mitomycin treated autologous tumor cells or allogeneic SW742 colorectal carcinoma cell line. The expression of CD3, CD56, NKG2D and NKp44 were detected with flow cytometry and reverse transcription-PCR. NK activity of PBMCs against K562 target cell line was measured by MTT colorimetric assay. **Results**: Stimulation with autologous tumor cells and allogeneic SW742 colorectal carcinoma cell line augmented CD56+ and CD56+CD3+ cells and up-regulated NKG2D and NKp44 expression. NK activity of PBMCs after co-incubation with autologous tumor cells or SW742 was significantly raised. **Conclusion**: Our results demonstrated that stimulation of PBMCs by SW742 can significantly improve NK activity as much as by autologous tumor cells which was confirmed by the higher expression of NKp44 and NKG2D. Since the separation of autologous tumor cells is difficult andtime consuming the allogeneic tumor cell line could be a good replacement for large scale short term generation of activated NK cells. These data may help to improve cancer immunotherapy protocols.

Keywords: Tumor immunotherapy, Natural Killer activity, Autologous Tumor cells, SW742, NKp44, NKG2D

1510P

IL-17 serum levels in patients with Gastric Cancer

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Background: Stomach cancer (excluding skin cancer) is the most prevalent cancer of the five most common cancers among males and females in Iran. Previous studies have tried to determine the role of cytokines in malignancies and exploit them as diagnostic or prognostic markers. IL-17 is an important cytokine in inflammation which promotes tumor growth and angiogenesis. The frequency of Th17 cells increases in tumoral tissue of stomach cancer patients. We evaluated the level of IL-17 in sera of patients with stomach cancer in south of Iran. **Methods:** In this study, we recruited 57 stomach cancer patients and 31 healthy individuals as control group. The blood was collected by venipuncture method after informed consent. Sera were separated by centrifugation and stored at -20 until used. The level of IL-17 in serum was measured using a commercial ELISA assay. The optical density results were then converted to concentration by a standard curve and were analyzed using SPSS software. **Results:** In this study, no difference was observed in IL-17 concentration between patients and controls. No correlation was observed between the stage of the disease and IL-17 level. However 5 (8.8%) patients in higher stages of the disease showed elevated IL-17 levels in their sera. Also, we did

not find any correlation between tumor grade, type, depth of invasion and other clinical and pathological data with IL-17 levels. **Conclusion:** Our result indicated that IL-17 level in serum is not elevated in stomach cancer patients in southern of Iran. This observation contradicts with another report in stomach cancer patients who had a higher proportion of Th17 cells in peripheral blood and increased level of serum and tumoral IL-17 cytokine. This discrepancy may be due to the few numbers of samples included in both studies. On the other hand, there is the possibility of differences in the risk factors and inflammation associated infections along with genetic differences between the two populations.

Keywords: IL-17, Gastric Cancer, ELISA

1615P

Evaluation of IL-17A in sera of patients with Colorectal Cancer

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Background: Colorectal cancer (CRC) is one of the deadliest cancers in the world. It is the third most common cancer among men and the fourth among women in Iran. The disease is associated with immune system disturbances such as changes in cytokine balances. Cytokines are part of the pro-inflammatory response to tumor which can be produced by cancer cells as well, thereby controlling angiogenesis and tumor growth. One of the inflammatory cytokines produced by both immune cells and tumor cells is IL-17A. **Methods:** We recruited 122 CRC patients and 50 healthy subjects. The level of IL-17A in serum was measured using a commercial ELISA assay. The optical density results were then converted to concentration by a standard curve and were analyzed using SPSS software. **Results:** We observed a significantly higher level of IL-17A in sera of patients compared to controls. Among 122 patients with CRC, 14 (11.5%) showed elevated IL-17A in their sera compared to controls (p=0.003). A significant association between IL-17A levels and vascular invasion was observed (p=0.008). However, there was no correlation between IL-17A in serum and stage grade, type, volume, site and depth of tumor. **Conclusion:** The elevation of IL-17A in sera of patients with CRC and its correlation with vascular invasion indicates a role for this cytokine in CRC development, progression and invasion.

Keywords: IL-17A, Colorectal Cancer, Tumor cells, ELISA

1610P

IL-17 does not increase in the Head and Neck Squamous Cell Carcinoma

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Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world. The disease is associated with immune system disturbances such as

changes in cytokine balances. One of the inflammatory cytokines produced by both immune cells and tumor cells is IL-17. The aim of this study was to investigate the serum levels ofinterleukin-17 in patients with HNSCC in comparison to healthy controls. **Methods:** In this study, 134patients with HNSCC and 42 healthy individuals were investigated. Every participant was interviewed and examined for inclusion and exclusion criteria. IL-17serum levels were measured by a commercial ELISA assay and the results were analyzed by SPSS software version 11.5. **Results:** Mean levels of IL-17 were 0.29±2.2 pg/ml in patients group but it was below the detection limit of the assay in healthy controls. Serum levels ofinterleukin-17 in the3.73% of patients was increased. Among those patients for whom the site of tumor was known, laryngeal cancer in men were more prevalent (93.75%) than women (6.25%), while the other types were more in women. HNSCC among men were in higher stages (stages 3 and 4) in comparison to women. Laryngeal cancers were found to have a well differentiated grade in comparison to HNSCCs in other sites. **Conclusion:** Unlike in other solid tumors, serum levelsofIL-17inpatients with HNSCC did not increase. However, previous studies in other ethno-geographic areas reported increasedIL-17 levels in head and neck cancer which in laryngeal cancer is mostly restricted to tumor associated macrophages (TAM).Therefore, we cannot rule out the role this cytokine may play in the tumor establishment and progression. The discrepancy in the results may have also arisen from the difference in the environmental and genetic risk factors in our population as well as the type of HNSCC evaluated.

Keywords: IL-17, Head and Neck Squamous Cell Carcinoma, ELISA

1777P

The chemokine receptor CXCR4 is associated with the staging of gastric cancer

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Background: CXCR4 is a cognitive receptor for stromal-derived factor-1 (SDF-1) and has been previously shown to be associated with tumor growth and invasion of many cancers. However, its expression and function in gastric cancer has not been well clarified. **Methods:** Herein, we studied the expression of CXCR4 on gastric samples from patients with gastric adenocarcinoma in comparison with precancerous lesions by employing qRT-PCR. **Results:** Our qRT-PCR data show that CXCR4 is highly expressed in tissue samples from patients with gastric cancer compared to precancerous lesions (2.4 times higher, *P* value< 0.05). When we correlated the level of CXCR4 with clinicopathological findings, we observed that CXCR4 expression in significantly elevated in tissue samples from patients with stages III and IV of the disease compared to lower stages (I & II). In addition, CXCR4 expression is higher is patients who had lymphatic invasion. **Conclusion**: We present evidence that CXCR4 level is significantly elevated in later stages of gastric cancer. Thus, CXCR4 may play a crucial role in gastric cancer progression.

Keywords: CXCR4, Staging of gastric cancer, PCR, Gastric cancer

2144P

Culture supernatant from normal breast cell line can induce differentiation and inhibit proliferation of malignant breast cell lines

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Background: Microenvironment of cancer has an important role in tumor cell growth and metastasis. Recent studies indicate that normal cells of the tumor microenvironment secret molecules (cytokines, chemokine, etc.) which affect on the interaction the immune system and tumor cells. This may cause provides supportive signals and poor prognosis or helps the immune system to eradicate the malignant cells. Culture supernatant from MCF10-A as a breast non tumorigenic epithelial cell line is used in this study to evaluate the maturation effect and growth inhibitory potential. **Methods:** MCF10-A, MCF7, MDA-MB468,T-47D cell lines are adapted in cell culture medium. Supernatant of MCF10-A cell line was collected in 90% confluence and expose to malignant cell lines while they were in 70% confluence, afterwards differentiation status of tumor cells was investigated and MTT proliferation assay have been performed. **Results:** MCF10-A culture supernatant after 48 hours leads to differentiate and reduced proliferation rate of malignant cell lines. The malignant cell lines showed spheroid phenotype, similar to the normal differentiated cells and the proliferation assay showed significant reduction as indicated by MTT assay. **Conclusion:** Based on our data, secreted biomolecules from normal epithelial cell around the tumors very important for the fate of cancer cells. This study inline with others finding indicates that normal cell surrounding tumor tissue may have important role in providing supportive or inhibitory signals for tumor cell growth and differentiation.

Keywords: Normal breast cell line, MCF10-A supernatant, Breast cancer cell lines

1765P

Inhibitory effect of dexamethazone on proliferation of lymphoblastic leukemoid cell line (C121) increases by pterostilbene

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Background: Acute lymphoblastic leukemia (ALL) is the most common malignancy in pediatric patients. Pterostilbene (trans-3, 5-dimethoxy-4-hydroxystilbene) is an antioxidant that is primarily found in blueberries. The aim of this study was to investigate the effect of pterostilbene with Dexamethasone on lymphoblastic leukemia cell line. **Methods:** Human T lymphoblastic leukemia Jurkat cells were treated with increasing doses of pterostilbene, Dexamethasone, or combinations of them. The viability of Jurkat cells were determined by MTS assay after Pterostilbene with or without dexamethazone treatment after 48-hours incubation. Cells were incubated with various concentrations of drugs in different time points and then analyzed for apoptosis with annexin V–FITC/PI double staining and flow cytometry. **Results:** The viability of cells decreased to 95%, 87% and 77% at the concentration of 1 µm, 10 µm and 100 µm respectively. Cell viability did not reach to 50% even in the higher concentration of dexamethazone. The cells had 50% viability at 60 µm concentration of pterostilben. Combination of this concentration with the above concentration of dexamethazone lead to

cell viability less than 50% in a dose dependent manner. Pterostilbene with dexamethazone treatment increased apoptosis in Jurkat cell line more than pterostilbene alone. **Conclusion:** As cell viability could not reach to 50% in the presence of dexamethazone alone, combination with pterostilbene can help to inhibit more cell proliferation. Pterostilbene can augment the effect of dexamethazone.

Keywords: Acute lymphoblastic leukaemia, Dexamethasone, Pterostilbene, Apoptosis, Proliferation

1764P

Effect of pterostilbene with or without Asparaginase on lymphoblastic leukemoid cell line (C121)

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Background: Acute lymphoblastic leukemia (ALL) is the most frequently occurring cancer in children and adolescents. Pterostilbene, a naturally occurring stilbenoid, is present in blueberries and has been found to have anti-oxidant, anti-inflammatory, and anti-proliferative properties. The aim of this study was to investigate effect of pterostilbene with or without asparaginase on lymphoblastic leukemia cell line. **Methods:** Human T lymphoblastic leukemia Jurkat cells were treated with increasing doses of pterostilbene, asparaginase, or combinations thereof. The proliferation of Jurkat cells before and after Pterostilbene, with or without asparaginase treatment was determined by MTS assay after 48 hours incubation. Apoptosis was evaluated by the annexin V/propidium iodide assay by flow cytometry. **Results:** Pterostilbene decreased cell viability in 60 µm concentration to 50%. By increasing the concentration to 70 and 100µm, the cell viability fell to 40% and 10% respectively. Asparaginase decreased cell viability in 0.5 IU/ml concentration to 50%. Pterostilbene with asparaginase decreased Jurkat cell viability in a concentration- and time-dependent manner. Pterostilbene with or without asparaginase treatment was increased apoptosis in Jurkat cell line. **Conclusion:** Pterostilbene can inhibit cell proliferation and increase cell apoptosis in Jurkat cells. Considering the fact that in combination with a lower concentration of asparaginase, pterostilbene can inhibit cell proliferation as much as a higher concentration of asparaginase alone, the side effect of asparaginase can be declined. In experimental models, this effect can be evaluated *in vivo* and may be a step to help treatment progress.

Keywords: Acute lymphoblastic leukaemia, L-Asparaginase, Pterostilbene, Proliferation, Apoptosis

1434P

Development of novel mouse anti-CD20 monoclonal antibody based on chimeric protein

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Background: CD20 has been an effective target for immune therapies that use mAbs to treat non-Hodgkin's lymphoma (NHL) because it neither internalizes nor dissociates from the

plasma membrane upon Ab binding. The anti-CD20 chimeric monoclonal antibody rituximab is the most widely used therapeutic antibody in these patients. It has also been shown that almost 60% of them respond to rituximab therapy and others become 'resistant' to repeat rituximab therapy. **Methods:** The aim of this research was investigating a further tool to develop a new anti-CD20 monoclonal antibody. Therefore, in addition of two usual CD20 antigens we used our own newly chimeric protein of CD20 which synthesized in our lab. Three separate groups of BALB\c mouse were immunized with all above mentioned antigen in usual manner to have enough plasma cells before fusion with SP2/0 cells. The supernatant of all hybridoma cells were analyzed against used antigen **Results:** Our Elisa results showed a significant monoclonal antibody of IgG1 against antigen used. The immune blotting results showed, monoclonal antibody produced against former antigens has some cross reaction with other non-related cells. Therefore, we followed our experiments with monoclonal antibody induced against newly CD20 chimeric protein. Our primary results showed no cross reaction with other two non-related cells. **Conclusion:** Based on our results of monoclonal antibodies against two usual CD20 antigen it seems work on new antigen of CD20 is required and our newly chimeric protein might be a good candidate for immunotherapy of NHL.

Keywords: Monoclonal antibody, CD20, Chimeric protein

1504P

Effect of the Royal jelly on necrosis of the Erythroleukemia cancer cell co-cultured with peripheral blood mononuclear cells

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Background: Royal jelly (RJ) is a substance synthesized by the common worker bee for the feeding of the Worker bees was shown to exhibit various biological activities on different cells and tissues of the human body. **Methods**: In this Experimental study human PBMCs (10^6 cells/ml) were separately cultured in standard conditions. The cells were then incubated with different concentrations of Royal jelly (5mg/ml, 10 mg/ml, and 25mg/ml) for 72 hours. Subsequently, cytotoxic effect of PBMCs pulsed with RJ on K56 (10^4 cells/ml) for 5 hours was evaluated by Anexin PI test (Flow cytometry). **Results:** In the Anexin PI test, Necrosis property and cytotoxic effect of PBMCs pulsed with RJ on K562 were remarkably up-regulated compared to control group. **Conclusion:** PBMCs pulsed with RJ increased Necrosis percent of K562 cell, this compound can be used as a useful strategy to control cancer. However this is a preliminary study and further studies should be designed.

Keywords: Royal jelly, K562, Necrosis, Peripheral blood mononuclear cell.

2100P

Antitumor effects of a novel glycoconjugate based vaccine $(Globo)_3$-DTPA-KLH in nude mice

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Background: There have been many attempts to produce and evaluate glycoconjugated vaccines against cancer in developed countries based on the most prominent MCF7 cell surface polysaccharides that is called Globo. Vaccination with tumor specific glycoconjugate complexes may induce a strong immune response against cancer cells. In this regards, many attempts have been made to develop a vaccine called Globo-H-KLH. **Methods:** We attempted to develop a novel vaccine complex based on the natural appearance of the polysaccharides on the cells using 3 Globo residues and conjugation with Keyhole limpet hemocyamin (KLH) protein to create a $(Globo)_3$-H-KLH complex using a DTPA as a linker. The final complex was then called $(Globo)_3$-DTPA-KLH. $(Globo)_3$-DTPA-KLH was injected to CB6F1 mice 4 times with 2 weeks intervals. Two weeks later, blood was drawn, centrifuged and the sera were collected. The immunized animals with $(Globo)_3$-DTPA-KLH showed a relevant and strong antibody titer at 1/900 dilution. **Results:** The ability of sera from immunized mice to prevent the formation of tumors was examined in nude mice by injecting MCF7 cells under the mice skin followed by injection of collected sera. In comparison to the control group, there was no progressive tumor formation in mice injected with anti-$(Globo)_3$-DTPA-KLH antibody. In addition, the splenocytes from immunized or control mice were incubated with MCF7 cells and injected under the skin of nude mice. Interestingly, no tumor was developed in injected mice. **Conclusion:** All together, these data could be promising for development of an effective, protective and/or therapeutic vaccine against human breast cancer.

Keywords: Globo, MCF-7 cells, Glycoconjugated vaccines, Nude mice, Splenocytes

1512P

Soluble CD138/Sdc-1 increases in sera of patients with moderately differentiated bladder cancer

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Background: We assessed the level of soluble CD138/Syndecan-1 (Sdc-1) in sera of patients with adenocarcinoma, papillary and urothelial carcinoma, transitional cell carcinoma and squamous cell carcinoma of bladder and compared it with healthy individuals. While the expression of CD138/Syndecan-1 molecule in the urothelial carcinoma and other types of bladder cancer is reported, the reports on soluble form of the molecule are scarce. **Methods:** The study population included 86 bladder cancer patients and 42 healthy individuals. The objectives of this study were to determine if soluble CD138/Syndecan-1 increases in bladder cancer and whether such an increase (if existed) is associated with grade, stage, invasion, size, location and type of tumors. Serum samples from patients and controls were gathered after consideration of inclusion and exclusion criteria and the level of CD138/Syndecan-1 was measured by a commercial ELISA assay .Clinical and pathological data for each patient was extracted from patients' files and also by using a questionnaire at the time of sampling. **Results:** Soluble CD138/Syndecan-1was significantly increased in the sera of patients with

bladder cancer (138.42 ± 81.85 vs. 76.81 ± 79.55 ng/ml, *P* = 0.000). Patients over 70 had higher levels of CD138/Syndecan-1 in their sera (*P* = 0.025). Soluble CD138/Syndecan-1 was higher in sera of patients with moderately differentiated tumors as compared to poorly differentiated ones (170.47 ± 85.06 vs. 101.79 ± 68.24 ng/ml, *P* = 0.01). Soluble CD138/Syndecan-1 was significantly higher in muscle invasive (154.45 ± 83.60 vs. 89.99 ± 55.02 ng/ml) but not lymphatic invasive (106.25 ± 52.10 vs. 123.43 ± 63.76 ng/ml) tumors (*P* = 0.027 and *P* = 0.45, respectively). A non-significant trend of increase in the level of CD138/Syndecan-1 in sera of male patients compared to female patients was observed (145.38 ± 85.47 vs. 110.20 ± 59.04 ng/ml, *P* = 0.054). **Conclusion:** To our knowledge, this is the first study to investigate the levels of soluble CD138/Sydecan-1 in sera of patients with bladder cancer and its correlation with clinicopathological criteria of the tumors. Our data suggest that elevated soluble CD138/Sdc-1 in bladder cancer patients may be considered a non-invasive marker of muscular invasion in males and elder patients.

Keywords: Soluble CD138/Sdc-1, Moderately differentiated bladder cancer, ELISA

1542P

Reduced levels of soluble CD138/Sdc-1 in the Squamous Cell Carcinoma of the Tongue compared to healthy controls

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Background: CD138 or Syndecan-1 (Sdc-1) is a transmembrane molecule which is expressed in epithelial tissues and mediates cell adhesion to extracellular matrix and cell-cell adhesion. **Methods:** We investigated the serum levels of CD138/Sdc-1 in 43 patients with Squamous Cell Carcinoma of the Tongue and compared it with its level in healthy age/sex matched controls using a commercial ELISA assay. The patients group consisted of 18 females and 25 male individuals. The control group consisted of 19 females and 27 male individuals. **Results:** CD138/Sdc-1 levels were decreased in patients with Tongue SCC compared to healthy controls (p=0.001). Interestingly, this decrease was more prominent in males (p=0.012) compared to females (p=0.06). The level of CD138/Sdc-1 in patients was 91.03 ± 88.52 pg/ml while its level in control group was 157.97 ± 99.05 pg/ml. There was no significant difference in the level of soluble CD138/Sdc-1 between males and females in general or males and females in each group. **Conclusion:** CD138/Sdc-1 levels are reduced in patients with Tongue SCC compared to healthy controls in southern Iran. Losing membranous (shedding) CD138/Sdc-1 is suggested to increase tumor motility and metastasis and this is one of the mechanisms involved in progressive loss of its expression during tumor development. ElevatedSdc-1 levels in sera of lung cancer patients is reported to correlate with poor prognosis in these patients, however, to our best knowledge, this is the first report concerning the levels of sCD138/sSdc-1 in sera of patients with Tongue SCC.

Keywords: Soluble CD138/Sdc-1, Squamous Cell Carcinoma, Tongue Carcinoma, ELISA

1652P

Mesenchymal stem cells do not suppress lymphoblastic leukemia cell line proliferation

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Background: Several studies have demonstrated the immunosuppressive effects of mesenchymal stem cells in allogeneic or mitogenicintractions. Cell-cell contact inhibition and secretion of suppressive soluble factors have been suggested in this regard. To investigate if adipose derived MSCs could inhibit Jurkat lymphoblastic leukemia T cell proliferation during coculture was observed. **Methods:** Adherent cells with the ability of cellular growth were isolated from normal adipose tissues.Initial characterization of growing cells by flow cytometry suggested their mesenchymal stem cell characteristics. Cells were maintained in culture and used during third to fifth culture passages. Jurkat or allogeneic peripheral blood mononuclear cells (PBMC) were labeled with CFSE dye and co-cultured with increasing doses of MSCs or MSC culture supernatant. Proliferation of PBMCs or Jurkat cells under these conditions was assessed by flow cytometry after 2 and 3 days of coculture, respectively. **Results:** Results showed the expression of CD105, CD166 and CD44, and the absence of CD45, CD34 and CD14 on the surface of MSC like cells. Moreover, initial differentiation studies showed the potential of cell differentiation into hepatocytes. Comparison of Jurkat cell proliferation in the presence and absence of MSCs showed no significant difference, with 70% of cells displaying signs of at least one cell division. Similarly, the highest concentration of MSC culture supernatant (50% vol/vol) had no significant effect on Jurkat cell proliferation ($p<0.6$). The same MSC lots significantly suppressed the allogeneic PHA activated PBMCs under similar culture conditions. **Conclusion:** Using Jurkat cells as a model of leukemia T cells, our results indicated an uncertainty about the suppressive effect of MSCs and their inhibitory metabolites on tumor or leukemia cell proliferation. Additional systematic studies with MSCs of different sources are needed to fully characterize the immunological properties of MSCs before planning clinical applications.

Keywords: Mesenchymal Stem Cells, Jurkat Cells, Suppression, Proliferation

1704P

The effect of Phytolacca's fruit extract into MCF-7 cell line

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Background: The Phytolacca plant can be found in huge amounts at the northern district of Iran (Caspian Sea zone). Despite being toxic, this plant especially its foliage has herbal usage. The Phytolacca's seeds are full of extract when they are ripped that make them black in color. This plant's seeds are one of the nighthingales's favorite meals. **Method:** In this research we dilute Phytolacca's fruit extract and incubate them with MCF-7 cell line in culture plate for 1.5 hours, and perform MTT test. **Results:** We showed that, this extract in low diluted can decrease function of MCF-7 cell line for reduction of MTT test. **Conclusion:** In this research, we showed that when the concentration of Phytolacca's extract is increased, cytotoxic effect is

not observed on MCF-7 cell line.
Keywords: MCF-7cell line, Phytolacca

1729P

Effect of Genistein on the expression of MMP2 in brain tumor-derived cells

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Background: In spite of advances in medical surgery, adjuvant radiation therapy and chemotherapy strategies, malignant gliomas continue to be associated with poor prognosis. Attempts have been made to study the role of angiogenic factors such as matrix metalloproteinase-2 (MMP-2), in brain tumor progression particularly in glioblastoma multiform (GBM). Genistein has previously been introduced as an anti-angiogenic agent for different cancers. To evaluate the effect of Genistein on the angiogenic cascade, we investigated the expression of MMP-2 transcripts in mesenchymal stem cell like cells from high grade and low grade brain tumors before and after in vitro treatment with Genistein. **Methods:** Brain tumor tissues were obtained from patients diagnosed with GBM and low grade glioma in sterile condition, washed with PBS, cut in to small pieces, digested enzymatically and cultured in tissue culture flasks contain DMEM and 10% FBS.As cells reached to passage 3, they were treated with Genistein and then MMP2 gene expression was determined by quantitative real time PCR in treated and untreated cells. **Results:** Genistein caused up-regulation of MMP2 transcripts in GBM treated cells up to 5-fold higher than untreated cells; whereas low grade glioma treated cells showed 200-fold lower expression of MMP2 mRNA compared to untreated cells. **Conclusion:** Our data showed that Genistein has an outstanding effect on down-regulation of MMP2 in low grade glioma compared to its effect on cells from GBM patients. Therefore, Genistein have some important advantages for treatment of low grade brain tumors, however further investigation with more works are required to see if Genistein has similar function under in-vivo condition for low grade glioma.

Keywords: Genistein, MMP2, Brain tumor

1583P

Suppression of MDR1 (ABCB1) gene expression by small interference RNA (siRNA) and evaluation of drug resistance in oxaliplatin resistant human colon cancer cell line (SW 480)

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Background: One of the most challenging aspects of colon cancer therapy is rapid acquisition of multidrug resistant (MDR) phenotype.The multidrug resistance gene 1 (MDR1) product, p-glycoprotein (p-gp), pump out a variety of anticancer agents from the cell, generallydrug resistance to chemotherapeutic agents is one of the major obstacles in the treatment of human cancers. One effective approach would be to identify and downregulate resistance-causing

genes in tumors using small interfering RNAs (siRNAs) to increase the sensitivity of tumor cells to chemotherapeutic challenge**. Methods:** Human colon cancer cell line (sw480) used in this study. Drug-resistant cells were generated by continuous incubation of cell line (sw480) with stepwise increases in drug (oxaliplatin) concentration over a period of 3 months. Resistance cell were transfected with-specific siRNA using lipofectamine 2000 (invitrogen) for knock Down of MDR1. Quantitative real time PCR and RT PCR were performed to determine the mRNA expression of MDR1 .Western blot was performed to determine the protein expression of MDR1. Drug sensitivity was analyzed by MTT assay and the number of apoptotic cells was determined with the TUNEL assay. **Result:** SiMDR1s delivery effectively inhibited MDR1 expression at both mRNA and protein levels and decreased p- glycoprotein function. Silencing MDR1 led to decreased cell viability and drug IC50. **Conclusion:** Our data demonstrates that the RNA interference could knock down gene MDR1 and reduce the P-glycoprotein expression, and partly reverse the MDR of sw480 cells in vitro. These results suggest that knockdown of MDR-1 is an effective therapeutic target for human colon cancer treatment.

Keywords: Colon cancer, SiRNA, MDR1 gene, Oxaliplatin, Sw480

2256P

Production of recombinant immunotoxin using diphtheria toxin and interleukin

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Background: Immunotoxin is a biological substance containing two distinct sections covalently bond by specific linkers. It includes an immunological section with diagnostic role to connect the immunotoxin to the specific receptors and a toxic section with cytotoxic role that leads to the cell death. One of the treatment strategies of cancers is protein therapy by protein toxins, especially immunotoxins. The aim of this study was to design a lymphoma cancer protein therapy method using a recombinant immunotoxin produced by connecting diphtheria toxin to human interleukin 2. **Methods:** The design and synthesis of the immunotoxin gene sequence was done in the pET expression system. pET-IDZ plasmid was transformed to *E. coli BL21(DE3)* bacteria and then was induced. The protein purification was accomplished by nickel affinity chromatography system and then the function of the produced recombinant immunotoxin was evaluated on K-562 cancerous cell line by MTT biological assay. **Results:** The expression of the produced recombinant immunotoxin in *E. coli BL21 (DE3)* bacteria was validated by electrophoresis and western blot methods. The purification yield was above 95%. Then K-562 cells were treated by different concentrations of the produced immunotoxin. The results showed the proper function of the produced immunotoxin. Also, IC_{50} amount was determined 6×10^{-6} M. **Conclusion:** Designing and production of recombinant immunotoxins can be a novel strategy for cancer treatment. Therefore, this produced immunotoxin is beneficial for the future studies.

Keywords: Immunotoxin, Diphtheria, Interlukin, Expression, Purification.

1840P

Combined heated 4T1 cells and naloxone improve outcome of breast cancer induced in mouse

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Background: Cellular immunity may play a useful role in defense against tumor cell.In this line, evidence indicates that naloxone (NLX) promote immune responses toward T helper 1 profile.The present study was carried out to investigate the efficacy of a new vaccine against breast cancer madeby mixingheated 4T1 cells and NLX,as an adjuvant. **Methods**: Breast cancer was induced by injection of $10^6$4T1 cells into the mammary fat pad of female BALB/c mice. After tumor growth, the mice were twice with one week interval vaccinated by a mixture of 10^5 heated 4T1 plus propranolol (6mg/Kg). **Results:** In mice vaccinated with heated tumor cells and NLX, decreased tumor growth rate and increased survival were seen. Furthermore, in these mice, the production of IFN-γ in splenocytes was increased and conversely, the production of IL-4 and IL-10 was decreased. Moreover, the frequency of FoxP3+Treg cells in splenocytes of vaccinated mice was significantly decreased. **Conclusion:** Combined NLX and heated 4T1 cells promote beneficial outcome in mouse model of breast cancer.

Keywords: 4T1, Naloxone, Breast cancer, Tumor vaccine

1917P

Assessing changes expression and presentation of NKG2D under the influence of serum factors in breast cancer patients

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Background: Breast cancer is the most common cancer in women worldwide. Nk cells play an important role in tumor cells killing by NKG2D binding to the MICA proteins on tumor cells surface. Accumulated soluble MICA from tumor cell surface in serum may lead to the down regulation of NKG2D expression that has been proposed to be a novel mechanism used by cancer cells to evade the immune system. In this study we assessed influence of sMICA on changes expression and presentation of NKG2D on NK cells from breast cancer patients. **Methods:** In heparinised PB from 49 healthy and 49 breast cancer persons before surgery and chemotherapy, NKG2D expression and presentation were assessed by qRT-PCR and Flow cytometry methods respectively and to determine the serum sMICA we used ELISA. In Flow cytometry, whol blood Stained by Anti-CD56/NKG2D/CD3. Then obtained Results analyzed with WinMDI software. In order to Statistical analysis of data we used SPSS software. **Results:** In contrast to healthy volunteers Significant amounts of sMICA was detected in sera from majority of patients. The expression and presentation of NKG2D was significantly lower than of healthy person with an inverse correlation to sMICA and direct correlation to tumor stage. **Conclusion:** Our study reveals that sMICA might be act as effective factor in diminish

NKG2D in breast cancer patient in compare to healthy person, and sMICA can be candidate as target for elevate expression and presentation of NKG2D that directly contributing in tumor cell killing. But need more work to determine other confounding factors in cancer patient.
Keywords: Breast cancer, NKG2D, sMICA, Flow cytometry, qRT-PCR

2215P

The effects of Plant Flavonoid, Chrysin on Proliferation and Apoptosis of a Gastric cancer cell line (AGS)

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Background: Cancer results from structural and quantitative alterations in molecules that control different aspects of cell behavior. Flavonoids are polyphenolic compounds that are ubiquitously in plants. They have important effects on cancer chemoprevention and chemotherapy. Chrysin is a natural flavonoid and has been shown recently to have anticancer effects. However, the mechanisms that chrysin inhibits cancers are not well known. In this study, we investigated the effects of chrysin on viability, proliferation and apoptosis of human gastric adenocarcinoma cell (AGS). **Methods:** Cells were cultured in DMEM medium and treated with different concentrations of chrysin for three consecutive days. Cell viability was quantitated by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) assay. The percentage of apoptotic cells was determined by flow cytometry using Annexin V fluorescein isothiocyanate. **Results:** The MTS assay revealed that chrysin had an antiproliferative effect on AGS cells in a dose- and time-dependent manner. **Conclusion:** Our results suggest that Chrysin has anti-proliferative effects on gastric cancer cells and is a powerful inhibition of growth of AGS cells in vitro. Therefore, chrysin may be a potential compound for both cancer prevention and treatment.
Keywords: Chrysin, Gastric cancer, Apoptosis.

1667P

The AA genotype of IL-17F rs763780 SNP is associated with reduced risk of colorectal cancer

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Background: IL-17 family of inflammatory cytokines playimportant and sometimes contradictory roles in autoimmune and inflammatory diseases as well as human malignancies. We investigated the association of IL-17A and IL-17F single nucleotide polymorphisms (SNPs) with susceptibility to colorectal cancer (CRC). **Methods:** The IL-17A rs2275913 (G197A) andIL-17F rs763780 (A7488G) SNPs in 202 patients with CRC and 203 healthy age/sex matched controls were detected by PCR-RFLP. For evaluation of the functional relevance of IL-17A and IL-17F SNPs on their production, the serum levels of IL-17A and IL-17F were investigated in 107 and 109 patients as well as33 and 52healthy individuals, respectively, by ELISA assays. **Results:** The AA genotypes and Allele [OR=0.46, 95% CI: 0.21-1.1] fromIL-17F were associated with a decreased risk of CRC compared with the AG genotype and G allele

(P=0.03). Moreover, IL-17F AA genotype was significantly associated with well-differentiated tumors (P=0.02).The G/G haplotype of IL-17A G197A/IL-17F A7488G was associated with CRC (OR=2.047,95% CI: 0.979-4.281, P=0.05). TheIL-17A concentrations in the sera of patients with CRC were significantly increased compared to healthy individuals (P=0.008), and serum levels of IL-17Awas significantly related to tumor size (P=0.043). However, the genotype distribution of IL-17A rs2275913 SNP was not significantly different inpatients and controls. No IL-17F was detected in patients and only one healthy individual had IL-17F in his serum. **Conclusion:** Our finding suggested that theIL-17F A7488Gpolymorphism may be involved in reduced risk of CRC and IL-17A may be a candidate tumor marker in colorectal cancer.

Keywords: Colorectal cancer, Single nucleotide polymorphisms, Haplotype, IL-17A, IL-17F

1668P

IL-23R gene haplotypes are associated with the risk of colorectal cancer

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Background: IL-23 is one the major drivers of Th17 response which exerts its effect through its cellular receptor. The human IL-23R gene is placed on the chromosome one at 1p31. **Methods:** The IL-23R rs11209026 and IL-23R rs1088967SNPs were investigated in 202 patients with colorectal cancer and 203 healthy age/sex matched controls by PCR-RFLP method. The association of these alleles with the levels of IL-17A in the sera of the 107 patients and 33 healthy controls was investigated by a commercial ELISA assay. **Results:** There was no significant difference in the frequencies of A and G alleles of IL-23R rs11209026 SNP and frequencies of A and C alleles of IL-23R rs10889677 SNP between patients and controls. Interestingly, the G/C haplotype of IL-23R rs11209026/rs10889677 was significantly associated with increased risk of colorectal cancer (OR=4, 95% CI: 1.323-2.086,P=0.008). On the other hand the G/A haplotype was associated with a decreased risk of colorectal cancer (OR=0.116, 95% CI: 0.28-0.521, P=0.0006). There was no significant correlation between these SNPs with the clinical characteristics of the patients. IL-23R rs10889677 polymorphism was marginally associated with increased IL-17A levels in the sera of patients (P= 0.06). In this regard, patients with CC genotype had higher IL-17A levels in their sera compared to patients with AA and AC genotypes (Mean ± SD = 3.41 ± 8.91 vs. 1.24 ± 0.722, and 1.43 ± 1.68 pg/ml). **Conclusion:** Our data suggest that different IL-23R haplotypes are differentially associated with the risk of colorectal cancer.

Keywords: Colorectal cancer, Haplotype, IL-23R, Iran

2129P

Differentiation of Naïve CD4+ T cells to T regulatory cells after co-culturing with either adipose derived stem cells (ASCs) or breast cancer cell line (MDA-MB468)

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Background: Mesenchymal stem cells (MSCs) have the ability to localize in breast carcinoma. They inhibit both innate and adaptive immune cells through cell to cell contact and release of soluble mediators. The aim of present study is to survey the plasticity of Naïve CD4+ T cells toward T regulatory cells in co-culture with either ASCs from breast cancer patients or invasive breast cancer cell line (MDA-MB468). **Methods**: ASCs were isolated from breast cancer adipose tissue and digested with collagenase type I. In passage 3 of cell culture, expression of mesenchymal stem cell specific markers was checked using flow cytometry method. Naive CD4+ T cells were isolated from normal individuals using magnetic activated cell sorter and then co-cultured with ASCs. T cells were then checked for expression of CD4, CD25, CD127 and FoxP3 by flow cytometry method. **Results:** Results showed that Naïve CD4+ T cells had a shift toward Tregs, to both $CD4^{+}CD25^{high}CD127^{low}$ $FoxP3^{+}$ and $CD4^{+}CD25^{-}CD127^{low}$ $FoxP3^{+}$ Phenotypes, after co-culture with both breast cancer ASCs and MDA-MD468 ($P < 0.05$). **Conclusion:** These data collectively conclude that besides breast cancer cells, resident ASCs may have crucial roles in breast tumor growth and progression by inducing regulatory molecules and promoting anti-inflammatory reaction within the tumor microenvironment.

Keywords: CD4+ T cells, T regulatory cells, Adipose derived stem cells (ASCs), Breast cancer cell line (MDA-MB468)

1709P

Effect of Licorice protein fractions on growth of gastrointestinal cancer cell lines

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Background: Colon cancer is one of the most common cancers which lead to death in men and women. It is believed that herbal therapy is safe and has fewer side effects. Licorice is an ancient plant in herbal medicine. There are many medical properties such as anti-cancer and anti-inflammatory in Licorice. In this study we investigate effects of licorice extract and its fraction proteins on colon cancer cell lines (HT-29 and CT-26) in compare normal cell line. **Methods:** The proliferation and apoptosis assay of HT-29 and CT-26 cells treated with different concentrations of licorice extracts fractions were assessed by MTT assay and AnnexinV apoptosis assay respectively. **Results**: Our results indicate that licorice protein fractions treated tumor cells such as protein band 30 with concentration 5 µg/ml and protein band 60 with concentration 8 µg/ml significantly can inhibit the growth of tumor cell lines (p value < 0.05) while they have little effect on normal cells. **Conclusion:** Different concentrations of the protein fractions of licorice have inhibitory effect on colon cancer cell lines.

Keywords: Licorice, Apoptosis, Colon cancer

1829P

Evaluation of microbial load, tumor markers variation and hematological parameters in patients with gastrointestinal cancer compared

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Background: The current study aimed to evaluate the microbial crowding in gastrointestinal tracts together with sera level of tumor marker variations and hematological parameters to see the interactions of these in GI cancer patients compared with healthy individuals. **Methods**: Totally 40 patients with GI cancer together with 40 healthy individual enrolled in this study. One stool and one blood sample was collected from each individual. PCR for microbial genes, ELISA for tumor markers, autoanalyzer and hematological procedures for other parameters were employed. Data were analyzed using different descriptive and analytical methods. **Results**: The mean level of CA19-9, CA15-3, AFP, CEA and CA125 in case group compared to healthy individuals was 57:8, 17:10, 14:3, 17:2, and 20:3 respectively. The mean level of CF was 0.59 for the healthy compared to 0.43 for the patients. *H.pylori*, *Ecoli*, Campylobacter and Enterococci were all significantly more prevalent in case than the control groups. **Conclusion**: Microbial load together with tumor markers and haematological parameters all had significant variations in case and control groups indicating the dynamic interactions of these variables in GI cancer patients. More studies are necessary to evaluate the preceding cause of each among patients suffering from this kind of cancer.

Keywords: GI cancer, Microbial load, Tumor marker, AFP

1863P

Enrichment and amplification of tumor-specific antigens using advanced molecular technique

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Background: The subtractive hybridization technique is the highly effective approach for isolating and identifying the specific and novel genes. This method is capable to enrich the unknown and known differentially expressed genes in tumor cells. In this study, subtractive hybridization was utilized to separate tumor-specific transcripts of esophageal squamous cell carcinoma samples. **Methods:** Total RNAs from the tumor and non-tumor tissue were extracted and purification of their mRNAs was carried out by using magnetic beads. The related cDNAs of normal mRNAs were synthesized on magnetic beads and followed by hybridization with tumor mRNAs. In order to enhance efficiency of subtraction, hybridization step was repeated three rounds. Finally amplification of subtracted tumor mRNA was performed by using SMART technique. The amplified subtracted tumoral mRNA then subjected to both tumor-specific and housekeeping genes analyzing by real-time PCR. **Results:** RT-PCR assessment on subtracted mRNA as tumor-specific mRNA pool was not showed any amplification on housekeeping genes such as GAPDG and β_2-microglubolin. Furthermore, the existence of tumor-specific antigen

such as MAGE-A4 was confirmed in subtracted mRNA. **Conclusion:** Removal of common normal mRNA sequences from tumoral transcripts through the subtractive hybridization, leads to the enrichment of tumor-specific transcript sequences. Introducing of this approach in the field of cancer therapy can help us to identify tumor-specific genes as immunotherapeutic targets. Elimination of the normal antigens can reduce auto-immune response for designing of the future cancer vaccines.

Keywords: Subtractive hybridization, Esophageal squamous cell carcinoma, Tumor-specific antigen, SMART

1834P

Development of therapeutic HPV DNA vaccine using MPG as an efficient delivery system

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Background: The development of a safe and effective vaccine against human papillomavirus (HPV) is important for the control of cervical cancer. Among various vaccination approaches, the potent DNA vaccine strategies were applied to enhance antigen-specific tumor immunity. Recently, cell-penetrating peptides (CPPs) have been designed to overcome the lipophilic barrier of the cellular membranes and improve cellular uptake of naked plasmid DNA. In the present study, a novel short peptide vector termed MPG was proposed for evaluation of immunostimulatory effects of DNA vaccine expressing HPV E7 as a model antigen *in vivo*. **Methods:** MPG peptide/DNA complexes were prepared, and DNA binding capability was evaluated by gel retardation assay. Particle size, surface charges and stability of the nanoparticles were investigated in different conditions. After determination of cell viability by MTT assay, the immunostimulatory activity of peptide/DNA complex was evaluated in C57BL/6 tumor mice model. **Results:** Our results showed that rapid self-assembly between MPG and plasmid DNA forms stable nanoparticles (~ 200 nm) with positive zeta potential. Stability assays demonstrated that the MPG peptide protects DNA during formulation and preserves its bioavailability. MTT assay showed that the peptide/ DNA complex decreases the toxicity of high concentrations of MPG (> 50 μM). In addition, we indicated that E7 DNA vaccine delivered by MPG was immunologically more effective than E7 DNA alone. **Conclusion:** The transfection efficiency of the MPG-based nanoparticles supports the potential of MPG for utilizing as an effective non-viral gene carrier in DNA based cancer vaccines.

Keywords: Immunotherapy, DNA vaccine, Human papillomavirus, Delivery system, Cell penetrating peptide

1960P

Whole recombinant *Pichia pastoris* expressing HPV16 L1 antigen induces potent L1-specific immune responses

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Background: The development of an efficient vaccine against high-risk HPV types can reduce the incidence rates of cervical cancer by generating anti-tumor protective responses. Inactivated vaccines especially heat-killed yeast cells have emerged as a promising approach for generating antigen-specific immunotherapy. In the present study, heat-killed whole recombinant *Pichia pastoris* expressing HPV16 L1 capsid protein (*Pichia*-L1) was generated and the immune responses were investigated in tumor mice model. **Methods:** A whole recombinant *Pichia pastoris* expressing HPV16 L1 protein, termed *Pichia*-L1 was engineered (pPICZB-L1) and its ability was evaluated to induce B- and T-cell mediated immune responses and to eradicate C3 tumor cells *in vivo*. In this system, the expression cassette including L1 gene was integrated in *Pichia* genome through homologous recombination. C57BL/6 mice were subcutaneously vaccinated three times at 2-week intervals with *Pichia*-L1, *Pichia*, Gardasil and PBS. Two weeks after the last vaccination, mice were subcutaneously challenged with C3 tumor cells. Tumor growth was monitored twice a week for 60 days following tumor challenge. **Results:** We found that *Pichia*-L1 and Gardasil not only enhance the levels of IgG1 and IgG2a, but also increase the IgG2a/IgG1 ratio, indicating a relative preference for the induction of Th1 immune responses. Furthermore, subcutaneous injection of killed *Pichia*-L1 generated the significant L1-specific IFN-γ immune response as well as the best protective effects as compared to killed *Pichia pastoris* and PBS groups. **Conclusion:** Whole recombinant *Pichia pastoris* could protect mice challenged with C3 tumor cells. These data suggest that *Pichia*-L1 may be a candidate for the control of HPV infections.

Keywords: *Pichia pastoris* expression system, HPV16, L1 capsid protein, Anti-tumor effects, Killed vaccine

1904P

Expression Analysis of Isoforms (V3, V6) and standard form of CD44 Gene in normal and tumoral tissue in Esophageal Squamous Cell Carcinoma

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Background: CD44 is a member of the cell adhesion molecules family. In normal cells, CD44S, along with CD44V3 and CD44V6 have some effects on the cell motility, migration, and adhesion, while in tumor cells they play roles in tumor invasion, progression, and metastasis. Our aim in this study was to evaluate the CD44S, CD44V3 and CD44V6 expression in esophageal squamous cell carcinoma (ESCC) and to reveal their correlations with clinicopathological features of the patients. **Methods:** The expression of CD44S, CD44V3 and CD44V6 was compared in tumoral and distant tumor–free tissues of the esophagus in 50 ESCC patiants using comparative real-time PCR. **Results:** Significant over expression of CD44S, CD44V3 and CD44V6 mRNA was observed in 13 (26.0%, p = 0.03), 11 (22.0%, p = 0.007) and 9(18.0 %, p= 0.0001) tumor specimens, respectively. The expression of the genes was significantly associated with each other. The expression of the genes (S/V3) were significantly associated with grade of tumor differentiation (p = 0.033), stage of tumor progression (p = 0.003), and depth of tumor invasion (p = 0.00) and (S/ V6) with grade of tumor differentiation (p= 0.033)

and (V3/ V6) with grade of tumor differentiation (p=0.02), stage of tumor progression (p = 0.011) and depth of tumor invasion (p = 0.04). **Conclusion**: Simultaneous expression of these genes has an important role in tumor genesis.

Keywords: CD44 Gene, Isoforms V3, V6, Esophageal squamous cell carcinoma, Real-time PCR

1857P

Comparison of HTLV-I Proviral Load in Adult T Cell Leukemia/Lymphoma (ATL), HTLV-I-Associated Myelopathy (HAM-TSP) and Healthy Carriers

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Background: Human T Lymphocyte Virus Type one (HTLV-I) is a retrovirus that infects about 10-20 million people worldwide. Khorasan province in Iran is an endemic area. The majority of HTLV-I-infected individuals sustain healthy carriers but small proportion of infected population developed two progressive diseases: HAM/TSP and ATL. Proviral load could be a virological marker for disease monitoring, therefore in the present study HTLV-I proviral load has been evaluated in ATL, HAM/TSP and healthy carriers. **Methods:** In this case series study, 47 HTLV-I infected individuals including 13 ATL, 23 HAM/TSP and 11 asymptomatic subjects were studied. Peripheral blood mononuclear cells (PBMCs) were investigated for presence of HTLV-I DNA provirus by PCR using LTR and Tax fragments. Then in infected subjects, HTLV-I proviral load was measured using real time PCR Taq-Man method. **Results:** average age of patients in ATL was 52±8, in HAM/TSP 45.52±15.17 and in carrier's 38.65±14.9 years which differences were not statistically significant. The analysis of data showed a significant difference in mean WBC among study groups (ATL *vs* HAM/TSP and carriers P=0.0001). Moreover, mean HTLV-I proviral load was 11967.2 ± 5078, 409 ± 71.3 and 373.6 ± 143.3 in ATL, HAM/TSP and Healthy Carriers, respectively. The highest HTLV-I proviral load was measured in ATL group that had a significant correlation with WBC count (R=0.495, P=0.001). The proviral load variations between study groups was strongly significant (ATL vs. carrier P=0.0001; ATL vs HAM/TSP P= 0.0001 and HAM/TSP vs. carriers P< 0.05). **Conclusion:** The present study demonstrated that HTLV-I proviral load was higher in ATL group in comparison with HAM/TSP and healthy carriers. Therefore, HTLV-I proviral load is a prognostic factor for development of HTLV-I associated diseases and can be used as amonitoring marker for the efficiency of therapeutic regime.

Keywords: HTLV-I, Proviral Load, ATL, (HAM-TSP)

1892P

The evolution of Salinomycin effect on CT26 cell line

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Background: Salinomycin sodium is a coccidiostat ionophore that produced from Streptomycin Albus, that it's complex with sodium and potassium can be dissolve in fat, so it cause to increase cell membrane permeability to this ions. **Methods:** In this research we dissolve 0.1gr of Salinomycin in Ethanol. This antibiotic cannot be dissolve properly in Ethanol, so for solving this problem we centrifuged them in 2000 Rpm for 10 minutes. The supernatant is used for this research. We cultured CT26 cell line in complete medium of RPMI 1640 in flask. Then we poured our product in 24 wells and incubated them in 37 degree for 2 hours, and then we removed fluid on the cells and added them total RPMI 1640 and Salinomycin. After 24 hours we perform MTT test for evolution effect of Salinomycin on CT26 cell line. **Results:** We found that when we used stock of antibiotic Salinomycin can inhibit proliferation of cells and decrease of MTT reduction. **Conclusion:** We observed that when this antibiotic is diluted in abundant dilution, Salinomycin have not enough power for decrease of cell proliferation and reduction of MTT is near normal. The Ethanol is used as a control. We found that when Ethanol is used only, it cannot very decrease cell proliferation and MTT reduction. We conclude that Salinomycin in stock form can decrease cell proliferation and decrease MTT reduction.
Keywords: Salinomycin, CT26 cell line

2106P

Gene therapy of thyroid carcinoma using mda-7/IL-24 as an anticancer cytokine

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Background: The annual incidence of thyroid cancer worldwide is alarming. Despite current various treatments such as surgical resection, radioiodine therapy and chemotherapy/ radiotherapy, thyroidcarcinoma remains a lethal cancer. Assuredly, the operative and new treatment strategies were necessary to control this malignancy. Gene therapy is regarded as one of the most reliable novel therapeutic methods for hopeless cases of thyroid cancer and those who do not respond to the prevalent treatments. **Methods:** We utilized IL-24to induce apoptosis in nude mice. The human thyroid epithelial cell line (HTori) was grown in certain condition. Recombinant adenovirus plasmid pAd–mda-7 carrying human IL-24 cDNA was transfected into HTori, leading to the formationof Ad/IL-24. The recombinant adenovirus Ad-GFP carrying green fluorescent protein (GFP) was constructed as a control. RNA from cells was extracted and cDNA was synthesized to perform RT-PCR. The cytotoxic activity of Ad/IL-24 is determined based on cytotoxicity to HTori cells, using the MTT assay. IHC was performed to measure level of IL-24 in tissue. TUNEL test was used to assay DNA fragmentation. **Results**: Obtained data indicated the significant increase in levels of IL-24 in tumoral tissue. Induction of IL-24 resulted in increase of apoptosis in cancerous cells. **Conclusion:** It could be advantageous to evaluate the anti-tumoral effect of IL-24 in a mouse xenograft model of thyroidcancer. Performing clinical trials would be the next suggestion.
Keywords: Thyroid carcinoma, mda-7/IL-24, Xenograft mouse model, HTori cell, Immune gene therapy

2105P

Role of IL-25 in immunogene therapy of pancreatic cancer

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Background: Pancreatic cancer is an aggressive type of malignancy. Certainly, loss of counterbalance betweengeneration and cell death will lead to the tumoral mass development in various tissues, such aspancreas.From earlier decades, a variety of treatments as chemotherapy, radiation and surgery have beenemployed in order to pancreatic cancer remedial purposes, which are not satisfying. This research aimed at using IL-25 for treatment of pancreatic cancer. **Methods:** We designed to select four groups of C57BL/6 mice, for IL-25 gene inoculation, via mesenchymal stem cells as a vector. The first group (n=10) contained control animals with no injection. The second, third and fourth groups (n=10) included for Panc02 cell line inoculation, IL-25 gene injection via bone marrow-derived cells (MSCs) and MScs injection of without the IL-25, respectively. To measure levels of IL-25 in blood, in the four groups via ELISA, the apoptotic tumor cells in the tumor tissues are characterized by the TUNEL test to assay DNA fragmentation, as a marker of apoptosis. **Results:** Results in this study demonstrated increased levels of IL-25 via mesenchymal stem cells which led to apoptosis. Apoptosis induced by IL-25 could successfully restrict pancreatic cancer promotion. **Conclusion:** IL-25 could activate apoptotic mediators including tumor necrosis factor receptor associated factor (TRAF6), Fas-Associated protein with Death Domain (FADD) and caspases consequently. This research results demonstrated that this method could be efficient in pancreatic malignancy treatment, via inducing apoptosis in pancreatic tumoral cells.

Keywords: Pancreatic cancer, IL-25, Mesenchymal stem cells, Apoptosis, Syngeneic mice model, Immunotherapy

2104p

Anti proliferative effect of rmIL-27 protein on 4T1 breast cancer cell, as a candidate for cancer immunotherapy

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Background: Breast cancer is the most common malignancy among women. Up to several decades, significant advances in breast cancer immunotherapy and tumor immune biology have been achieved. In vitro studies of the effect of various cytokines are conducted in breast tumor. Among interleukins, IL-27-a novel cytokine- is associated with interesting properties. In addition to contributing to the Th1 reaction, IL-27 acts as a pro- inflammatory cytokine. **Methods:** In this study, mIL-27 gene cloning and production of recombinant protein were conducted, and then anti proliferative effect of IL-27 on 4T1 breast cancer cell line was evaluated. **Results:** Our study, showed that IL-27 could eliminate 4T1 cells proliferation

significantly (p<0/01). Cell to cell interactions and also morphology of cells have changed remarkably. **Conclusion:** Our data for the first time showed that IL-27 in in vitro studies, poses potential to suppress tumor by means inducing of P21 gene without any essential cells and biologic factors of tumor matrix. Therefore, rmIL-27 may be an attractive candidate protein as an antitumor agent, applicable to breast cancer immunotherapy.

Keywords: Recombinant murine IL-27, Anti proliferative, Breast tumor, Immune therapy.

2108P

Induction of apoptosis in Hodgkin's Lymphoma using IL-24 via Adenovirus vector

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Backgrounds: Hodgkin Lymphoma (HL) as a prevalent hematolymphoid malignancy begins in cells of immune system with deregulation in apoptosis. There is need for novel treatment strategies for absence of efficient therapeutical methods. Since apoptosis has an important mechanism in cancer treatments, novel anti cancer molecules to induce apoptosis are required. IL-24 interestingly, has antiangiogenic, immunomodulatory and bystander antitumoral activities. It can also induce GADD family. The authors present a novel treatment for HL, regards to anti-tumoral and immunomodulatory effects of the IL-24. **Methods:** Total of 40 female severe combined immuno deficient mice (SCID) model was prepared including control group with any injection, the group with HLinfected mice(L540Cy cell line), the group with HLinfected mice receiving IL-24 gene via Ad.5/3 recombinant adenovirus and the group contains mice receiving adenovirus without the IL-24 gene. Real-time PCR is applied to measure levels of IL-24 in cells. Apoptosis assay was performed using the TUNEL. Tumor Necrosis Factor-α (TNF-α) and Interferon gamma (IFN-γ) production were measured using ELISA. **Results:** The results indicated that IL-24 induced apoptosis in HL cell lines. Levels of immunological factors, TNF-α and IFN-γ were increased in HL infected mice which had received IL-24 via Adv. **Conclusion:** This study suggests that adenovirus vectors expressing mda-7/IL-24 may help for effective immunotherapies of HL.

Keywords: Hodgkin Lymphoma, IL-24, Apoptosis, Immune system up-regulation, Gene therapy.

2033P

A new murine monoclonal antibody for screening of human epidermal growth factor receptor-2 (HER2) in breast cancer patients

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Background: Breast cancer is a major health concern and a leading cause of death among

women. HER2 is over-expressed in 20-30% of breast cancer patients who are candidates for mAb-based therapy. Thus, HER2 detection is critical for therapeutic intervention with humanized anti-HER2 antibody. Monoclonal antibody (mAb) is the most important tool for HER2 detection and also targeted-therapy. In the present study we assessed suitability of our new mAb against HER2 (clone 1H9) for detection of HER2 expression in tumor cells. **Methods:** Reactivity of the 1H9 mAb was checked with the immunizing HER2 peptide by ELISA and also with HER2 positive and negative cell lines by flow cytometry (FACS). HER2-binding activity of this mAb was further investigated on normal and malignant breast tissues by Western blotting (WB) and immunohistochemistry (IHC) techniques. **Results:** The mAb 1H9 reacted strongly with the immunizing peptide in ELISA. Further characterization showed that the mAb could specifically recognize native HER2 protein on breast cancer cell lines as assessed by FACS. Immunoblotting results demonstrated detection of HER2 protein in breast cancer samples without reactivity with normal breast tissues. This mAb displayed a positive reactivity to HER2 protein in both frozen and paraffin-embedded breast cancer tissues in IHC assay. **Conclusion:** The mAb 1H9 seems to be a suitable candidate for screening of HER2 molecule in breast cancer tissues by IHC method.
Keywords: Breast cancer, HER2, Immunohistochemistry, Monoclonal antibody

2103P

Microenvironment supports tumor cell survival in patients with chronic lymphocytic leukemia

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Background: Communication between the tumor cells and the surrounding microenvironment are of importance for the survival of Chronic Lymphocytic Leukemia (CLL) cells. T-cells seem to bea key player in the pathogenesis of CLL contributing to the development of a microenvironment in which the leukemic clones evade apoptosis. In this microenvironment the leukemic cells exhibit an increased proliferative activity that sustains the growth of the malignant clone of B-cells. The Krüppel-like transcription factor (KLF) family has been identified as important regulator of proliferation, differentiation, cell signaling, and tumor genesis and cell death. **Methods:** In the present study we examined the expression of KLF6 and the KLF6-splice variant 1(SV1 isan oncogenic splice variant of the KLF6 wild type tumor suppressor gene) at the mRNA and protein level in $CD4^+$ (purity≥96%) and $CD8^+$ (purity≥94%) T-cells from39 patients with CLL and 10 patients with multiple myeloma as well as 10 normal donors. siRNA technology was used to down-regulate KLF6-SV1 mRNA. Sequence specific siRNA treated purified T cells and non-treated leukemic B cells were cultured together or alone for 5 days. Apoptosis of tumor cells was analyzed using Annexin V/PI staining and flow cytometry. **Results:** Western blot showed the expression of KLF6 wild-type in purified $CD4^+$ and $CD8^+$ T-cells. Real time PCR revealed a significant over expression of KLF 6-SV1 in $CD8^+$ T- cells ofCLL patients compared to normal donors (p=0.002). siRNA KLF6-SV1 transfection of T cells induced a significant down-regulation of KLF6-SV1 and in co-culture experiments a significant increase of apoptosis of B cells was noted. **Conclusion:** The results may indicate that the KLF6-SV1might be involved in a dysregulated microenvironment supporting the growth of CLL cells.
Keywords: CLL, siRNA, KLF

1628p

Evaluation of crude extracts of sea cucumbers *Holothuria lecospilota*Genus in persian gulf effects on COX-2 and VEGF genes expression and PGE2, VEGE secretion levels in comparison with celecoxib on colorectal cancer cell line.

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Background: The sea cucumber is an important food and traditional medicine in Asian countries But the Persian Gulf Sea cucumber *Holothuria lecospilota* is largely un-known for its cytotoxic and anti-inflammatory activities. The aim of this study was determine cytotoxic and anti inflamatory effect of persian sea cucumber and compare with celecoxib. **Methods:** Colorectal cancer cell line SW 742 were expose to Body wall, Culomic fluide, Internal viscera extraction of sea cucumber, (10,50,100 µg/ml) for 18 h. Following exposure to the sea cucumber expression level of COX-2 and VEGF was determined with Real-Time PCR and PGE2 and VEGF secretion levels was determined with ELIZA. The capacity of extracts to inhibit SW 742 growth was tested by MTT assay. **Results:** Body wall *extract of Holothuria lecospilota* significantly inhibited the secretion of VEGF and PGE2 by inhibiting VEGF and COX-2 at their protein and gene levels. All three fractions showed no significant ($p < 0.05$) cytotoxicity on SW 742. **Conclusion:** In general, in this study we found that *Holothuria leucospilota*can acts as an anti-inflammatory and anti cancer agent and no toxicity and could be entering it to food chain and taking advantages of this food

Keywords: Sea cucumber, COX-2, VEGF, Real Time PCR, ELIZA, Colon cancer cell line.

1605P

Induction of tumor-specific immunity against Her2-derived p5 and p435 peptide by CpG adjuvants in breast cancer vaccine

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Background: One approach to improve the immunogenicity of peptide vaccines is employment of synthetic peptide containing CTL epitopes with an adjuvant. The immunostimulatory properties of CpG motifs may be key in inducing consistent long term immunity to tumor-associated Ags when using peptides or proteins as T cell-inducing vaccines. We targeted to test the combination of effective rHER2/neu-specific CTLs epitopes (p5 and p435) with immunostimulatory ODNs containing unmethylated cytosine-guanine (CpG) motifs as adjuvant to enhance the antitumor effects and specific CD8+ T cell immune responses in vaccinated mice. **Methods**: In this study BALB/c female mice were vaccinated 3 times with p5 and p435 peptide in combination with or without CpG and PBS as control.14 days after last vaccination 3 mice per group were euthanized and Immune responses were studied in

their spleens for assessment TCD4 and TCD8 subpupulation by flowcytometry and IFN-g by Elispot. 6 mice per group were challenged by live TUBO cell line and followed for tumor size and survival. **Results**: Our result show that mice vaccinated with p5 and p435 peptide in combination with CPG adjuvant induced higher antigen-specific T-cell responses compared group without CPG. Also tumor in these mice grew slowly compared group without CPG and survival rate was significantly improved. **Conclusion**: The combination of CpG with antigenic peptide (p5 and p435) is capable of generating potent antigen-specific CD8+ T cell immune responses and antitumor effects in vaccinated mice.

Keywords: Tumor-specific immunity, Breast cancer, Vaccine

1870P

Study of the relationships between PD.1 and ICOS gene variants with colorectal cancer

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Background: Positive and negative co-stimulatory molecules are important factors determining the outcome of immune response against tumors. Since co-stimulatory molecules expression may be affected by their gene polymorphisms, in this study aimed to investigate the association between PD.1 and ICOS gene polymorphism and susceptibility to colon cancer. **Methods:** Three polymorphisms were genotyped in a total number of 76 colon cancer patients and 73 healthy controls. ICOS (-693A/G), ICOS (+1720C/T) and PD.1 (-538G/A) gene polymorphisms were evaluated by PCR-RFLP method. **Results:** Frequency of GG genotype and G allele at position -693 of ICOS gene were significantly higher in patient group (P=0.014 and p=0.0002). On the contrary AA genotype at this position was significantly higher in controls (P=0.001). There were no significant differences between the frequency of genotypes and alleles of ICOS at position +1720 among control and patients. At position -538 of PD1.1, GG genotype and G allele frequencies were higher in patient group (P<0.0001and P<0.0001). On the contrary, AA and AG genotypes at this position were significantly higher in controls (P<0.0001 and P=0.012). Frequencies of GCG and GTG haplotypes were higher in patients compared to those of controls (P=0.016 and P<0.0001). While, frequencies of GTA, ATA and ATG haplotypes were higher in controls (P=0.0017, P<0.0001 and P=0.015). GTG/GTG and GTG/GCG double haplotypes were more frequent in patients compare to controls (P=0.0147 and P=0.0071). **Conclusion:** Our study clarified the role of PD-1 (-538G/A) and ICOS (-693A/G) gene polymorphisms as one of the genetic risk factor for the development of colon cancer among Iranian patients.

Keywords: Colon cancer, PD.1, ICOS, Polymorphism

1609P

The induction of metformin inhibitory effects on tumor cell growth by hypoxic condition

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Background: Emerging evidence suggests that metformin, a widely used anti-diabetic drug, may be useful in the prevention and treatment of different cancers. Metformin acts as proliferation inhibitor in hypoxia condition. Due to lack of previous experiments in separating hypoxia and normoxia conditions, in the recent study we provide hypoxia condition in order to access the anticancer effects of metformin on cancer cells comparing with normal cells. S6K1 is one of the most important proteins in the metformin signaling pathways and its phosphorylation rate could affects activation of metformin signaling cascade. **Methods:** We cultured normal cells (HEK239) and cancer cells (MCF-7) in both hypoxia and normoxia conditions and treated with different concentrations of metformin. Their proliferation rate and apoptosis assessed using MTT test and Annexin V kit respectively. The phosphorylation of S6K1 assessed using western blotting. **Results:** Metformin inhibits proliferation more effectively in hypoxia condition compared with normoxia in cancer cells, while it has no significant difference between normoxia and hypoxia conditions in normal cells. Statistical analysis indicates that metformin causes an increase of apoptosis percent in cancer cells with hypoxia condition (p value< 0.05), while there is no acceptable increase in normal cells. In addition metformin cause a significant decrease in S6K1 phosphorylation and activation in cancer cells under hypoxia condition compared to normoxia. But there was no significant difference between normoxia and hypoxia condition in normal cells. **Conclusion:** Our results indicate that in hypoxia condition metformin shows its anti-cancer function more effectively than normoxia condition.

Keywords: Metformin, Hypoxia, Cancer

3189P

The role of Carbohydrate Antigen 19-9 (CA19-9), Carcinoembryonic Antigen (CEA), Cancer Antigen 125(CA125), Carbohydrate Antigen 15-3 (CA15-3), ferritin, and Human Chorionic Gonadotropin (hCG) Levels in diagnosis of Cancers

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Background: Today cancer is one of the most important causes of death in all over the world. So we decide to assay of the Carbohydrate Antigen 19-9 (CA19-9), Carcinoembryonic Antigen (CEA), Cancer Antigen 125(CA125), Carbohydrate Antigen 15-3 (CA15-3), Ferritin, and Human Chorionic Gonadotropin (hCG) in serum to patient with different cancers to find better diagnosis test for each cancer. **Methods:** This study included patients who are suffering of various cancers between 2010 and 2012 in Zanjan. The sera of both patients (benign and malignant) are assay and compare with control group. , the samples were centrifuged at 1000 rpm (Universal 16A- 1601; Hettich, Tuttlingen, Germany) for 10 min and the separated serum

.We utilize the Chemiluminescence and ELISA method for investigate the results. **Result:** This study included 106 patients. Of 106 patients, 40 (37.73%) male and 66 (62.26%). The mean age was 57 ± 22 years (range 24 – 83 years(P value< o.oo5). Patient selection for the study was random. Patient were divided three groups, and there were no significant gender differences between the their groups in each cancers. The first one is control group without any cancers another one was benign and the third group was malignant patient and then level of CEA, CA19.9, CA125, CA15-3, Ferritin, hCG was assay. **Conclusion:** we found that CEA levels and Ferritin in patients with malignant were significantly higher than and in patients with benign. An elevated serum CA125 level increased more specifically in the ovarian cancer group than those of other tumor makers in the serum. Furthermore, CEA was able to identify only 48.7% patients with metastatic Breast Cancer, whereas CA 15.3 detected _74% of these cases. This parameter was considered to be more useful for the diagnosis of ovarian cancer than the levels of the other tumor markers. The level of CA19.9 malignant patient in Gastrointestinal Cancer was increased compare of benign of patient. We found that CA15-3 levels in 23 patients with Lung Cancer were significantly increased compared the control group. We found that there were not any different of hCG level in malignant and benign group. So there are relation between tumor marker and various cancers that help to good monitoring the patient of suffering various cancers.
Keywords: CA19-9 ,CEA, CA125, CA15-3, Ferritin, hCG.

2734P

Correlation between the sera levels of tumor markers and the intestinal flora related parameters and hematological indices

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Background: Tumor markers in human are constantly stimulated by intestinal flora. Variation in colonization and bacterial translocation can change the pathology of the digestive tract, particularly in digestive cancer, that may change the pattern and level of tumor markers and the severity of the disease. To evaluate the relation of the level of serum tumor markers and intestinal flora and hematological parameters in gastrointestinal cancer patients the current study was designed. **Methods**: The level of tumor markers was analysed in the serum of patients with gastric (n = 37) and colorectal (n = 41) cancers using ELISA. The presence of intestinal flora was analysed using PCR and the other parameters were evaluated using autoanalyser BT3000. To evaluate the correlation of tumor markers with flora the Pearson method and the T-test model were used. **Results:** A significantly positive correlation was observed: (1) between the tumor markers and Ureas test in patients with gastrointestinal cancer and (2) tumor markers with age,(3) tumor markers with campylobacter and chlostridum (4) tumor markers with severity of the disease and (5) intestinal flora with severity of the disease and hematological indices. The highest level of tumor marker was found for CEA and CA19.9 with 16 and 10 times more than the mean levels of these markers in control group. **Conclusions**: The presence of intestinal flora can affect the pathology of the disease and is proportional to the increased level of tumor markers in serum. The severity of the disease is in direct correlation with the indices of intestinal flora. Gastrointestinal cancer and some

intestinal microbes have an effective interaction.

Keywords: Gastrointestinal cancer, Tumor markers, Intestinal Flora, colorectal cancer

1747P

Combined heated tumor cells and heated *Lactobacillus* species cause beneficial outcome in mouse model of breast cancer

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Background: The 4T1 mouse mammary tumor cell line is one of only a few breast cancer models with the capacity to metastasize efficiently to sites affected in human breast cancer. This survey was carried out to investigate the efficacy of a new vaccine against breast cancer madeby mix in gheated 4T1 cells and heated *Lactobacillus* species, as an adjuvant. **Methods:** 4T1 cells were used to induce breast cancer in BALB/c mice. After tumor growth, the mice were twice with one week interval vaccinated by a mixture of 105 heated 4T1 plus heated *Lactobacillus* species (108 colony-forming units). **Results:** In mice vaccinated with heated *Lactobacillus* speciesplus heated tumor cells, decreased tumor growth rate and increased survival were seen. In addition, in these mice, the production of IL-17 and IFN-γ in splenocytes was increased and conversely, the production of interleukin-10 was decreased. **Conclusion**: This new vaccination method cause beneficial outcome in mouse model of breast cancer.

Keywords: Breast cancer, 4T1, *Lactobacillus*, Tumor vaccine.

1673P

Reduced frequency of NKT-like cells in patients with progressive chronic lymphocytic leukemia

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Background: Natural killer-T (NKT) cells are a subset of innate immune cells displaying a limited repertoire of antigen specificities and CD1d restriction. Little is known about contribution of NKT cells in cancer initiation and progression. **Methods**: The frequencies of NKT-like cells, B cells expressing CD1d molecule and $CD4^+$ regulatory (Treg) cells were analyzed in 40 chronic lymphocytic leukemia (CLL) patients and 15 healthy subjects by flow cytometry. **Results**: Our results showed that the frequency of $CD3^+CD56^+$ NKT-like cells is significantly decreased in progressive (4.9±0.8% of total $CD3^+$ T cells) compared to indolent (8.1±1.2%, p=0.036) patients and healthy subjects (10.6±1.7%, p=0.003). However, no association was found between NKT-like cell frequency and immunoglobulin heavy chain variable region

gene (IGHV) mutation or CD38 and ZAP70 expression. On the other hand, expression of CD1d molecule was significantly higher in leukemic B cells of CLL patients (75±1.5 % of total $CD19^+$ B cells) compared to B cells from healthy subjects (59.6±2.2%, $p<0.001$), with no significant difference between progressive and indolent patients. Interestingly, the frequency of Treg cells was inversely correlated with that of NKT-like cells in CLL patients ($r=-0.4$, $p=0.002$). **Conclusion**: Our results suggest a protective role for NKT-like cells in CLL patients which seems to be downregulated presumably by Treg cells.

Keywords: Natural killer-T cells, chronic lymphocytic leukemia, IGHV mutation, CD38, ZAP70

2805P

Dendritic cell based immunotherapy using melanoma stem cells increases the survival rate of vaccinated mice

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Background: The existence of cancer stem cells (CSCs) in many cancers including melanoma and their resistance to conventional regimens of cancer therapy results in post treatment relapse and metastasis of cancer, so targeting the CSCs population is the goal of new researches. In this study we used the cancer stem cells antigens for dendritic cell pulsing and targeting the induced immunresponse to cancer stem cells. **Methods:** The melanoma tumors were induced subcutaneously in C57BL/6 mice using B16F10 cell line. DCs drived from mice bone marrow were pulsed with melanoma CSCs lysate and used for immunotherapy of cancer bearing mice. The mice immunized with tumor cell lysate pulsed DCs were used as control. **Results:** Vaccination of tumor bearing mice by CSCs lysate pulsed DCs increased the survival rates and caused the reduction of tumor volume compared to the control groups. **Conclusion:** Our results showed that the immune response could be directed to cancer producing cells (CSCs) and CSCs targeted DC based immunotherapy might also be a good choice for cancer prophylaxsis and treatment.

Keywords: Dendritic cell, Melanoma stem cells

2807P

Dendritic cell based immunotherapy using melanoma stem cells induced potent cellular immunity against melanoma

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Background: Many therapeutic modalities are being investigated for treatment of malignant tumors including melanoma with divers results. The main problem after common rotes of cancer therapy is the relapse of cancer wich is proposed to inatiate from a rare population of drug resistance cells named cancer stem cells (CSCs). This study was done to target the CSCs by the induced immune response through DCs based immunotherapy. **Methods:** Melanoma stem cells were prepared using anti-CSCs antibodies and cell sorting. DCs derived from mice bone

marrow and pulsed with CSCs or tumor cell lysates was used for mice immunization. In vivo cell cytotoxicity assay was carried out to measure the cell mediated immune responce against CSCs and tumor cells. IFN-γ and IL-4 were also masured in the supernatant of immunized mice lymphe node cells culture in the presence of antigen. **Results:** The mice immunized with CSCs lysate pulsed DCs showed higher cytotoxic activity against CSCs coated splenocytes compared to tumor cell lysate coated cells. The ratio of IFN-γ to IL-4 also showed a shift of cytokine profile to Th1 responses in mice immunized with CSCs pulsed DCs. **Conclusion:** Our resuls showed that cancer stem cells could be targeted using DC-based immunotherapy. Considering the important role of CSCs in resistance of cancers to convenetional methods of cancer therapies and the tumor relapsing, their targeting is the main goal of new strategies of cancer treatment.

Keywords: Dendritic cell, melanoma stem cells

2272P

Construction of a scFv phage display library against CD123, a stem cell antigen on acute myeloid leukemia cells

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Background: The CD123 antigen is the alpha subunit of the human interleukin-3 receptor (IL-3Ralpha) which is strongly expressed in various leukemic blasts and leukemic stem cells and seems to be an excellent target for the therapy of leukemias. The aim of this study was construction of an antibody phage display gene library to find some clones with high specificity for human CD123 antigen. **Methods:** A hybridoma cell line producing anti-CD123 monoclonal antibody was cultured in RPMI medium and assayed for antibody production by ELISA and Western blotting. Then total RNA was extracted from 10^7 cells and transcribed into cDNA. The cDNA pool was used as template for PCR amplification of VL and VH domains using specific primers. The amplified VL and VH fragments were assembled by SOE-PCR into the scFv format. Then, scFv genes were digested with *Sfi*I, ligated into pComb3X vector and electroporated into *Escherichia coli* TG1competent cells. Ten clones were picked randomly and sequenced. **Results:** Antibody expression by hybridoma cells was confirmed by ELISA and Western blotting. The VH, VL and scFv DNA fragments were about 340 bp, 320 bp and 750 bp, respectively. The DNA sequencing data confirmed the correct cloning of the scFv fragments into the phage display vector. **Conclusion:** Phage display technology is a widely used method for the selection of novel antibodies with high affinity and specificity for the target antigen. From our scFv gene library, we can obtain phages that bind to CD123 antigen. This procedure facilitates the successful cloning of functional anti-CD123 single-chain antibodies from hybridoma cells.

Keywords: CD123, Leukemia, scFv, Phage display, Hybridoma

2580P

Evaluation the correlation between VEGF and EGFR expression and tumor stage in ovarian cancer

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Background: Ovarian cancer is the fifth most common cancer among females. The symptoms are often vague. So by the time that cancer diagnosed the tumor has often spread to other organs. VEGF is one of the most important vascular growth factor of tumor. EGFR is one of the regulator factors in physiologic and also pathologic conditions. In the present study we intended to evaluate the correlation of these two factors with the tumor stage in ovarian cancer. **Methods:** 50 cases of ovarian cancer which had available histologic biopsy entered in this study. Immunohistochemical staining was performed using monoclonal mouse anti-human VEGF and EGFR antibody. The slides were reviewed by a pathologist. The data were analyzed using Fisher's Exact test. **Results:** Among 43 slides which were stained for VEGF only 7 slides were positive for this marker (16.3%) and 36 slides were negative (83.7%). In low stage tumors 20% of cases stained positive for VEGF and in high stages 14.3% became positive. Among 32 slides which were stained for EGFR, 13 slides became positive for expressing this marker. 33.3% were positive in lower stages and 45% of high stages were positive for expressing EGFR. **Conclusion:** No correlation was found in VEGF and EGFR expression and the stages of ovarian tumors. Low expression of VEGF in our study might be due to epithelial types of ovarian cancers. It is suggested that a comparison between expression of these markers in tumor tissues and normal tissues should be done to determine the role of these two markers in ovarian cancer.

Keywords: Ovarian cancer, VEGF, EGFR, Tumor stage

2802P

Investigation of CCR4 and CCL22 genetic variants, respectively at positions C1014T & C16A, with prostate cancer

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Background: Genetic factors have been already reported to be involved in development and progression of Prostate cancer. We aimed, in the present study, to investigate the association between genetic variants of CCR4 and CCL22, respectively at the positions C1014T and C16A, with prostate cancer. **Methods:** One hundred patients with prostate cancer (mean age 66.10±7.61) and 100 age and sex matched healthy subjects were recruited. Genotypes were investigated by PCR-RFLP methods. Data was assembled and analyzed in SPSS software package and P value less than 0.05 were considered significant. **Results:** The percentage of CC, CT and TT genotypes at position 1014C/T in CCR4 gene were 43.6%, 39.4% and 17% in patients and 45.3%, 42.1% and 12.6% in controls respectively. Distribution of the genotypes and alleles at this locus observed not to be significantly different between patients and controls (P = 0.694, P = 0.539, respectively). The percentage of CC, CA and AA genotypes at position 16 C/A in CCL22 gene were 83.3%, 15.6% and 1.1% in patients and 80.4%, 17.5% and 2.1%

in controls respectively. Distribution of the genotypes and allele at this locus observed not to be significantly different between patients and controls (P = 0.830, P = 0.516). Unlike the polymorphism in CCR4 gene, A allele at the position 16C/A in CCL22 gene was observed to be associated with the increase of Gleason score (P = 0.021). **Conclusion:** The association of CCL22 gene polymorphism with Gleason score suggests the effect of this genetic variation on the prognosis in patients with prostate cancer.

Keywords: Polymorphism, Prostate Cancer, Gleason Score, CCL22, CCR4

2331P

Effects of combination treatment with radiotherapy and chemotherapy drugs (MLN4924and2DG) on MCF-7 breast cancer cell line

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Background: Breast cancer is the most malignant disease worldwide that is characterized by uncontrolled growth and local tissue invasion with sometimes distant metastasis.Despite advances in diagnosis and treatment modalities, breast cancer remains a major public health problem and a major cause of death in women worldwide. Ionizing Radiation represents one of the most clinically effective therapies of breast cancer and frequently applied as a single treatment modality with curative intent or, more often, combined with surgery and/or chemotherapy to maximize the therapeutic effect. The aim of this study is to investigate the effect of radiation therapy combined with MLN4924 and 2- deoxy - D - glucose (2DG) in increased induction of cell death (apoptosis) in breast cancer cell line (MCF7). **Methods**: The MCF-7 cells were seeded in 96 well culture plates in treated with different concentrations of MLN4924 (30, 100, 200nM) and a single dose of 2DG(0.5mM) for 72 hours and 24 hours, respectively, and also irradiated with gamma radiation from Co-60 (1,1.5,2 Gy) following the treatments. viability and apoptosis of the cells for the treatments were determined by MTT assay, TUNEL assay and also by Cell Death Detection. **Result**: The obtained results showed that combination treatment with radiotherapy and chemotherapy (MLN4924 +2DG) have cytotoxic effect on MCF-7cell line and induced apoptosis. **Conclusion**: MLN4924 and ionizing radiation reduce the breast cancer cell viability compared to single modality treatments. This study suggests the possibility of reducing the common dose of each treatment modality and consequently the reduction of related side effects.

Keywords: Breast cancer,MCF-7, Radiation therapy, Apoptosis ,2DG, MLN4924

2367P

Investigating the cytotoxic and anticancer activities chloroform fraction of *Verbascum speciosom* on AGS cancer cell line

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Background: Gastric cancer is one of the most causes of death in the world. As the age of

modern medicine, single pure drugs emerged, and plant-derived active principles, their semi-synthetic and synthetic analogs have served as a major route to new pharmaceuticals. The aim of this study was to investigate the the cytotoxic and anticancer activities of chloroform fraction of *Verbascum speciosom* on AGS cancer cell line. **Methods:** Cultivated gastric cancer cell line (AGS) in standard conditions was incubated with different concentrations of chloroform fraction of *Verbascum speciosom* for 48 hours and then cytotoxic activity was determined by MTT assay and cell death induction was determined by using flow cytometry. **Results:** The results demonstrated that the chloroform fraction decreased AGS cell viability in a concentration-dependent manner. This fraction effectively increased apoptosis in AGS cell line. **Conclusion:** The cytotoxic and anticancer activities of chloroform fraction of *Verbascum speciosom* on AGS cancer cell line may indicate the beneficial effect of this plant in prevention or treatment of gastric cancers. Given the importance of new herbal compounds to treat cancer and reduce the side effects of chemical treatments, increase in our knowledge regarding the therapeutic mechanism of natural compounds is recommended.

Keywords: Adenocarcinoma gastric cancer, *Verbascum speciosom*, Cytotoxic activity, Apoptosis

2418P

Cloning and expression of human L- Asparaginase from PC-3 cell line and study of its antineoplastic activity

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Background: L-Asparaginase catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonia. L-Asparaginase has an antineoplastic activity selectively reduces the level of L-asparagine in blood and decreases the proliferation of cancerous cells. Consequently, it have been widely used as a therapeutic agent in the treatment of acute lymphoblastic leukemia. In the present study, we aim to produce of human l- Asparaginase enzyme and investigate of its antineoplastic effect on leukemia cell line. **Method**: Total RNA was extracted from PC3 cell line and converted to cDNA. L- Asparaginase gene was amplified by RT-PCR and cloned into the PGEM- T vector. The positive clones were digested and finally confirmed by sequencing. The product was expressed in E. Coli using pET-22b expression vector. Recombinant protein contained hexahistidine tag was purified using nickel nitrilotriacetic acid chromatography. C105 cell line were treated by purified enzyme. The cytotoxic effect of enzyme were measured by MTT assay. **Result**: The sequence of cloned L- Asparaginase gene consisted of 937 bp showed 100% identity with previously reported sequence of this gene. Expression of L- Asparaginase gene in E. coli resulted in high levels of protein with molecular weight of 32kDa in SDS-PAGE. The result of MTT assay showed that the C105 cell line is considerably sensitive to deduced protein. **Conclusion**: In this study the recombinant L- Asparaginase was expressed and the anti proliferative effect of this product on leukemia cell line was confirmed. Hence, it would be of interest to employ this recombinant protein as anti lucemic drug.

Keywords: L- Asparaginase, Antineoplastic effect, Recombinant protein, Cloning, Expression

2562P

Single nucleotide polymorphisms on Osteopontin gene are relevant with disease aggression in breast cancer

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Background: Osteopontin (OPN), a secreted integrin-binding glycophosphoprotein, is associated with progression and metastasis in a variety of cancers and has been studied as a prognostic marker. Several studies have represented that SNPs might act as genetic contributions in tumor stage, disease progression, and aggression in cancer mechanisms. The aim of this study is to evaluate the frequency of two variants, rs11730582, and rs2728127 on promoter region in OPN gene in breast cancer patients in comparison with the healthy subjects in Iranian population, eastern Azerbaijan province. **Methods:** In a case-control study, genomic DNA from 5 ml blood sample from 350 patients and 360 healthy people was extracted and the presence of genetic polymorphisms was examined by HRM-PCR. Then agarose Electrophoresis was done in order to see the specific bond. Then statistical analysis was performed using SPSS 18.0. **Results:** Our data shows a significant relationship the between the frequency of two variants and progression and metastasis in case group. **Conclusion:** Statistical analysis demonstrated undeniable relationship between OPN genetic variant and breast cancer progression in breast cancer patients in comparison with the healthy control which might provide new methods for cancer diagnosing.

Keywords: Osteopontin (OPN), Single nucleotide polymorphisms (SNPs)

2229P

Relationship between cytokine combination with CD26+ cord blood hematopoietic stem cells

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Background: Umbilical cord blood (UCB) has been used as transplantable source of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs). CD26+ cells are a big population in UCB cells which negatively regulate *in vivo* homing and engraftment of HSCs. In this study we tried to find out relationship between cytokines and CD26+ cells as well as the effects of cytokines on proliferation of CD26+ cells. **Method:** In mentioned work, we used combination of cytokines including: SCF, Flt3 ligand, IL2, IL7 and IL15 for cord blood CD26+ hematopoietic stem cells and in different time point we analyzed the effect of cytokines by flocytometry. **Result:** The data indicated that percentage of CD26+ cells has been increased by time point. Also evaluation of different combination of cytokines showed that IL7 is more effective than other cytokines to increase CD26+ cells. **Conclusion:** According to the recent findings, cytokines are essential to increase cord blood hematopoietic stem cells to transplantation. Our findings suggest that blocking of CD26+ cells may be a promising

strategy to improving homing and clinical outcomes.
Keywords: Umbilical cord blood, Cd26+ cells, Cytokine, Flow cytometry.

2232P

Assessing the cytotoxic and apoptotic effect of methanolic fractions of Scrophularia oxysepala on MCF-7 cancer cell lines

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Background: Cancer is a major public health problem in both developed and developing countries. It is the second largest common disease spread world-wide. Drug toxicity and resistance to chemotherapeutic agents make a struggle to treat cancer. For this reason, initial research focuses on traditional medicines or herbal formulations. As we know, breast cancer is the most commonly occurring cancer among women (about 25%). So, the cytotoxic effect of methanolic fractions of Scrophularia oxysepala was investigated in human breast cancer MCF-7. Scrophularia oxysepala plant was collected from Eastern Azarbaijan province, Iran. This is the first time that cytotoxic effect of Scrophularia oxysepala fractions and cell death mechanism of it, is studied. **Methods:** The MCF-7 and L929 cells were seeded in 96-well culture plates in the presence of different concentrations (30, 50, 100, 200, 300 µg/µl) at 12, 24, 36 hours and then results were followed to determine cytotoxic effects on viability and apoptosis by MTT, TUNEL and DNA fragmentation. **Result:** The obtained results declared that certain concentrations have cytotoxic effect on MCF-7 cell line and induce apoptosis while L929 cells, as normal cell line, remained intact. **Conclusion:** Increased concentration of the fractions and treating time reduced cell viability. Our data showed that methanolic fractions of Scrophularia oxysepala have an apoptotic effect on MCF-7 cells and it might be an effective agent in cancer treatment.

Keywords: Scrophularia oxysepala fractions, MCF-7, cytotoxicity, apoptosis, MTT assay

2347P

Expression of EMSY in breast carcinomas in Iranian patients

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Background: *EMSY* gene encodes a BRCA2- binding partner protein that represses the DNA repair function of BRCA2 in non-hereditary breast cancer. Although amplification of *EMSY* gene has been proposed to have prognostic value in breast cancer, no data is available concerning EMSY tissue expression pattern and its association with clinicopathological features. **Methods:** In current study, we examined the expression and localization pattern of EMSY protein by immunohistochemistry and assessed its prognostic value in a well-characterized series of 116 unselected breast carcinomas with a mean follow up of 47 months

using tissue microarray technique. **Results:** The immunohistochemical expression of EMSY protein was detected in 76% of primary breast tumors and localized to the nuclear (18%), cytoplasmic (35%) or both cytoplasmic and nuclear sites (23%). Univariate analysis revealed a significant positive association between EMSY expression level with lymph node metastasis (p =0.045), larger tumor size (p= 0.027), as well as a relative association with increased risk of recurrence (p=0.088), whereas no association with patients' survival (log rank test, p=0.482), tumor grade or type was observed. **Conclusion:** Herein, we demonstrated for the first time the immunostaining pattern of EMSY protein in breast tumors. Our data imply that EMSY protein may have impact on clinicipathological parameters and could be considered as a potential target for breast cancer treatment.

Keywords: Cancer, BRCA2, EMSY

2297P

Comparison of human CCRL1[1] expression level in normal and neoplastic brain tissue

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Background: Chemokines and chemokine receptors belong to a superfamily of small molecules that control the migration of leukocytes as well as many other cell types. A typical receptor can bind to its ligand with high affinity and specificity and elicit a cellular response. However, a group of special receptors that do not signal in response to binding of their ligands has been described in the chemokine system, including CCX-CKR (CCRL1) and so on. CCX-CKR receptor is a scavenger of CCL19, CCL21, CCL25, and CXCL13 chemokines. This chemokines and their typical receptors are involved in cancer growth and metastasis. However, CCX-CKR plays a negative role in the growth and metastasis. The aim of this study is to compare the expression level of CCRL1 gene in normal and neoplastic brain tissue. **Method**: In this study the normal and tumor brain tissue were collected and total cellular RNA was extracted. Then complementary DNA synthesis reactions were performed using 1 µg DNase treated total RNA from each sample and cDNA synthesis kit with random hexamer priming in a 20 µl reaction according to the manufacturer's instructions. Using specific primers for CCRL1, the expression level of mRNA were evaluated using Real time PCR. The expression level of CCRL1 protein was also evaluated using western blot. **Results and Conclusions**: There were difference in expression level of CCRL1 gene in both mRNA and protein level between normal and neoplastic brain tissue. The results of our study show that CCRL1 may have important role in brain cancer and it could be a novel target for brain cancer therapy.

Keywords: CCRL1, Brain tissue, Real time PCR, Western blot

2430P

Up-regulation of Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) by TNF-α and IFN-γ in mouse adipose tissue derived mesenchymal stem cells

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Background: It is well demonstrated that mesenchymal stem cells are recruited to tumor sites using different chemokine and receptors. One of the most promising candidates for molecular cancer therapy is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) that express by mesenchymal stem cells and leads to activate apoptosis pathways. The goal of present study is to up-regulation of TRAIL in mouse adipocyte derived mesenchymal stem cells (AD-MSC). **Methods:** Balb/c AT-MSCs were cultured in DMEM, 15% heat-inactivated adult fetal bovine serum (FBS, Gibco), 100 U/ml penicillin and 100 mg/ml streptomycin. Flowcytometry analysis were conducted using monoclonal antibodies against mouse CD73, CD105, CD44, CD29, CD90, VEGFR, CD31, CD11b, CD45, CD34 and TRAIL (All from eBiosciences). Cells were seeded in 6-well plates and were treated by TNFα, IFNγ and both of mentioned cytokines in doses 10 ng/ml, 20 ng/ml, 5+10 ng/ml and 10+20 ng/ml respectively. Expression level of TRAIL in different groups was conducted using real-time PCR. **Results:** Our results showed that pre-activation of AD-MSCs with TNFα, IFNγ and both of TNFα and IFNγ significantly increased expression level of TRAIL. These data were approved by flowcytometry analysis and surface expression of TRAIL remarkably increases in TNFα, IFNγ and combination of TNFα and IFNγ. **Conclusion:** Our data showed that culturing AT-MSCs with TNF-α and IFN-γ in combined and separate manner enhances their tumor-suppressive properties and may represent a useful strategy to develop MSC-based approaches for the treatment of cancer.

Keywords: Mesenchymal stem cells, TNF-α, IFN-γ, TRAIL, Cancer

2581P

Evaluation the Expression of Ki67 and Bax in endometrial carcinoma and their association with tumor stage.

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Background: Endometrial cancer describes as several types of cancers that start from the endometrium of the uterus. The cancer is one of the major malignancies in the world. Bax is one of the apoptotic members of the Bcl-2 group of genes that regulate program of cell death. Ki67 gene makes a nuclear protein that is related with cellular proliferation **Methods:** A total of 50 patients with endometrial cancer were entered in this study. Immunohistochemical staining was performed on the sections provided from paraffin embedded tissues. A rabbit anti-human Bax and a monoclonal mouse anti-human Ki67 were used for immunohistochemical staining and the pathologist reviewed the staining slides.Fisher Exact test was used to analyze the data. **Results:** 70 percent of low stage patients expressed Bax. But in high stages 57 percent of cases expressed the marker. No correlation between expression of Bax and stages of the cancer was found ($P > 0.05$).Also no correlation between stages with index of Ki67 was detected ($P > 0.05$). **Conclusion:** The results of study demonstrated no correlation between expression of the markers and the stages of the tumor. Less expression of the marker could be greater if more samples were available. As Bax promotes apoptosis, the downregulation of this marker occurs in high stages when proliferation and metastasis is upregulated. Although the reduction of Bax and upregulation of Ki67 in cancerous endometrium were not statistically significant but these

data might have important role for treatment in clinic.
Keywords: Endomerial carcinoma, Ki67, Bax, Tumor stage

2732P
No impact of NOD2 gene polymorphisms on incidence of acute and chronic GVHD in acute myelogenous leukemia patients

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Background: This study was conducted to evaluate the association of NOD2 gene polymorphisms with the occurrence of GVHD in acute myelogenous leukemia patients who underwent HSCT from their HLA-matched sibling donors. Methods: We examined retrospectively NOD2 genotypes by PCR-SSP both in 124 patients who **Underwent** HSCT and in their donors; then, the association of the genetic polymorphisms on acute and chronic GVHD was evaluated. Median follow up of patients was 60 months (range of 48-97 months). Statistical analyses were performed using Chi-square test and SPSS software. **Results:** Mutation incidence were the same between donors and recipients as 12.1%. In three of the patient–donor pairs (2.4%) SNPs occurred in both resulting in an overall frequency of 21.8% in patient–donor pairs. There weren't any significant differences between aGVHD and cGVHD incidence rates when donor/recipient pairs with SNPs were compared with the pairs without SNPs. aGVHD and cGVHD incidence rates in the former pairs were 52% and 56% and in the latter pairs 50.5% and 55%, respectively. Conclusions: No impact of NOD2 SNPs on incidence of acute and chronic GVHD was observed. Further studies are required to ascertain whether the findings of this study can be extended to other disease groups. In addition, further studies are required to identify the relevance of other SNPs.
Keywords: Hematopoietic Stem Cell Transplantation, Graft vs Host Disease, PCR

2676P
Imbalance of Th17 and FoxP3$^+$ regulatory T cells in benign and malignant salivary gland tumors

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Background: Salivary gland tumors are relatively uncommon lesions of head and neck cancers. The relationship between these tumors with immune system has not been well inspected. Treg cells accumulate in tumors and suppress anti tumor responses but specific roles of Th17 cells remain unclear. We investigated the distribution of Th17 and Treg cells in peripheral blood of benign and malignant salivary gland tumors. **Methods:** EDTA blood samples were obtained from 27 patients with salivary gland tumors (19 benign and 8 malignant; mean age of

49.25±18.31) and 19 age-sex matched healthy donors (mean age of 47.21±17.46). The mean percentage of $CD4^+$ $IL\text{-}17^+$ (Th17) cells and $CD4^+$ $CD25^+$ $FOXP3^+$ (Treg) cells were detected by flow cytometry after staining for surface markers and intracellular cytokines. **Results:** Patients with malignant tumors observed to have significantly reduced percentage of Th17 cells (0.84±0.38) and an increased level of Treg cells (7.74±3.11) in comparison to patients with benign tumors (2.09±1.09 for Th17 cells, 4.38±2.44 for Treg cells), as well as control subjects (2.31±0.91 for Th17 cells, 2.34±1.25 for Treg cells) ($pv \leq 0.001$ in all cases). In addition, the ratio of Th17/Treg cells was significantly lower in both malignant (0.13±0.86) and benign (0.49±0.31) tumors compared with controls (1.3±0.92) ($pv < 0.001$). There was also a positive correlation between Th17 cell percentage and tumor size in malignant tumors. **Conclusion:** Our results imply that the imbalance of Th17/Treg ratio may contribute to progression of salivary gland tumors. From Th17 and Treg cells point of view, these data also suggest that benign salivary gland tumors might fall between healthy and malignant condition.

Keywords: Th17, Salivary gland tumors

2154P

The inhibitory effect of ethylacetate fraction of *Tribulus terrestris* (TT) on gastric cancer cells via apoptosis induction

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Background: Gastric cancers are amongst the most common malignancies of upper intestine and are the second leading causes of cancer death in the world. Chemoprevention of cancer especially by natural compounds is a promising strategy against cancer initiation. In this regard natural compounds with antioxidative and anti-inflammatory effects are interesting candidates to evaluate their abilities in influencing the initiation and growth of tumors. In this study we investigated the anticancer effect of ethylacetate fraction of *Tribulus terrestris* on gastric cancer cells. **Methods**: Cultivated gastric cancer cell line (AGS) in standard conditions was incubated with different concentrations of ethylacetate fraction for 24 and 48 hours. The proliferation of AGS cells was determined by using MTT assay, Apoptosis was evaluated by the annexin V/propidium iodide assay by flow cytometry. **Results:** The results demonstrated a strong and dose dependent inhibition of cancer cell growth by ethylacetate fraction of *TT*. The data indicate that this fraction effectively induced the apoptotic death of AGS cells. **Conclusion**: The inhibition of cancer cell growth by ethylacetate fraction of *TT* may indicate the beneficial effect of this plant in prevention or treatment of gastric cancers. Due to importance of the new herbal compounds to treat cancer and reduce the side effects of chemical treatments, more investigations are recommended to increase our knowledge regarding the therapeutic mechanism of natural compounds in the treatment of cancer.

Keywords: Adenocarcinoma gastric cancer, *Tribulus terrestris*, Proliferation, Apoptosis

2222P

Comparison of miR-24 and miR-107 expression in cord blood of identical twins

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Background: MicroRNAs have critical functions across various biological processes. They are short single stranded noncoding RNAs that suppress gene expression through either translational repression or degradation of target mRNAs. miR-107 targets cyclin-dependent kinase 6 expression, induces cell cycle G1 arrest and miR-24 inhibit cell proliferation by targeting E2F2, MYC and other cell cycle genes. The question arise is that does the newborn identical twins have the same expression of miRs? **Methods:** The expression of this cell cycle regulating miRs was measured in cord blood samples of three pairs of identical twins using Real time RT-PCR technique. **Results:** Statistically significant differences in expression levels of miR-24 and miR-107 between each pairs was observed. (miR-24: ratio= 2.04 fold (P-value =0.0259), 2.68 (P-value =0.0052) and 2.021 fold (P-value =0.0149) respectively in pair 1, 2 and 3) (miR-107: ratio= 5.55 fold (P-value =0.0014) , 12.10 (P-value =0.0010) and 7.15 (P-value =0.0011) respectively in pair 1, 2 and 3). **Discussion:** Intrauterine environment and stochastic factors can be responsible for different miR expression in twins with the same genomic DNA at the birth time.

Keywords: miR-24, miR-107

2441P

Construction of recombinant transgenic retrovirus expressing human DPPA2 gene

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Background: Cancer/testis antigens (CTAs) are a subgroup of tumor-associated antigens which are expressed normally in germ line cells and trophoblast, and aberrantly in a variety of malignancies with different histological types. One of the most important CTAs is DPPA2 with unknown biological function yet. Considering the importance of DPPA2 in developmental events and cancer, preparing a suitable platform to analyze DPPA2 roles in the cells seems necessary. **Methods**: The coding sequence of DPPA2 gene was amplified, cloned and sequenced. The coding fragment then sub cloned into the pRUF expression vector of retroviral gene expression system. To produce recombinant retrovirus, pRUF-DPPA2 vector was cotransfected with pVSV-G to GP293 cells, the packaging cell line. The virus particles were enriched from the medium and used to transduct target cells. The stable transducted cells were selected and confirmed for ectopic expression of DPPA2 gene by real-time PCR. **Results**: The appropriate sequence of DPPA2 coding region was successfully cloned and sub cloned in desired vectors. After transduction of target cells, the enriched virus particles were obtained at a final concentration of 10^5 TU/ml. **Conclusion**: According to the critical characteristics of retroviral expression system such as stable and long time expression of interested gene and also being safe due to the deletion of retroviral pathogenic genes, we used this system to induce expression of DPPA2 gene and prepared a valuable platform to analyze the biological function

of the gene. Also the recombinant DPPA2 protein can be used in production of recombinant vaccines and serological tests.

Keywords: Cancer, DPPA2 protein, Retroviral vector, Vaccine

2804P

Identification and characterization of cancer stem like cells in mouse malignant melanoma

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Background: Recent evidence suggests that tumors arise from a small subpopulation of cells, the cancer stem cells (CSCs) or tumor initiating cells.These so called cancer stem cells have been proposed to originate either from malignant transformation of a normal somatic tissue stem cell or a progenitor cell. However there are some investigatins concerning the characterization of cancer stem cells of melanoma but the reported results are controversial, so we decided to characterize cancer stem like cells in mouse melanoma tumors for our further investigations. **Methods:** The murine model of melanoma was induced in C57BL/6 mice using the B16F10 cell line, the tumor bulk was dissociated and sorted using anti-CD44 and anti-CD24 antibodies. The obtained subpopulations ($CD24^+$, $CD44^+$), ($CD24^+$, $CD44^-$), ($CD24^-$, $CD44^+$) and ($CD24^-$, $CD44^-$) were compared according to their ability to sphere formation and tumor induction. **Results:** The sphere formation assay showed that all subpopulations were almost clonogenic but the double positive cells produced more and larger spheres and were more tumorigenic, supporting that they might be the cancer stem cell populations in mouse melanoma tumor. **Conclusion:** Considering the higher potential of tumorogenicity of double positive cells we suggest that double positive cells may be considered as CSCs, whereas other subpopulations show somehow change to transient amplifying cells.

Keywords: Malignant melanoma, Cancer stem like cells

2809P

PD1 genetic variations is associated with susceptibility to bladder cancer

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Background: The association between PD-1 gene variation and auto immune diseases has been frequently reported. The data regarding cancer is rare. In this study, we aimed to investigate the possible association between two single nucleotide polymorphisms in PD-1 gene, +7146 G to A (PD-1.3) and +7785 C to T (PD-1.5), with susceptibility to bladder cancer. **Subjects and Methods:** 150 patients with confirmed bladder cancer (Age mean 64.8±1.8) and 150 age and sex matched healthy individuals (Age mean 61.9±13.4) without history of autoimmune diseases or malignancies in the first-degree relatives were enrolled. DNA was extracted using salting out method. Genotypes were determined using PCR-RFLP methods. Data were analyzed by SPSS and Aarlequin software packages. **Results:** The percentage

of CC, CT and TT genotypes at position +7785 C/T in PD-1.5 gene were 48%, 37.3% and 14.7% in patients and 33.3%, 52.7% and 14 % in controls respectively. The frequency of CC genotype was observed to be significantly higher in patients than control subjects. No difference was, however observed in allele frequencies between patients and controls. The percentage of GG, GA and AA genotypes at position +7146G/A in PD-1.3 gene were 78%, 20.7% and 1.3% in patients and 83.3%, 14.7% and 2% in controls respectively. Distribution of the genotypes and alleles at this locus observed not to be significantly different between patients and controls (P>0.05). Haplotype analysis did not show any significant differences between two groups. **Conclusion:** This study, for the first time, revealed the association of PD-1.5 gene polymorphism with increased risk of bladder cancer in Iranian population.

Key word: Bladder cancer, Polymorphism PD-1

2567P

Protease activity of *Allium* genus extract on different substrates using zymography method.

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Background: A lot of data support medicinal advantages of Liliaceae especially *Allium spp.* Among different constituents of these plants, some cysteine proteases played a role in initiation and execution phases of apoptosis and activity of these proteases recognized as a key element in the apoptotic process. The purpose of this study was to investigate the protease activity of *Allium spp.* **Methods:** The underground organs of garlic, onion, chive, and Iranian shallot crushed in a squeezer and centrifuged to obtain their extracts. Protease activities of the extracts were identified using zymography method containing four substrates including collagen, casein, gelatin and fibrin. **Results:** Collagen zymography showed the existence of different clear bands for chive (at 48, 78, 90, 140 and 150 kDa), onion (at 95 and 105 kDa), one band at 70 kDa for garlic and one band at 90 kDa for Iranian shallot. Based on these results, Iranian shallot, chive, garlic and onion showed protease activity on collagen and gelatin but they could not digest fibril and casein. In addition, chive extract showed higher protease activity than the other extracts. **Conclusion**: The results of this study showed significant differences in zymogram patterns of the studied plant. These differences can guide more quantitative studies in order to find new application of the proteases in medicine and food industry.

Keywords: Protease, *Allium*, Proteome, Zymography.

2221P

Evaluation of the p16 and CDK6 genes expression in cord blood of identical twins

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Background: The $p16^{INK4a}$ tumor suppressor protein functions as an inhibitor of CDK4 and CDK6, the D-type cyclin-dependent kinases that initiate the phosphorylation of the

retinoblastoma tumor suppressor protein, RB. Thus, $p16^{INK4a}$ has the capacity to arrest cells in the G1-phase of the cell cycle. Here we compared expression of p16 and CDK6 in newborn identical twins with the same genome. **Methods:** The expression of p16 and CDK6 genes in cord blood samples from three pairs of newborn identical twins were studied using Real time RT-PCR technique. **Results:** The expression level of p16 gene was different between each pair but not statistically significant. CDK6 gene expression have statistically significant difference (more than 7.3, 4.87 and 4.04 fold respectively in the first, second and third twins) (P-value<0.01). **Discussion:** Different expression levels of p16 and CDK6 genes, confirmed the important effects of epigenetic even on newborn identical twins.

Keywords: p16, CDK6

2452P

Comparison of Toll-like Receptor 9 Expression in 1321N1 and U87 Cell Lines

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Background: Toll-like receptors (TLRs) detect pathogen associated molecular patterns and promote immune response against them. Each member of TLR family recognizes a specific ligand, e.g. TLR-4 and TLR-9 detect LPS and unmethylated CpG motif in DNA, respectively. Unfortunately the expression of TLRs by tumors enables them for metastasis. In this study, we investigated the expression of TLR9 in human tumor cell lines; 1321N1, an astrocytoma grade II, and U87MG, a glioblastoma grade IV. **Methods**: RT-PCR and Immunocytochemistry were used to determine the expression of TLR9 in these cells. **Results:** RT-PCR results showed lower expression capacity of the receptor in 1321N1 cell line compared with U87. Further, Immunocytochemistry did not detect the receptor expression at the protein level in 1321N1 cell line. **Conclusions:** Our data indicated that TLR9 expression is lower in low-grade 1321N1 cell line.

Keywords: Toll-like receptor 9, 1321N1, U87MG

2786P

Synthesis and potent MDR reversibility of 10 imidazolylacridinediones in breast cancer cell lines

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Background: Breast cancer is the most common cancer in women worldwide. Nowadays multidrug-resistant breast cancer cell strains have become a worldwide problem. There is clearly a need for a pipeline of novel anti-cancer drugs to combat the MDR strains. **Methods**: Four novel 10-imidazolyl acridine 1,9diones derivatives (cyanide, nitro, thioethyl and thiomethyl derivatives) have been synthesized by the reaction of 5,5-dimethyl-1,3-cyclohexanedione with aromatic aldehydes (2a-d) in the presence of ammonia in methanol. In vitro cytotoxicity of 4 imidazolylacridinedione derivatives in combination with doxorubicin on T47D (human ductal breast epithelial tumor cell line and TAMR-6 (tomoxifen-resistant T47D) breast cancer cell

lines were investigated using MTT test. Drug resistant index (DRI), which is equal to the ratio of IC50 in drug-resistant cells over IC50 in drug-sensitive cells, was calculated for each substance. Flowcytometry experiments were also implemented to distinguish cells undergoing apoptosis from those undergoing necrosis (subG1 method). **Results**: Synthesized cyanide derivative (1 nM) along with doxorubicin significantly increases the doxorubicin cytotoxicity in T47D and TAMR-6 breast cancer cell lines. Synthesized thioethyl and thiomethyl compounds in concentrations of 1 nM with doxorubicin can increase the cytotoxicity of doxorubicin on T47D and TAMR-6 breast cancer cell lines. **Conclusion**: The nitro derivative with doxorubicin did not exhibit good synergistic effect on cytotoxic activity of doxorubicin, and can slightly increase doxorubicin cytotoxicity in both cell lines. The most effective synthesized imidazolylacridinedione derivative was cyanide which exhibited rational synergistic effect in combination with doxorubicin in both normal and tamoxifen resistant breast cancer cell lines.
Keywords: MDR, 10-imidazolyl acridine 1,9diones derivatives, Cytotoxicity, subG1

2787P

Socioeconomic Status and Other Characteristics in Childhood Leukemia

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Background: Leukemia is the most prevalent childhood cancer and Acute Lymphoblastic Leukemia (ALL) constitutes 75% of all cases. Some epidemiological studies have found a relationship between socio-economic status (SES) and some childhood cancers. In the present study, an attempt was made to assess socio-economical class in a case-control study. **Methods**: A case- control study conducted on 100 case of acute lymphoblastic leukemia aged 1-14 years in Department of Pediatric Oncology of Dr sheikh Hospital in Mashhad – Iran and matched on age and sex to 400 healthy controls. Data was collected by interview using a questionnaire. Data analyzed by chi-square test and Regression analysis. 95% confidence intervals were used to measure the relation between childhood Acute Lymphoblastic Leukemia (ALL) and parental education, income status, father's job (Socioeconomic status), number of children, birth score and paternal smoking. **Results:** There was a significant difference in parental education level, income status, number of children, birth score, father's job and paternal smoking between two groups. Regression analysis showed the risk of childhood ALL associated with paternal smoking, and father's high risk job. Most of cases and control groups located in upper lower and lower middle class of socioeconomic status, respectively. There is a meaningful different between Socioeconomic status in two groups. But the risk of childhood ALL did not associate with socioeconomic status. **Conclusion:** The results suggest that paternal smoking and father's high risk job are related to risk of childhood leukemia. It should be considered for planning support.
Keywords: Child, Leukemia, Social Class

3350P

Regulatory T Cells and Myeloid-Derived Suppressor Cells in Patients with Peptic Ulcer and Gastric cancer

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Background: To clarify the effect of Myeloid-Derived Suppressor Cells (MDSCs) and regulatory T Cells (Tregs) in pathophysiology of dyspeptic disorders and gastric cancer, the number of these two cells in patients with non-ulcer dyspepsia (NUD), peptic ulcer disease (PUD), and gastric cancer (GC) were compared. **Methods:** Patients with dyspepsia who were positive for *Helicobacter pylori* infection were selected and divided into three groups of NUD, PUD, and GC according to their endoscopic and histopathological examinations. *H. pylori* infection was diagnosed by rapid urease test and histopathology. The number of peripheral blood $CD4^+CD25^+FoxP3^+$ Tregs and $CD14^+HLA\text{-}DR^-$ MDSCs were measured in all patients, by flow cytometry. The number of $FoxP3^+$ regulatory T cells was also determined by immunohistochemistry (IHC). **Results:** Twenty two patients with NUD, 25 with PUD, and 27 with GC were enrolled in this study. The percentage of peripheral blood Treg cells in both PUD (0.81±0.39, $p<0.001$) and GC groups (0.98±0.65, $p<0.001$) were significantly higher than in NUD group (0.46±0.10). These results were also confirmed by IHC. A significant increase in the percentage of MDSCs in patients with PUD (0.73±0.19, $p<0.001$) and GC (0.73±0.16, $p<0.001$) compared to NUD group (0.46±0.16) was also observed. There was no difference in percentage of these two cells between the PUD and GC groups. The increase of Tregs and MDSCs in two groups of PUD and GC was not significantly correlated. **Conclusions:** Increased number of MDSCs and Tregs may contribute in the pathogenesis of PUD and GC.

Keywords: Myeloid-derived suppressor cell, Regulatory T cells, Gastric cancer, Peptic ulcer

2284P

Cytotoxic Effect of Dichloromethane Fractions of Scrophularia_*oxysepala* on MCF-7 Human Breast Cancer Cells

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Background: Breast cancer is the most common malignancy in women, especially in developing countries. It has been observed that some of the plants contain a rich source of anti-cancer drugs ingredients. This paper tends to evaluate the effect of cytotoxic activity of Scrophularia oxysepala fractions, which is located at induction of apoptosis in MCF-7 breast cancer cell line on the breast cancer. Hence, a herbal based treatment for breast cancer is mostly stipulated in this work. **Methods:** As a matter of fact, Dichloromethane fractions were examined based on MTT (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Trypan-blue assays were performed in MCF-7 breast cancer cell line to analyze the cytotoxic activity of the extract of Scrophularia oxysepala. In addition, the apoptosis inducing action of the extract was determined by TUNEL (terminal deoxy transferase (TdT)-mediated dUTP nick- end labeling) test and DNA fragmentation. **Results:** The results revealed that the dichloromethane fractions effectively inhibited cell growth and viability in doses (30, 50, 100 ,200, 300 µg/µl) and times at (12, 24 and 36 hours) dependent manner, regardless the inducing

damage to non-cancerous cell lines. Furthermore, cell death assay and DNA fragmentation analysis using TUNEL indicated induction of apoptosis by dichloromethane fractions of Scrophularia oxysepala in MCF-7 cell. **Conclusions:** The obtained results illustrate for the first time that fractions of Scrophularia oxysepala may cause of the apoptosis in breast cancer. The findings indicate that fractions of Scrophularia oxysepala contains potential anti-cancer components are against breast cancer cell proliferation through DNA damage.

Keywords: Scrophularia oxysepala, Cytotoxic, MCF-7 cells, Apoptosis, Fractions

Cellular Immunodeficiency

Oral Presentations:

3385O

Present status of chronic granulomatous disease in Kerman

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Chronic granulomatous disease (CGD) is characterized by defect in killing of catalase positive microorganisms due to a genetic defect in the generation of NADPH oxidase.The aim of this paper is to introduce 17 patients with this disease. Eleven males and 6 female with medium age of 11.3 years were studied. The most common clinical manifestations were lymphadenitis (64.7%), non-mycobacterial pneumonia (52.9%), recurrent skin abscess (41.1%), mycobacterial pneumonia (29.4%), liver abscess (11.7%), disseminated BCGiosis (11.7%), and fungal infection (5.8%).Two patients died to severe corpulmonale. One patient suffered from corpulmonale needs Oxygen frequently for daily activities. Other patients have nearly normal activity under chemoprophylaxis e.g. co-trimoxazole, ketoconazole, folic acid, gamma interferon. CGD can potentially induce severe life threatening infections, however early diagnosis and proper management can retained a normal and healthy life for the patients.

3151O

Hereditary angioedema a neglected disease in Iran (A report from Iranian Hereditary angioedema registry)

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Background: Hereditary angioedema (HAE) is a rare autosomal dominant disorder caused by C1INH (C1 esterase inhibitor) deficiency and leads to subcutaneous and submucosal edema attacks. The aim of this study was to investigate the clinical and laboratory findings of patients

with HAE that were registered them in Iranian Hereditary Angioedema Registry (IHAER). **Methods:** The qualitative and quantitative levels of C1inhibitor were evaluated in all patients suspected to HAE who had been referred to Immunology, Asthma and Allergy Research Institute (IAARI) between Jan 2006 and Sep 2013. The patients with confirmed hereditary angioedema diagnosis were enrolled in this study. **Results:** Of 47 patients with HAE from 29 different families (23 female and 24 male) 62.2 % had type I HAE and 37.8 % had type II. Fourteen patients were under 18 years and thirty three were adult. The average age of symptoms onset and diagnosis were 12.07±10.28 years (1 to 53 years) and 24.90±14.76 years (3.5 to 72 years), respectively. Hand, face and genitalia were the most locations of edema. Moreover, laryngeal edema was observed in 60% of patients that led to death in two patients during this study. Most of patients (48.8%) received danazol and/or tranexamic acid for long prophylaxis and 30.2% of them had no treatments. **Conclusion:** Hereditary angioedema is a neglected disease in Iran with considerable morbidity and risk of mortality. Therefore, it is necessary to promote the awareness of physicians, patients and their families about hereditary angioedema and its appropriate control.
Key word: Hereditary angioedema, C1esterase inhibitor, Laryngeal edema

3124O
TREC/KREC assay for screening the SCID newborns

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Introduction: Recent advances in bone marrow transplantation have led to long-term success rates of up to 95%, when transplants are done before 3.5 months of age in SCID infants. Thus, early diagnosis of patients can be applicable using a standard screening test. PCR-based detection of T-cell receptor excision circles (TRECs) and κ-deleting excision circles (KRECs), has proven to be a valuable tool for identifying the patients. In this project, we report a pilot study conducted on evaluation of TREC/KREC copy numbers in newborns and SCID patients. **Methods:** We investigated 100 blood samples of newborns taken during the first 72 hours after birth on Guthrie cards and 14 frozen DNA samples of SCID patients referred to IAARI. DNA was extracted from the paper's punches. After running on Real time PCR, copy numbers were deduced based on the standard curve obtained from plasmid standard dilutions. **Results:** Zero copy numbers of TREC and KREC were not seen among newborn samples. But for the samples suspected to SCID, we interestingly found zero copy numbers of TREC and KREC in 10 out of 14 patients revealing SCID T-B- phenotype. The zero number of TREC and higher but not normal number of KRECs were also found in the remaining samples which may be related to SCID T-B+ phenotype of them. These results were in accordance with the genetic analysis of the related genes in some patients. **Conclusion:** This molecular method can easily be used for early screening the newborns for PID specially SCID and further evaluating.
Keyword:TREC/KREC, screening, SCID, newborn

3170O

Prenatal diagnosis of primary immunodeficiency disorders; a report from Iranian Primary Immunodeficiency Registry (IPIDR)

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Background: Primary immunodeficiency disorders (PIDs) are characterized by recurrent or unusual infections. Regarding the identification of molecular defects of many PID disorders, prenatal diagnosis can be done as a preventive tool in pregnant women with a family history of PID. The aim of this study was to investigate the outcomes of prenatal diagnosis in couples registered to Iranian Primary Immunodeficiency Disease Registry (IPIDR).**Methods:** Forty-seven couples with a family history of PID and a tendency for prenatal diagnosis entered this study. CVS was performed in the first trimester of pregnancy to analyze molecular defects of the fetus based on gene defect of the affected person in the family. Also postnatal confirmation was carried out using lab tests and molecular analysis. **Results**: Of 47 families, we had cases with family history of SCID (21), CGD (8), Cystic fibrosis (6), LADs (5), Wiskott–Aldrich syndrome (3), congenital Neutropenia (2) and Hemophagocytic lymphohistiocytosis (2). As a consequence of prenatal diagnosis, 37 cases were healthy, four were suspected to PIDs and six were determined as carriers. In addition, we could confirm the prenatal diagnoses with the postnatal evaluations. **Conclusion:** Prenatal diagnosis is highly recommended to families with a positive family history of PID. This can be a promising tool to reduce government, family and personal burden of these diseases by early treatment of infants or making a decision for fetus abortion. Moreover DNA banking which is applicable in all primary immunodeficiency disorder centers can help to find out the genetic defect of neonatal in early gestational age.

Keywords: Primary immunodeficiency disorders, prenatal diagnosis, fetus, early treatment, abortion

Poster Presentations:

2776P

NBT reduction and Killing activity in patients with thalassemia major

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Background: There are different reports about abnormalities in CMI and defective Neutrophil functions in patients with thalassemia. The aim of this study was to evaluate NBT reduction and C. albicans killing activity by neutrophils of patients with thalassemia major. **Methods:** PMNs was separated from whole blood of 30 patients with thalassemia major in 6% dextran saline. Sixteen patients were splenectomized. Thirty healthy controls were included in the study. Candida Albicans killing and nitro blue tetrazolium (NBT) slide test were used to evaluate neutrophil activity in these patients. **Results:** There was no different in neutrophil count and NBT test but C. albicans killing result was significantly different between two groups.

Groups	NBT %	p Value	C. albicans killing%	p Value
Patients	261.9	0.07	24.6	0.001
Controls	285.9		261.6	

Conclusion: With normal NBT reduction and impaired C. albicans killing results, our data showed a probable myeloperoxidase deficiency in these patients.

Keywords: Thalassemia, NBT, Killing activity

3399P

The results of lymphocyte transformation test in patients suspected to cellular immunodeficiency in Mofid children's hospital, Tehran, Iran

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Background: LTT (lymphocyte transformation test) is a useful method for evaluation of cellular immunity. If the patientsand the stimulatory agents are selected correctly and the test is done in desirable condition, the test can give precious results to physicians.This study was done to evaluate the results of lymphocyte transformation test in patients suspected to have primary cellular immunodeficiency referring to Mofid children hospital. **Methods:** The lymphocytes were isolated from heparinized peripheral blood of the patients, usingficollhypaque gradient centrifugation. The cells were stimulated with PHA (Phytohaemagglutinin), as amitogen and BCG (Bacillus CalmetteGuerin) and candida albicans, as antigens. The cells were incubated in humified CO2 incubator at 37 c for 2-5 days. The proliferation was measured using Brdu proliferation assay kit (Roche, Germany).The stimulation index was calculated by dividing

OD of stimulated to unstimulated wells. **Results:** Sixteen patients were evaluated in this study. They were suspected to have cellular immunodeficiency according to the patients' history and primary evaluation. Among them, 2 (12.5%) had impaired proliferation to PHA, 6 (37.5%) to BCG, and 10 (62.5%) to candida. **Conclusions:** The most common proliferation impairment was after stimulation with candida. The results should be interpreted in the context of clinical presentations.

3162P

Pediatric hereditary angioedema: a report from Iranian Hereditary Angioedema Registry (IHAER)

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Background: Hereditary angioedema (HAE) is an uncommon, autosomal dominant disorder resulting from C1esterase inhibitor deficiency. It is also characterized by recurrent subcutaneous and submucosal edema especially in respiratory and gastrointestinal tracts. The aim of this study was to investigate the clinical and laboratory findings in Iranian children with HAE. **Methods**: In this study, we evaluated children suspected to HAE who were referred to Immunology, Asthma and Allergy Research Institute (IAARI) from 2006 to2013. Thereafter, we registered all pediatric patients with definite diagnosis of HAE base on clinical manifestations of HAE and deficiency in C1esterase inhibitor quantity(type I) and/or quality(type II), in Iranian hereditary angioedema registry (IHAER). **Results**: in this study, we found 16 pediatric patients with definite diagnosis of HAE that 56.3% of them, had type I and 43.8% had type II of HAE. The mean age of diagnosis was 9.74±4.71 years with a minimum age at onset of 15 months. The mean of delay diagnosis was 2.59±3.07 years. In addition, positive family history of HAE was observed in 62% of patients. The most affected locations were hands, feet and face. Laryngeal edema was also reported in 31.3% of patients. **Conclusion:** Based on the early age of onset the symptoms, it is highly recommended to precisely evaluate the children with positive family history of HAE to start the appropriate treatments if needed. Training the physicians and raising the awareness of the patients' family would be of interest to prevent life threatening attacks especially laryngeal edema.

Key word: Hereditary angioedema, C1 esterase inhibitor, laryngeal edema.

2150P

Assessment of cell-mediated immunity by measuring ATP levels in mitogen-stimulated CD4+ T lymphocytes

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Background: Current *in vitro* methods for assessing cell-mediated immunity (CMI) include measuring cell activation signals, lymphoproliferation, cytotoxicity, and cytokine production. Stimulation of lymphocytes causes an influx of ions and increased ATP synthesis that followed by RNA synthesis, cytokine production and release, and DNA replication.The presented assay measures the early response to phytohemagglutinin (PHA) stimulation by detecting intracellular ATP synthesisin CD4+ T cells isolated from whole blood by magnetic beads. **Methods:** A total of 33 healthy individuals (Men=23 and Women=10) with mean age of 38±10 years (range: 22-68) were included in this study. Whole blood sample collected in tube containing sodium heparin was diluted 1:4 with RPMI-1640 medium and cultured (100µL, duplicate) in the presence or absence of PHA (25 µL, 5.0 µg/mL). After incubation for 15-18 hours at 37◦C/5% Co2, CD4+ cells were separated by antibody-coated magnetic beads and lysed with a hypotonic solution containing detergent. Then, ATP content in unstimulated and stimulated conditions was measured by firefly bioluminescence assay using a log-log standard curve. The quality of cell separation and CD4+ cell-specific ATP release were validated by flow cytometric and depletion experiments. **Results:** The mean ofATP levels in stimulated condition was 565±131 ng/mL compared to 115±55ng/mL in unstimulated CD4+ cells (*P*=0.0001). The maximum and the minimum levels of ATP release from stimulated CD4+ T cells were 810 and 374 ng/mL, respectively. **Conclusion:** This rapid and low-cost assay reflects the degree of immune cell function through assessment of CD4+ T cell activation. Thus, it can be used for evaluation of CMI in immunodeficient individuals as well as in immunosuppressed transplant recipients who needs drug adjustment.

Keywords:ATP, Cell-mediated immunity, Immunodeficiency, Immunosuppression, Transplantation

1907P

Skin fibroblast radio sensitivity assay in severe combined immunodeficiency patients

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Background: Severe Combined Immunodeficiency Disease (SCID) is the most serious and rare but important primary immunodeficiency disorder. The defining characteristic of SCID is the absence of T cells and lack of functional B cells. This disease can be inherited in two forms of X-dependent and autosomal recessive. X-linked SCID blocks T cells by mutations of the genes encoding the γc cytokine receptor subunit of interleukin-2, -4, -7, -9, and -15 receptors. Genes involved in autosomal recessive form of SCID are RAG_1, RAG_2, JAK_3, ARTEMIS, CD_{45}, and IL7R. Autosomal recessive SCID mutations impairs a common V(D)J recombination during lymphocyte development and are also involved in the repair of DNA

double-strand breaks (DSB) by the non-homologous end joining (NHEJ) pathway.**Methods:** In this study, fibroblast cells of children suspected to SCID were cultured and were used in comet assay technique followed by comet score analysis by software freeware v1.5.**Results:** In this study, fibroblast cells of children suspected to autosomal recessive SCID showed tranquil rate of DNA damage repair relative to X-link form as evaluated by alkalin comet assay(The Single Cell Gel Electrophoresis assay) technique and finally comet score software.**Discussion:**This technique is fast, inexpensive and accurate for sub typing children suspected to SCID disease into autosomal recessive and X-linked without the common clinical testing.

Keywords:SCID, comet assay, γc cytokine receptor, V(D)J recombination, (NHEJ)

2467P

Dysregulation of T cells in hindlimb unloading rats

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Background: Hindlimbunloading (HU) is a ground-based model which has been used to study the effects of space flight onphysiological systems such as immune system. Studies on HU have shownalterations of the immune system similar asthose observed in spaceflights. At the present research, we studied dysregulation of T cellsin HU rats.**Methods**: Seven Wistar male rats (weight 285 ± 15 gr) were randomlyselected and unloaded. Blood was taken on days 0, 12, 24 and 48 andTcells of CD4+ and CD8+ were measured by flow cytometery.Data for control group obtained from sample on day0 and reviewing the literature. Statisticalanalysis of the recorded data was done with SPSS (version 21).**Results**:Analysis showed decrease of CD4+ on days 12, 24, and 48. CD8+increased on days 12 and 24 but decreased on day 48. CD4+/ CD8+ ratiodecrease in all 3 samples.**Conclusions**: Microgravity and stress experienced during spaceflights result in immune system aberration. It has been reportedthat HU model causes abnormal cell proliferation and cytokineproduction. In our study, cellular immunity was dysregulated in HUmodel (CD4+, CD8+, and their ratio).Reviewing the literature, Wesuggest some countermeasures (such as micronutrients, vitamins,antioxidants, and/or nutritional nucleotides) which may be applied toovercome the alterations of immune system in short and long spaceflights.

Keywords:Hindlimb unloading,Tcell,CD4+, CD8+

2762P

Psoriasis in hyper IgE syndrome – a case report

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Background: Hyper IgE syndrome (HIES) is a rare primary immune deficiency, described as Job`s syndrome characterized by increased serum levels of IgE, eczema, recurrent cutaneous and pulmonary infections. In this paper, we presented a case of Hyper IgE syndrome. **Method:** A 16-year-old Iranian boy presented with a one year history of skin lesions in knees

and elbows was diagnosed of psoriasis disease. He had a history of recurrent infections including otitis media, pneumonia, diarrea and skin infection. **Results:** Laboratory results showed increased level of total IgE and normal in other immunoglobulin. Histologic finding showed hyperkeratosis, parakeratosis of acanthotic epidermis with regular elongation of rete ridges diagnose psoriasis disorder. **Conclusion:** In conclusion, this is the first case of hyper IgE patient with psoriasis disorder. We addressed the important laboratory findings and actual theories explaining possible association between hyper IgEimmunoglobulinemia and psoriasis disorder.
Keywords: Hyper IgE syndrome, Psoriasis, Immune deficiency

3018P

Malignant external otitis as a first clinical presentation of leukocyte adhesion deficiency 1 disease

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Background: Leukocyte adhesion deficiency (LAD) is a rare inherited primary immunodeficiency disorder characterized by defect of phagocytic function resulting from a lack of leukocyte cell surface expression of β_2 integrin molecules (CD11 and CD18) that are essential for leukocyte adhesion to endothelial cells and chemotaxis. **Method and Results:** The 8month-old female infant referred with chief complaint of pussy discharge from left ear canal and admitted in otolaryngology ward. She received antibiotics for external otitis caused by pseudomonas aeuroginosa and alsotympanostomy and mastoidectomy were done. Because of severity of infection, history of delayed cord separation and neutophilia, we suspected to leukocyte adhesion deficiency and very low number of CD11b/CD18 adhesion molecules on the patient's granulocytes confirmed this diagnosis. **Conclusion:** Malignant external otitis usually occurs in middle aged or elderly diabetic patients and it is uncommon in children and rare in infants.This presentation in every infant emphasizes the need of immunology work up for rule out immunodeficiency syndromes such as leukocyte adhesion deficiency.
Keyword: LAD, immunodeficiency syndrome.

3321P

Mismanagement of severe congenital neutropenia with G6PC3 mutation in an adult case with enterocolitis

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Background: Severe congenital neutropenia (SCN) is a rare primary immunodeficiency disease. Different genes are found to be associated with SCN, including ELA2, HAX1, WAS, GFI1, G6PC3 and G-CSFR. G6PC3 deficiency is a syndromic variant of SCN associating congenital neutropenia with various developmental defects including cardiac or urogenital malformations. Inflammatory bowel disease like colitis is uncommon complication of G6pC3 deficiency. **Method**: Here we report a 20 years old man with enterocolitis . He was referred to Immunology, Asthma & Allergy Research Center with congenital neutropenia. His main

symptoms were recurrent aphtousulsers, pneumonia, and gingivitis in early childhood and history of cardiac abnormality (MR and TR). His chronic diarrhea and enterocolitis lead to right hemicolectomy. Also in further assesment, he had AGC between 200-500. For many years he was treated as an IBD patient without any health improvement. Based on above evidences, G6PC3 deficiency was considered as a related SCN gene. Genomic DNA was extracted from his whole blood sample. Gene mutation was analyzed by PCR and followed by direct sequencing. **Result**: We found a reported mutation in G6PC3 exon 4 (R161X) in his DNA sample. **Conclusion**: It is important that patients with chronic enterocolitis should be considered for having SCN and G6PC3 gene mutation. Subspecialty collaboration and team working in diagnosis and treatment of complex disease such as immunodeficiency disorder is worthy.

Keywords: Severe congenital neutropenia, G6PC3

3152P

Laryngeal edema and death in four patients with hereditary angioedema

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Background: Hereditary angioedema (HAE) is an autosomal dominant disease caused by C1inhibitor deficiency. It is characterized by recurrent subcutaneous and submucosal edema and can be fatal if attacks involve upper airways. There are several trigger factors for hereditary angioedema attacks such as: emotional stress, physical injury, menstruation, infections and some drugs like oral contraceptives and angiotensin-converting enzyme inhibitors but the trigger factors of HAE attack may remain unidentified in many of cases.Laryngeal edema and deaths due to hereditary angioedema attack in four families are presented in this report. **Method:** Four families with history of HAE were evaluated by determination of C1 estrase inhibitor. Twenty two cases with definite diagnosis of HAE and four deaths due to HAE attack are founded in these families.**Result:** All of 4 cases had trigger factors for laryngeal edema attacks. These factors consist of: oral injury due to eating of toasted bread in case 1(53 year old man), severe cough due to hair bleaching in case 2(27 year old woman), tonsillectomy in case 3 (36 year old man) and emotional stress in case 4 (29 year man). None of the patients received regular and adequate treatment. They died during laryngeal attack (all of patients in emergency room) before appropriate medical treatment and/or procedures.**Conclusion:**Due to the risk of asphyxiation in HAE laryngeal attacks, urgent monitoring of airway patency and appropriate treatment are indicated. Access to new drugs for control of HAE attacks can be effective in mortality reduction of HAE patients.

Keyword: Laryngeal edema, hereditary, angioedema

Dendritic cells in Health & Disease

Oral Presentations:

2184O

Targeting of multi-epitope HIV-1 vaccine candidate to CD8+ dendritic cells enhances immune responses

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Background: Delivering of antigens directly to DCs enhances the immune responses to the antigen and is an attractive approach for eliciting cellular immune responses against pathogens like HIV virus. The aim of this study, therefore, was to evaluate immune responses elicited by delivered multi-epitopic HIV-1 tat/pol/gag/env recombinant protein to DCs in situ using αDEC-205 mAb (NLDC-145). **Methods:** HIVtop4 sequence ($Gag_{158-186}$, $Pol_{150-190}$, $ENV_{296-323}$, $ENV_{577-610}$, Tat_{1-20} and Tat_{44-61}) was designed based on computer analysis, cloned into pET23a plasmid. Expression was induced in BL21 E. coli cells by addition of IPTG and purified by IMAC and confirmed against HIS-Tag in western-blotting. To exploit DCs properties for immunization purposes, we coupled this recombinant protein chemically to αDEC-205 and immunized Balb/c mice subcutaneously (s.c.) with conjugated multi-epitopic peptide or purified peptide (as control) simultaneously with Poly I: C for DC maturation. Lymphocyte proliferation was measured with Brdu, IL-4, IL-17 cytokine with ELISA and IFN-g cytokine with ELISPOT. Total antibody and IgG1, IgG2a isotypes with indirect ELISA methods. **Results**: Immunization by anti DEC-205 conjugated peptide led to a significant increase in the proliferative responses of lymphocytes, IFN-γ cytokine and total antibody titer in comparison with the control groups (None targeted). **Conclusion**: We concluded targeting of peptide antigens to DEC-205+DCs significantly enhances immune responses in compare with non-targeting strategies.

Keywords: Dendritic cell targeting, DEC-205, Multi epitopic, Vaccine

2470O

Immunotherapy with tumor cell lysate-pulsed CD8α$^+$ dendritic cells modulates intra-tumor and spleen lymphocyte subpopulations

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Background: Using cellular adjuvants including dendritic cells (DCs) has provided a promising approach in immunotherapy of cancer. Our previous study showed that mice immunization with tumor cell lysate-pulsed DCs (TL-CD8α+DCs) could significantly suppress the tumor growth and increase mice survival. The aim of the present study was to investigate the impact of TL-CD8α+DC vaccine on intra-tumor and spleen lymphocyte subpopulations in tumor-bearing mice. **Methods:** A BALB/c mouse model of fibrosarcoma was used and changes in various lymphocyte subpopulations including $CD4^+$, $CD8^+$ and $CD4^+CD25^+Foxp3^+$ T cells in mice immunized with TL-$CD8\alpha^+$ DCs were studied. The cytotoxic activity of the lymphocytes and tumor growth inhibitory rate were also measured. **Results:** Immunotherapy with TL-$CD8\alpha^+$ DCs significantly enhanced both CD4+ and CD8+ lymphocytes, whereas decreased $CD4^+CD25^+$ $Foxp3^+$ regulatory T cells as well as the tumor growth rate. There was also a decrease in the ratio of regulatory T cells to $CD4^+$ and to $CD8^+$ lymphocytes in both the tumor and spleen tissues as compared to that in the non-immunized control mice. Immunization with TL-$CD8\alpha^+$ DCs as well as $CD8\alpha^+$ DCs significantly increased the splenocytes cytotoxic activity by 45.1% and 18.2% of control, respectively. **Conclusion:** The current study indicated that TL-$CD8\alpha^+$ DCs can enhance tumor immunity against the fibrosarcoma by enhancing both the $CD4^+$ and $CD8^+$ lymphocytes and reducing regulatory T cells. This finding suggests the usefulness of TL-$CD8\alpha^+$DCs vaccine for cancer treatment.

Keywords: $CD8\alpha^+$ dendritic cells, Intra-tumor and spleen lymphocytes, Immunotherapy, Tumor cell lysate

1695O

Peripheral blood dendritic cell subsets in methamphetamine abusers

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Background: Human circulating blood dendritic cells (DCs) are the most potent APCs and divided into two major subsets: myeloid DCs ($CD11c^+$DC); and plasmacytoid DCs ($CD123^+$DC). The percentage of circulating DCs might be a good indicator of immune status or polarization. We investigated whether in vivo chronic methamphetamine exposure alters the peripheral blood DCs subsets percentage. **Methods:** The present study was done on 20 male methamphetamine abusers and 40 controls of the same sex and age (20–40 years). The control group was healthy individuals with no lifetime history of drug abuse or dependence. All of the methamphetamine abusers were selected from those who had a history of methamphetamine use, for at least one year, with a daily methamphetamine dosage not less than 500 mg. Addicts known to abuse alcohol or other drugs were excluded. Peripheral blood DC subsets were analyzed with flow cytometry according to expressions of CD11c, and CD123. DCs were defined as $lin^-HLA\text{-}DR^+$ cells. **Results:** The methamphetamine abusers showed decreased total DC compared to controls (0.84±0.29 and 1.32±0.46, respectively) (P=0.024). The proportion

of CD11c+ peripheral blood DCs was significantly decreased in methamphetamine abusers [(0.272±0.039) %] in comparison with that in healthy controls [(0.775±0.076) %](P=0.001). There was no significant difference of CD123+ DC proportions between methamphetamine abusers [(0.167±0.030) %] and the controls [(0.397±0.066) %](P>0.05). **Conclusions:** In the present study, methamphetamine abusers showed decreased total DC and mDC counts compared to healthy controls. We conclude that decreased count of mDC may reflect the Th2-skewed immunity in methamphetamine abusers.

Keywords: Dendritic cells, Subset, Methamphetamine, Addiction

2136O

A new cell source for Dendritic cells: generation of Dendritic cells from programmable cells of Monocytic origin in the presence of TNF-α, GM-CSF and IL-4

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Background: TNF-α is a cytokine that has a wide variety of functions, it might be required in the early stage of dendritic cells (DCs) development from $CD34^+$ cells. However, there are several methods for DCs generation. Due to the limited accessibility of human $CD34^+$ cells, DCs development from this source is not common. We evaluated the differentiation potential of human programmable cells of monocytic origin (PCMO) into DCs with TNF-α. **Methods:** To obtain PCMO, the peripheral blood monocytes were dedifferentiated by IL-3 and M-CSF in 6 days. These cells were incubated with TNF-α, GM-CSF and IL-4 for 4 days, followed by Breast tumor antigen pulsing and the addition of MCM and TNF-α with poly (I-C) to generate mature DCs. The DCs generated from peripheral blood monocyte (IL-4+GM-CSF) were used as a control group (conventional DC). The PCMO were characterized by Light microscopy, immunocytochimestry, and flow cytometry. DCs phenotypic and functional analysis was carried out using anti CD14, HLA-DR, CD83, CD86, CD80 monoclonal antibodies, mixed lymphocyte reaction, phagocytic activity and cytokine release by DC stimulated T lymphocytes. **Results:** The monocytes in response to IL-3 and M-CSF regained proliferative activity and became confluent. Phenotype characterization revealed up-regulated of CD45, CD34, CD177, and CD15 in 6^{th} day. To generate DCs using TNF-α was shown, produced mature DCs with same survival rate and different phenotype and function properties in comparison with conventional DCs. **Conclusion:** We developed the new effective method to generate DCs from manipulated monocytes $CD34^+$ cells (PCMO) in this study.

Keywords: Conventional DC, TNF-α DC, PCMO.

2282O

Synergistic effect of Flt3ligand and M-CSF for plamacytoid dendritic cells development by in vitro niche

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Background: Plasmacytoid dendritic cells (pDCs), also known as IFN producing cells, are

playing central roles in antiviral immunity. Murine pDC are characterized by CD11c, B220, Gr-1, BST2, and siglec-H. The aim of this study was to assess the effect of Flt3 ligand (FL) alone, with L929 Feeder or supernatant on the differentiation of mouse bone marrow cells into pDCs in vitro. **Methods:** We describe a FL BM culture with L929 feeder or L929 supernantant that induce more pDCs. Murine BM cells cultured at 1×10^6/ml in FL (100ng/ml) or with L929feeder until 9 days, developed into pDC that double stained with PDCA-1-FITC and B220-APC and determined by flow cytometry. Then purify pDC by Isolation Kit II (MACS) by negative selection. **Results:** Surprisingly we have found that pDCs, better develop within l929 feeder with Flt3ligand (100ng/ml) up to 19% in bone marrow cultures. Indeed M-CSF express by L929 synergistic with Fl is able to derive pDCs from BM cells in vitro. Furthermore, L929 supernatatnt 30% with Flt3ligand was able to derive pDCs up to 8% in comparing with FL alone that was 6.5% in vitro. We purified up to 60 % PDC by MACS Negative selection. **Conclusion:** Thus, we present for the first time that the L929 feeder as a producer of M-CSF, promotes development of pDCs in vitro with FL. Suggesting that L929 feeder is probably not only as a Growth factor (M-CSF) but also as a Cell - Cell signaling, like in vivo niche that will be crucial for the Development of pDC.

Keywords: Plasmacytoid Dendritic Cell, Flt3ligand, L929 Feeder, M-CSF

3022O

Response of T cell induced by dendritic cells pulsed with myelin basic protein (MBP) and matured in the presence of histamine, interferon-β and 2, 3-dimethoxy-1, 4 naphtoquinone (DMNQ)

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Background: The key role of denderitic cells in skewing of immune responses causes that DC-based Vaccines have been used as a alternative treatment in diseases; including autoimmune Ones. Multiple sclerosis (MS) is a autoimmune diseases which is triggered by attacking of auto-reactive TH1/TH17 to Myelin Basic Protein(MBP).This study was carried out to evaluate the effects of Histamine, IFN-β and DMNQ on Polarization of autologous T cells in Co-culture with MBP-pulsed DCs. **Methods**: Plastic adherent monocytes were cultured with granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) for five days , three days with MBP as a antigen and two days with monocyte-conditioned medium (MCM) + Histamine , IFN-β and DMNQ in treatment group vs. only MCM in control one.. Phenotypic and functional analyses were carried out using anti-CD14, anti HLA-DR, anti-CD83 monoclonal antibodies. Phagocytic activity, cytokine production were also evaluated. T cell proliferation was tested by MTT test. **Results**: The expression of HLA-DR as a maturation marker of DC increased significantly, Except for histamine and DMNQ treatments. CD83 expression escalated in all groups except for DMNQ. The amount of IL-10 Produced in DC culture (at day 7) increased in all groups other than DMNQ .on the contrary, IL-12 decreased significantly in all treatments except for DMNQ. The proportion of IL-10 to IL-12 increased in compare with control. IL-4 produced in DC- T co culture increased significantly except for DMNQ and histamine+ DMNQ .But IFN-γ decreased in all groups other than DMNQ .The proportion of IL-4 to IFN-γ rised up in all treatments except for DMNQ.MTT assay revealed that T cell proliferation increased in all groups that contained IFN-β. **Conclusion**: it can be suggested that in this study DMNQ had no effect on T cell polarization and IFN-β and Histamine synergistically polarized T cell responses ex vivo toward Th2. These findings might provide a promising approach on making of DC- based vaccine against MS.

Keywords: Dendritic cells, Interferon-β, DMNQ, Myelin basic protein (MBP),

2966O

The frequency and phenotype of murine decidual dendritic cells during normal pregnancy and abortion

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Background: Dendritic cells (DCs) as the most important cells of the innate immune system and the intermediary of innate and acquired immunity possess a vital role in pregnancy maintenance. This study was performed to investigate the changes in the decidual dendritic cells (DCs) frequency and phenotype in abortion prone and non-abortion prone mice. **Methods:** We analyzed the decidual DCs in abortion and non-abortion-prone mice on gestation Days (GDs) 4.5, 7.5 and 13.5 using the immunohistochemistry method. Cryosections from the uterus and deciduas of pregnant mice were immunostained with CD11c-specific antibody. Two-color immunohistochemistry was carried out using anti-CD11c and one of the antibodies against CD11b or CD8α for estimation of the myeloid DCs (MDCs) and lymphoid DCs (LDCs) respectively. CD11c and one of the CD40, CD86 or I-A/I-E-specific antibodies were also used for the evaluation of DCs maturation. **Results:** Increased frequency of decidua basalis DCs and decreased frequency of decidua parietalis DCs were found in abortion prone mice, compared with non-abortion prone mice on GDs 7.5 and 13.5. The frequency of CD11c+ CD40+ cells on GDs 4.5 and 7.5 and CD11c+ CD86+ cells on GDs 7.5 were higher in abortion prone mice in comparison to non-abortion prone mice. The frequency of CD11c+ MHC-II+ cells was similar between abortion prone and non-abortion prone mice. **Conclusion:** Based on the key role of DCs in regulating the immune responses, we concluded that the changes in the decidual DCs frequency and phenotype may play an important role in fetal rejection by maternal immune responses leading to immune mediated abortion.

Keywords: Abortion, Decidua, Dendritic cells

2724O

Dendritic cell-based breast cancer vaccination by simultaneous targeting of alpha lactalbumin and autophagy pathway

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Background: The limited period of alpha lactalbumin (ALACTA) expression during lactation in the normal breast tissue and its overexpression in breast cancer make ALACTA a suitable target antigen in breast cancer. On the other hand, autophagy plays an important role in the pathways of antigen presentation. In this study, we evaluated the efficacy of an autophagy-related protein (LC3) in enhancement of ALACTA presentation by dendritic cells (DC) in a murine model of breast cancer. **Methods:** DCs were generated from mouse bone marrow

and transfected with ALACTA or ALACTA-LC3 fusion gene or pulsed with recombinant ALACTA protein. DCs were then injected subcutaneously into Balb/c mice twice with a 14 day interval. Five days following the second vaccination, mice were challenged with mouse breast cancer cell line, 4T1, transfected with ALACTA-EGFP fusion gene. Anti-tumor immune responses were analyzed using LDH assay, flow cytometery and ELISA. **Results:** In contrary to control mice, no palpable tumors were observed in the vaccinated mice of all groups 30 days following tumor inoculation. Mice vaccinated with ALACTA-LC3 gene-transfected DC significantly differed with those immunized with ALACTA gene-transfected DCs as judged by anti-tumor cytotoxic T lymphocyte (CTL) immune responses and IFNγ/IL-10 ratio. In this regard, there was no significant difference between the group vaccinated with recombinant ALACTA-loaded DCs and the group immunized with ALACTA-LC3 gene-transfected DCs. **Conclusion:** This is the first study exploring the role of autophagy in improvement the efficacy of dendritic-based anti-tumor vaccination. Moreover, our results provide further evidence suggesting ALACTA as a proper antigen to be targeted in prophylactic breast cancer vaccination.

Keywords: Autophagy, Alpha lactalbumin, Dendritic cell, Vaccine, Breast Cancer

2842O

Tolerogenic dendritic cells produced by lentiviral-mediated CD40- and IL-23p19- specific shRNA can ameliorate experimental autoimmune encephalomyelitis by suppressing Th17 cells

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Background Down-regulation of soluble or membrane-bound co-stimulatory molecules by RNAi in dendritic cells can prevent the activation of immune responses. **Methods**: Therefore, this study was designed to evaluate the therapeutic efficacy of bone marrow-derived DCs (BMDCs) transduced with lentiviral vectors to permanently expressed shRNA specific for CD40 (CD40LV-DCs) and/or p19 subunit of IL-23 (p19LV-DCs) mRNAs in experimental autoimmune encephalomyelitis (EAE). **Results:** In vitro studies showed that double-transduced BMDCs (CD40+p19LV-DCs) resemble tolerogenic DCs due to profound down-regulation of CD40, lower expression of pro-inflammatory cytokines (IL-6 and IL-12), increased IL-10 production, and stronger stimulation of MOG_{35-55}-specific T cells for production of IL-10 compared with CD40LV-DCs, p19LV-DCs and BMDCs transduced with control lentiviral vector (CoLV-DCs). Moreover, injection of transduced BMDCs in EAE mice revealed more reduction in clinical score when mice were treated with CD40-+p19LV-DCs, which correlates with significant reduction in IL-17 or increased production of IL-10 by mononuclear cells derived from the lymph nodes or spinal cord of CD40-+p19LV-DCs-treated EAE mice compared with CoLV-DCs-treated EAE mice. **Conclusion:** In conclusion, simultaneous knock down of CD40 and IL-23 production by BMDCs may represent a promising therapeutic tool for the treatment of IL-17-dependent autoimmune diseases, including multiple sclerosis.

Keywords: Dendritic cell, RNAi, Lentiviral vector, CD40, IL-23p19.

2796O

Early in vivo detection of 99mTc-HMPAO labelled CD4+ T cells after dendritic cells vaccination

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Background: T cells are one of the key cells of the adaptive immune system and their protective function in the immune system roles have made them a potential tool for immunotherapies for treating various diseases such as infection and cancer. However, key questions related to T cells behaviour *in vivo* once transferred remain unanswered: for example what is the biodistribution of adoptively transferred T cells at early time points? And do they go to the regions of interest? And how long does it take to reach their "destination"; can we measure the *in vivo* function of adoptively transferred cells non-invasively? **Methods:** To use non-invasive imaging single photon emission computed tomography and CT (SPECT/CT) to track antigen-specific $CD4^+$ T cells following adoptively transfer. Ovalbumin (OVA) specific $CD4^+$ T-cells were radiolabelled with ^{99m}Tc-Hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO). These radiolabelled cells were then imaged using small animal SPECT/CT in mice vaccinated with OVA pulsed DCs. **Results:** $CD4^+$ T cells were tracked early after adoptive transfer, using NanoSPECT/CT preferentially to the right draining inguinal lymph node in the leg of mice which had previously received OVA pulsed dendritic cells. These $CD4^+$ T cells proliferated significantly in the draining lymph node. **Conclusions:** We have demonstrated that murine $CD4^+$ T cells can be radiolabelled with ^{99m}Tc-HMPAO and imaged using NanoSPECT/CT. Consequently, this is a promising technique for pre-clinical studies tracking adoptively transferred T cells.

Keywords: T cell imaging, DC vaccination, non-invasive imaging, SPECT/CT, adoptive transfer therapy, ^{99m}Tc-HMPAO, pre-clinical

2899O

Tumor microenvironment can direct dendritic cells to promote tumor growth

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Background: Cancerous and non-cancerous cells in tumor microenvironment can suppress antitumor immune responses. Dendritic cells, the most potent antigen presenting cells, have the capability to trigger immune responses against cancerous cells. However, dendritic cells can also be tolerogenic in some microenvironment based on their maturation states. In this study the effect of tumor microenvironment on dendritc cells in tumor growth was examined. **Methods:** Dendritic cells were generated ex vivo from bone marrow precursor cells in the presence of GM-CSF (granulocyte-macrophage colony stimulating factor) and IL-4 (interleukin 4). Immuophenotype and antitumor activity of dendritic cells were tested in vitro. Dendritic cells were injected subcutaneously before tumor inoculation or directly injected into tumor. **Results:** Dendritic cells expressed high levels of MHC-II and costimulatory molecules CD40, CD80 and CD86 on their cell surface and had the capacity to secrete large amount of IL-12 and to induce proliferation of T cells after exposure to tumor cell lysate and maturation factors in

vitro. Injection of dendritic cells before tumor challenge led to antitumor effect while injection of dendritc cells directly into the tumor tissue did not. Indeed, the presence of dendritic cells in the tumor microenvironment led to prominent increase in tumor growth. **Conclusion:** These findings indicated that tumor microenvironment can promote tumor growth by induction of tolerogenic dendritic cells.

Keywords: tumor microenvironment, dendritic cells, tumor growth

Poster Presentations:

1903P

The study of tollerogenic effect of human amniotic epithelial cells co-culture with monocyte derived dendritic cells

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Background: Various studies indicated that Epithelial Cells derived from the amniotic membranes of human term placenta have inhibitory effect on components of the innate or acquired immune system. On the other part, during pregnancy one of the most inhibitory mechanisms of fetal survival maybe though to closely contact between AECs and maternal Myeloid Derived DCs that leads to generate immunological tolerance avoid from tissue graft rejection by maternal immune defense. **Methods:** In the presence study we look out for generating tollerogenic DCs from human monocytes derived iDCs by co-culturing with human term amniotic isolated epithelial cells to demonstrate inhibitory effect of AECs on phenotyping changes on maturation of cultured DCs. This change included: expression of immature (CD14, CD1a) or mature (CD80_ CD8 6_CD83) CD markers& MHC I, II by flow cytometer technique. The cultivation system improved by addition of LPS, IL4&GM-CSFgrowth factors and insert chamber contact manner during 7 days culture. **Results:** Not significant differences observed between two groups according to maturity of DCs but with obvious changes on MFI manifestation onCD14, MHC and some co stimulatory markers. **Conclusion**: By the side of immune phenotyping study, giving attention and consider of another investigation such as cytokine research and functional assessment are essential to evaluate immune regulatory mechanism of hAECs .This project are going to complete above mentions.

Keywords: Human Amniotic Epithelial Cells, Immunosuppression, Dendritic cell, Immunophenothyping

1885P

The phenotype of monocyte derived dendritic cells in reject and non reject liver transplant patients

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Background: The role of DCs (dendritic cells) has been widely described not only in viral and autoimmune diseases but also in liver transplantation. In the transplantation, it is well documented that DCs are key initiators of the immune response leading to acute rejection. In this study, we investigated and compared the maturation and phenotype of dendritic cells in reject and non reject liver transplant recipients. **Methods:** In this study, 15 reject and non reject liver transplant recipients were selected. After isolation of CD14+ monocytes by using a MACS(magnetic activated cell sorting)system, monocytes were cultured in culture plates in fresh complete medium supplemented with recombinant human GM-CSF , IL-4 and TNF-α. Monocyte derived dendritic cells were produced and analyzed for expression of cell surface markers by flow cytometry. **Results:** Results showed the expression levels of CD83 and CD1a in liver transplant patients with the symptoms of acute rejection were markedly decreased when compared with levels in non reject liver transplant recipients (median value of 18% and 17% versus 56% and 39% , respectively , p value < 0.05). But expression levels of CD86 and HLA-DR did not differ(Approximately 90%, p value >0.05).**Conclusion:** Our data suggest that immunosuppressive drugs in liver transplant patients with the symptoms of acute rejection can prevent the maturation step of DC and reduce the expression levels of DC markers.

Keywords: Dendritic cells, Phenotype, Reject and non reject liver transplant patients

1973P

The Immunomodulatory Effect of Bone Marrow Mesenchymal Stem Cells on Expression of TLR2 and TLR4 in Mice Dendritic Cells

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Background: Mesenchymal stem cells (MSCs) are multi potent cells with immunomodulatory effect on immune cells including T cells, B cells, and dendritic cells. Dendritic cells (DCs) are most potent antigen presenting cells. Maturation, Migration and differentiation of which can be affected by mesenchymal stem cells according to immunoregulatory properties of mesenchymal stem cells. Dendritic cells express a kind of surface receptors called Toll-like receptors which fall under the PRR family of receptors and play a key role in maturation process of dendritic cells. **Methods:** We evaluated expression of two surface receptors (TLR2 and TLR4) in dendritic cells after exposure to mesenchymal stem cell's supernatant in culture media containing LPS and devoid of LPS. Mesenchymal stem cells and dendritic cells were extracted from adult BALB/c mouse bone marrow and spleen respectively. MSCs supernatant were collected 24 and 48 h after passage 6. Isolated DCs were co-cultured with

MSCs supernatant, incubation time were 24 and 48 hours. TLR2 and TLR4 gene expression was evaluated using real time PCR technique**. Results:** Expressions of these two receptors were up regulated in culture media lacking LPS in comparison with the control group but the increase is not significant statistically. After LPS stimulation, expression of TLR4 was lower than the control group while TLR2 showed an increase. Both are non-significant statistically. **Conclusion**: Over all data was shown that both TLR2 and TLR4 gene expression in exposing to MSCs were modulated but it was not significant.

Keywords: Mesenchymal stem cells (MSCs), Dendritic cells (DCs), Toll like receptors (TLRs), Immunomodulation

2408P

Mesenchymal stem cells derived Wharton's Jelly of the Umbilical cord exhibit modulator effects on cytokine gene expression and secretion in dendritic cells

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Background: The Wharton's jelly (WJ) is a gelatin substance inside the umbilical cord .MSCs obtained from the Wharton's Jelly of umbilical cords (UC) have gained much attention over the last years since they can be easily isolated, without any ethical concerns, from a tissue which is discarded after birth. **Methods:** The umbilical cord mesenchymal stem cells were collected enzymatically and cultured in a suitable cell media. The initial induction of monocyte differentiation to immature dendritic cells in exposure to GM-CSF and IL-4 for 6 days and followed by TNF-stimulation for 48 hours to finally generate mature dendritic cells. Then mature dendritic cells were co-cultured with UC-MSCs. Finally, the cells contained in the flasks were collected to perform RNA extractions in Real Time PCR. The culture supernatants also were tested through ELISA to measure secreted cytokines in dendritic cell. **Results:** Ourdata showed that UC-MSCs decreased IL-12, TNF-α cytokine gene expression and secretion in mature dendritic cells at varying dilution ratios (1:1; 1:2 and 1:10). Also the UC-MSCs increased IL-10 secretion and cytokine gene expression in mature dendritic cells at all ratios. UC-MSCs compared to BM-MSCs have showed greater impact on increased the secretion of IL-10 in mature dendritic cells. **Conclusion:** The our data show that UC-MSCs can like BM-MSCs have a significant moderator effects on secretion and gene expression of IL-12, TNF-α and IL-10 cytokines in dendritic cells. Therefore, adjustment or decrease of gene expression and secretion of inflammatory cytokines from DC cells can inhibit inflammatory responses of the immune system.

Keywords: Dendritic cell, Mesenchymal stem cells, Wharton's jelly, Gene expression

2246P

In Vivo effects of 18α-Glycyrrhetinic acid on phenotypic and functional properties of dendritic cells

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Background: Dendritic cells (DCs) play a central role in the initiation of immune responses. The compounds which have the ability to induce immunomodulatory effects on DCs may be employed for the treatment of autoimmune diseases and allograft rejection. The aim of this study was to investigate the in-vivo effects of 18α-Glycyrrhetinic acid on DCs. **Methods**: 10 µg/gr 18α-Glycyrrhetinic acid was injected intra-peritoneally into BALB/c mice every other day within 4 weeks, and splenic DCs were isolated using nycodenz. The phenotypic and functional properties of DCs were studied by flow cytometry and mixed lymphocyte reaction (MLR), respectively. In addition, the percentage of lymph node isolated T-regs was compared in both groups and the level of IL-4 and IFN-γ in MLR supernatant and mice serum were measured by ELISA kits. **Results**: Flow cytometry analysis showed decrease in expression of CD40 ($P<0.0250$) and MHC II ($P<0.0048$). Although, expression of CD86 was not changed in test group compared to control group. The percentage of T-regs in control (2.350±0.8227) and test (1.276±0.4477) groups was not statically significant. Allogeneic T-cell stimulation in MLR was also significantly inhibited in comparison with the control groups ($p<0.05$). The level of IL-4 and IFN-γ were not different in MLR supernatant and serum of both groups. **Conclusion**: Our data indicate that in-vivo 18α-Glycyrrhetinic acid administration inhibits maturation and activation of DCs which this kind of cells is of particular interest in treatment of autoimmune disease and transplantation.
Key word: 18α-Glycyrrhetinic acid, Dendritic cells (DCs), In-vivo study, MLR assay, T-regs

1683P

Phenotypic and functional maturation of murine dendritic cells induced by 18 alpha- and beta-glycyrrhetinic acid

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Background: Various studies have described Glycyrrhizin (GL), an active triterpenoic saponin extract of licorice roots, as an anti-inflammatory, antiviral, antimicrobial, anti-tumor and immunomodulating agent. The activity of GL has been mainly attributed to its metabolites, 18 alphas- (GA) and beta-glycyrrhetinic acid (GB), In this study we have investigated the effects of GA and GB on the immune system by targeting dendritic cells and analyzing phenotypic and functional maturity of murine dendritic cells (DCs) after treatment with these components. **Methods**: DCs were isolated from mouse spleen using a three- step purification technique including collagen digestion of tissue, selection of low-density cells using nycoprep and plastic adherence. The effect of GA and GB on the viability of DCs was determined using MTT assay. Surface expression of CD11c, CD86, CD40 and MHCII molecules on DCs was determined by flow cytometry. The proliferation of allogenic T cells in mixed-lymphocyte reaction (MLR) in the presence of GA and GB -treated DCs was measured by BrdU incorporation assay. **Results**: Stimulation of DCs with GA and GB resulted in up-regulation of CD40, CD86 and MHC-II molecules indicating their effects on the maturation of DCs. These components induced the allogenic immunostimulatory capacity of DCs by stimulating the proliferation of T cells and production of the T helper (h) 1-promoting cytokine, IL-12. They also increased the production of IFN-γ by T cells in mixed-lymphocyte reaction. **Conclusion**: These results of this study demonstrated that GA and GB may insert their immunomodulatory effects by enhancing DC maturation and promoting Th1 immune response.
Keywords: maturation of murine, dendritic cells, induce

2135P

A new cell source for Dendritic cells: generation of Dendritic cells from programmable cells of Monocytic origin in the presence of IL-3 and IL-4

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Background: In order to Dendritic cells (DCs) generation, several laboratories have developed culture systems to obtain these cells from peripheral blood monocytes and $CD34^+$ cells. We evaluated the differentiation potential of human programmable cells of monocytic origin (PCMO) into DCs as a new cell source. **Methods:** To obtain PCMO, the peripheral blood monocytes were dedifferentiated by IL-3 and M-CSF in 6 days. These cells were incubated with IL-3 and IL-4 for 4 days, followed by Breast tumor antigen pulsing and the addition of MCM and TNF-α with poly (I-C) to generate mature DCs. The DCs generated from peripheral blood monocyte (IL-4+GM-CSF) were used as a control group (conventional DC). The PCMO were characterized by Light microscopy, immunocytochimestry, and flow cytometry. DCs phenotypic and functional analysis was carried out using anti CD14, HLA-DR, CD83, CD86, CD80 monoclonal antibodies, mixed lymphocyte reaction, phagocytic activity and cytokine release by DC stimulated T lymphocytes. **Results:** In response to M-CSF and IL-3, monocytes became confluent, which was the result of both an increase in cell size and proliferation. Phenotype analyses, indicated that CD45, CD34, CD177, CD15 were up-regulated after 6 days and other markers (CD68, CD1a, CD14) remained low. IL-3DCs evaluation confirmed the identity of these cells, but the phenotype and function of these cells were different with conventional DCs. **Conclusion:** Our data was proved that PCMO as a new cell source can be differentiated into DCs in the presence of IL-3 (instead of GM-CSF). This method can modify the yield and Th cell-inducing properties of DCs.

Keywords: Conventional DC, Dedifferentiation, IL-3DC, PCMO

2693P

Study of phenotype and function of monocyte-derived dendritic cells induced by FcγR in multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic immune-mediated disease of central nervous system (CNS). Myelin-reactive Tcells have been implicated in the initiation of an inflammatory cascade. Dendritic cells (DC) are key modulators of this immunopathological cascade. Ligation of FcγRs on monocytes induces cell activation and specific inflammatory response. We investigated whether activation of monocytes via FcγR induced DC differentiation and inflammatory responses in MS patients compared to controls. **Methods:** CD14+ monocyte isolated from PBMC of RRMS patient in active phase and controls were cultured with different concentrations of plate bound human IgG or GM-CSF & IL-4.After 5 days, the expression of immature DC (iDC) markers (CD14, CD1b, CD86) was measured by flow cytometry. Onday

7, LPS matured DCs were harvested and expression of DC markers (CD40, CD83, CD86) and IL-12p70 and IL-23 levels in culture supernatants were measured .**Results:** We found that immobilized IgG induced iDC in a dose-dependent manner. IgG- derived iDCat 100 µg/ml human IgG incomparison with GM-CSF & IL-4 iDC expressed lower levels of CD14(0.02% vs. 5%), CD1b(30% vs 60%) and CD86(40% vs 60%) in cases and controls. After maturation, IgG- derived DCsin comparison with GM-CSF & IL-4 DCs also expressed lower levels ofCD83 (25%vs 40%), CD40(25%vs 55%), CD86(40% vs. 60%) and higher levels of IL-12(65% vs. 50%) and IL-23(76% vs. 60%). **Conclusion:** Human IgG-differentiated DCs were phenotypically and functionally different from GM-CSF, IL-4 -derived DCs and may contribute to the immunopathogensis of immune complex mediated inflammatory response in MS patients.

Keywords: Multiple sclerosis, Central nervous system, Dendritic cells, Fcγ receptor

2905P

Nanoparticles treated- and gamma interferon gene transducted- mouse bone marrow derived dendritic cells inhibited melanoma tumor development *in vivo*

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Background: Cancer arises from the uncontrolled proliferation of transformed cells. Cancers development can be inhibited by specific immunotherapy. Cytotoxic T cells are the most important immune cells in tumor therapy. Dendritic cells (DCs) are able to stimulate naive T cells activation. **Methods:** DCs were derived from mouse bone marrow using GM-CSF- and IL-4-enriched media. Gamma interferon-adeno vectors (AdVs) were propagated in 293 cell lines. Chitosan tri poly phosphate (TPP) nanoparticles were synthesized by ionotropic gelation methods from chitosan and TPP salt. In days 7 derived DCs were harvested and cultured in media. Then 24h DCs transducted by AdVs-gamma interferon and loaded with chitosan-TPP nanoparticles, unmethylated CpG and tumor antigen. Afterward treatment group (melanoma tumor induced mice) and preventive group of mice treated with manipulated DCs. **Results:** Our results have shown that DC therapy decreased the growth rate of tumor as well as improved survival of melanoma tumor induced mice in compare of controls groups. So, manipulated DCs produced cellular immunity and type-1 immune responses in preventive tumor induced mice. **Conclusion:** Gamma interferon transduced tumor antigen-, unmethylated CpG- and chitosan-TPP nanoparticles-loaded DCs are able to induced potent specific immune response against melanoma tumor. Thereupon these cells can be utilized as an effective preventive and therapeutic tool in tumor therapy.

Keywords: Dendritic cells, Melanoma Tumor, Adenovector, Gamma-interferon, Chitosan-TPP nanoparticles, CpG

3297P

Multifunctional immobilized gold nanoparticles for targeting dendritic cell and treatment of Experimental autoimmune encephalomyelitis

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Background: Multiple sclerosis (MS) is a disabling inflammatory disease of the central nervous system. Experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model for MS. In this study, we prepared Myelin Oligodendrocyte Glycoprotein (MOG) and anti DEC-205 immobilized gold nanoparticles (AuNPs) and evaluated these particles for treatment of MS. **Method**: AuNPs were prepared by citrate reduction of $HAuCl_4$, after surface modification with PEG, the thiol-functionalized MOG peptide and anti DEC-205 (for targeting dendritic cell) were attached to AuNPs. 40 C57BL/6 female mice were divided into 5groups: normal, control and treatment groups. All Groups except normal were given MOG subcutaneously to induce EAE. Mice in treatment groups received intraperitoneal injection of 20ng/mice prepared multifunctional AuNPs. Clinical assessment was performed daily. On day25 animals were sacrificed and Brain was stained for histological studies. Splenocytes were analyzed using flowcytometer and Real-time PCR. **Results**: Our results showed significant Clinical score decrease in treatment groups. Histological studies revealed lower lymphocytic infiltration and demyelination in treatment groups. The percentages of spleen $Foxp3^+$Treg increase in treatment groups. Expression of transcription factor and cytokines related to Treg and Th2 showed significant increase and related to Th1 and Th17 showed significant decrease in treatment groups. **Conclusion**: Significant increase in spleen Treg cells and decrease in Th1 and Th17 demonstrate that MOG/Anti DEC-205/AuNPs may alleviate disease progression through reducing inflammatory immune responses and suppressed immune responses. Consequently, the balance between Treg and Th17 has got a shift to Treg as well as balance between Th1 and Th2 has got a shift to Th2.

Keywords: Multiple sclerosis, Experimental autoimmune encephalomyelitis, AuNPs, Myelin Oligodendrocyte Glycoprotein, DEC-205

2810P

The effects of decidual-secreted factors on antigen-presenting activity of dendritic cells in abortion prone mice

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Background: The decidual dendritic cells (DCs) are the most potent antigen presenters of the uterine mucosal immune system, inducing feto-maternal tolerance in normal pregnancy. The present study aimed to investigate the immunomodulatory activity of decidual-secreted factors on DCs antigen presenting capacity in abortion-prone mice compared with non-abortion-prone mice. **Methods:** The decidual cell supernatants (DS) were obtained from abortion and non-abortion prone mice. Splenic DCs were pulsed with a candidate antigen in presence and absence of DS. Ag-pulsed DCs were injected into mice palms. Draining lymph node cells of immunized mice were prepared 5 days post immunization and cultured in presence of cognate antigen. The proliferation of culture cells was measured by 3H-thymidin incorporation method. **Results:** Our results showed that DS from non-abortion-prone mice significantly decreased the DCs capacity for lymphocytes stimulation compared with DS from abortion-prone mice (Simulation index (SI) of 4.93 ± 0.34 versus 11.84 ± 0.79, $P < 0.01$).We, also found that DS prepared from non-resorption sites compared with DS from resorption sites in abortion-prone

mice had increased immunosuppressive activity on DCs function (SI of 7.31 ± 1.02 versus 2.67 ± 0.49, $P < 0.01$). **Conclusion:** Taken together, our data **suggest** that soluble factors within deciduas alter maternal immune responses to paternal alloantigens via modulation of DCs function that may be implicated in the complex processes of normal pregnancy or fetal rejection.

Keywords: Abortion, Decidua, Dendritic cell, Lymphocyte

1848P

Geo-genetic level of arsenic studied in Bijar, Kordistan, hazardously disrupts human monocyte-derived DCs in vitro

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Background:
Arsenic (As) appears in water, food and soil in Bijar, Kordistan province. As in drinking water and body alarmingly disregulates the immune system through immune-sensor molecules like TLRs. dendritic cells (DCs) are pivotal in the induction of immune responses and T-cell polarization. Information on the effect of As on immune-sensor molecules in DCs is little. To examine the effect of As on the gene and protein expression of TLR2 and TLR4 in monocyte-derived DCs (MoDCs) of healthy humans. **Methods:** The pure/viable MoDCs were generated from blood samples of 6 healthy young individuals. The MoDCs were exposed to 0ng/ml (control group) and 20ng/ml (treatment group) of As for 2 h. The RNA were extracted and subjected for cDNA synthesis and then used for qPCR. Protein expression of TLR2 and TLR4 in MoDCs upon stimulation w/wo As for 2 h was assessed by two-color flow cytometry method; The MoDCs stained with FITC-anti-TLR2 and PE-anti-TLR4. 10,000 MoDCs was acquired for each sample. Protein expression of the TLR2 and TLR4 was eventually expressed by mean fluorescence intensity. **Results and conclusion:** We focused the *in vitro* effects of a minimal geogenic level of As on human TLR2 and TLR4 in MoDCs. As significantly up-regulated expression of TLR2 and TLR4 mRNA and proteins in MoDCs. When exposed to As for 2h, the observed over-expression of each TLRs mRNA in the MoDCs was similar to what was observed in the TLRs proteins. In porcine *in vitro* model we also observed that As interferes with antigen presenting capacity and maturation of MoDCs (unpublished). Immunodisregulation, immunotoxicity and thus (non)infectious diseases in chronically As-exposed people are highly likely.

Keywords: Geogenic arsenic, Flow cytometry, TLRs, Dendritic cells, Immunotoxicity, qPCR

3135P

Investigation of Sodium Benzoate (NaB) Effects on Dendritic Cells (DCs) Maturation and Cytokine Production

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Background: Mature DCs have a crucial role in the activation and polarization of T helper (Th) cells. Sodium benzoate (NaB), a FDA approved food preservative, has been reported to be involved in Th cells deviation from Th1 to Th2 cells. Despite the pivotal role of mDCs in Th cells activation and differentiation, effects of NaB on DC maturation have not been investigated. The aim of the present study was to investigate whether NaB affects Th cell deviation through its effects on maturation of DCs. Since the role of LPS in DC maturation has been documented, in this study, the maturation (the levels of CD86 and MHCII expression) of mouse spleen-derived including LPS-treated and non-treated DCs were studied in the presence or absence of NaB. **Methods**: BALB/c mice spleen-derived DCs were isolated by MACS method and the effect of different concentrations of NaB (250, 500 and 1000µg/ml) on un-treated DCs or LPS-treated DCs were investigated. To reach these aim, surface expression of CD86 and MHCII on the DCs were measured by Flow Cytometry after an overnight incubation. **Results:** The results showed that fluorescent intensity of CD86 expression on the surface of DCs was decreased in the presence of 1000µg/ml NaB compared to those of untreated DCs ($p<0.02$). The results of the present study indicated that DC maturation can be inhibited by NaB. **Conclusion:** NaB may be useful for tolerance inducing in DCs by decrease of CD86 surface expression, as a maturation molecule.
Keywords: Sodium Benzoate; Dendritic cell; IL-6; IL-12.

3223P
Preparation of immunosuppressive Core Peptide-producing Dendritic Cells using Lentiviral vectors

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Background: Dendritic cells commence both immune tolerance and immune response. They are good targets for genetic manipulation with the aim of specific suppression of aberrant immune responses. Producing DCs able to express immunosuppressive molecules like trans-membrane region of TCR-alpha chain named core peptide (CP) is among different methods of genetic manipulation. CP (GLRILLLKV) is an effective immunosuppressant of T cells. A promising gene delivery tool is lentiviral vector due to its ability to insert the gene of interest in to the host genome for persistent gene expression. Considering the immunosuppressive activity of CP and suitability of lentiviral vectors, the aim of the present study is to manipulate DCs using bi-cistronic lentiviral expression vector to produce hygromycin resistant DCs with the ability to secrete CP and to check their immunosuppressive properties in an in vitro system. **Methods:** We inserted CP into Lentiviral Transfer Vector (LLTV) via the use of restriction enzymes and used LLTV with other plasmids for the production of psudolentivirus (PL). DCs transduction with PL and polybrene was done and Hygromycin resistant DCs were picked for MLR assay. MLR assay was performed; using manipulated and un-manipulated DCs confronted with T cells, with CFSE staining and was checked through flow cytometery. **Result:** Results showed that T cells in the presence of transduced (core peptide secreting) DCs were significantly less activated compared to those which were co-cultured with control un-transduced DCs (43.6% + 1.26 and 91.3 + 4.1 respectively; p=0 .0001). **Conclusion:** Transduced DCs are able to suppress T cells activation through the secretion of CP.
Keywords: Dendritic Cells, Lentiviral vectors, Core Peptide

2964P

Cytokines liberated of T & Fibroblast and Endothelial cells induced maturation Dendritic cells

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Back ground: Dendritic cells in the way of the tissue to lymph nodes and also in blood cells have direct contacts with T lymphocytes and endothelial and Fibroblast cells which can involve their maturation by cell to cell contacts or cytokines release. To address this matter, in this study we evaluated the effects conditioned media from T lymphocytes & Fibroblast & endothelial cells and as a basis of immunomodulatory cytokines on phenotypic and functional maturation of monoyte-derived Dendritic cells. **Method**: Dendritic cells were generated from peripheral blood monocytes of volunteers in two setup groups and maturation factors as well as Monocyte Conditioned Medium, TNF-α and polyI-C (control group) or above mentioned maturation factors in addition mixture of activated T cell, skin fibroblast and human umbilical vein endothelial cell supernatants were added on day five. **Result**: Our result show that cytokines released from mentioned conditioned mediums induced the phenotypic and functional maturation of DCs which, not only, reflected by expression of CD83, CD86, CD80, and HLA-DR, but, by decreased Ag endocytosis, and facilitated the differentiation of T cells and polarization of immune response, These effects possibly due to release proinflammatory cytokines such as TNF-α, GM-CSF and VEGI by endothelial, Fibroblast and activated T cells in the supernatants of Dendritic cells culture. **Conclusion**: Cytokines liberated of T Lymphocyte & Fibroblast and Endothelial cells Conditioned Medium can be modified for the production of large amounts of mature DCs as a source of immunotherapy from monocytes

Keywords: Dendritic cell, condition medium, maturation

2001P

Dendritic cells pulsed with heated tumor cells and heated yeast form of *C. albicans*cause beneficial outcome in mouse model of breast cancer

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Background: In recent years, dendritic cell (DCs) based immunotherapy has received increased interest in the treatment of specific malignancies including breast cancer. This study reports on the beneficial immunotherapy with DCs pulsed with heated tumor cells and yeast form of *C. albicans*. **Methods:** Highly metastatic 4T1 cells were used to induce breast cancer in BALB/c mice. Dendritic cells (DCs) were isolated from the spleen of normal BALB/c mice by enzymatic digestion and nycodenz centrifugation gradient. Isolated DCs were pulsed with heated tumor cells and heated yeast form of *C. albicans*. About 10^6 pulsed DCs were injected twice with one week interval around the tumors. Tumor growth rate, survival rate and cytokine production of spleen cells were evaluated in the studied groups. **Results:** In mice vaccinated with DCs pulsed with heated tumor cells and yeast form of *C. albicans*, decreased tumor growth rate and increased survival were seen. In addition, in these mice, the production of IL-

17 and IFN-γ in splenocytes was increased and conversely, the production of interleukin-10 was decreased. **Conclusions:** DCs pulsed with heated tumor cells and heated yeast form *of C. albicans*cause beneficial outcome in mouse model of breast cancer.

Keywords: Dendritic cells, 4T1, C. *albicans*, breast cancer

2771P

In vitro effect of thymus microenvironment on plamacytoid dendritic cell development

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Background: Plasmacytoid dendritic cells (PDC) are a haematopoietic cell population with a characteristic plasma cell-like morphology found in many tissues of the mouse, including blood, thymus, bone marrow, liver, and the T-cell areas of lymphoid organs. Murine pDC are characterized by CD11c, B220, Gr-1 and BST2. The thymus provides a specialized environment skillful to attract lymphoid precursor cells and to control their survival, expansion, differentiation, and selection to functionally mature T cells, which herein we investigate effective in PDC development too. The aim of this study was the evaluation effect of the thymic microenvironment on the differentiation of mouse bone marrow cells into pDCs in vitro. **Methods:** We describe a thymus cell culture as a feeder or natural niche can induce pDCs properly without any additive growth factors feasibly . Murine BM cells cultured at 1×10^6/ml on thymic feeder until 9 days, developed into pDC, that double stained with PDCA-1-FITC and B220-APC and determined by flowcytometry. **Results:** In this study, we have found that pDCs, have better development after co-culture of BM cells with Thymic feeder up to 29%. Probably cocktail of Growth factor that express by thymic cell in the synergistic manner with cell-cell interaction is able to derive pDCs from BM cells in vitro. **Conclusion:** Thus, we present for the first time that the thymic feeder as a producer of cocktail of Growth factors, promotes development of pDCs in vitro. Suggesting that in addition to soluble factor production by thymic feeder, cell-cell interaction is an important factor in PDC development.

Keywords: Plasmacytoid Dendritic Cell, Thymic Feeder, Thymus.

Environmental Pollution & Immunology

Oral Presentations:

1395O

Dusty air pollution are associated with an increase risk of allergic diseases in general population

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Background: Concerns have been raised about the adverse impact of dusty air pollution (DAP) in Iran on human health; but there is no study showing the effect of DAP on immune system toward allergic diseases. **Methods:** The effects of ambient DAP exposures (based on PM_{10}) on cytokine profiles and lymphocyte immunophenotypes in blood among 148 individuals of general population in hazardous (AQI >300) and good condition weather (AQI <50) was examined. We measured cytokine production (IL-4, IL-10, IL-13, IFN-γ) using ELISA as well as blood samples using a FACSort flow cytometer to determine phenotypes of T-lymphocytes (CD4+ and CD8+), CD19+ B-lymphocytes, CD25+ and CD4+ CD25+ cells. **Results:** The mean serum level of IL-4 (33.4± 2.9 *vs*0.85± 0.65 pg/dl) and IL-13 (15.1± 4.4 *vs* 0.12± 0.7 pg/dl) in subjects who exposure to ambient DAP were increased significantly than individuals in good condition weather (P= 0.001 for both). In addition, CD19+ B-lymphocytes (12.6± 4.9 *vs* 8.9± 3.2%) and CD4+ CD25+ cells (13.6± 4.6 *vs* 7.7± 3.8%) counts in peripheral blood were increased parallel with increased DAP exposure levels (P= 0.035 and P= 0.004, respectively). **Conclusion:** The study may suggest ambient DAP may affect immune system shifting allergic inflammation in general population.

Keywords: Dusty air pollution, immune system, allergic diseases, cytokine, lymphocyte

2161O

Mild to severe effects of radiation on human immune system: natural and occupational exposure vs. accidental overexposure

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Background: It is well known that ionizing radiation at high doses depresses immunity and depression or dysfunction of the highly radiosensitive cellular components of the immune system can lead to serious consequences, including increased risk for cancer. However, there is in vivo evidence for both enhancement and depression of immune responses after exposure to low-dose radiation. In order to examine the effects of high and low dose ionizing radiation on the immune parameters different groups of radiation exposed individuals were examined. **Methods:** The survey groups consisted of: 1) 35 naturally exposed individuals living in a high background radiation area with a mean exposure level of 13 mSv/y. 2) 37 medical radiation workers occupationally exposed to a mean value of 8.14 mSv/y and 3) one case of acute accidentally overexposed to 3.5 Gy gamma radiation. 4) In addition, 35 healthy age- and sex-matched subjects were included as a control group. Lymphocyte subpopulations and the concentration of different cytokines, serum immunoglobulins and components of the complement system and proliferative response and DNA damage in T cells were investigated in all groups. **Results:** Accidental overexposure case showed a typical hematopoietic syndrome with significant decrease of WBC, absolute number and percentage of lymphocytes, with remarkable decrease in $CD19^+$, $CD3^+$ cells and $CD4^+/CD8^+$ ratio. However, there was no statistical difference between study groups (1 and 2) and controls (4) in terms of the above mentioned factors, except significant increase of mitogenic response of the T lymphocytes in the first group and moderate increase of WBC in the second group. Significant increases of IgE in the first group and IgG in the second group identified. Significant increase in serum IL-2 and decrease in IL-10 concentrations were observed in the two groups as compared to the controls. DNA damage by using chromosome aberration analysis showed a dicentric yield of 68 in accidental case and 0.20 and 0.25 in the two groups, respectively that were significantly higher than 0.04 in the control group.**Conclusion:** As it is expected, acute, high-dose ionizing radiation had significant effects on the immune cell populations. However in the two low dose exposed groups, while cytogenetic results show higher chromosomal damage, humoral immunity and cellular responses are stimulated and some cytokine productions are immunomodulated.

Keywords: Natural radiation exposure, Occupational exposure, Human lymphocyte, Chromosomal aberration, Cellular and humoral immunity

1549O

Association of GSTM and GSTT polymorphism to allergic rhinitis among resident of Rawalpindi/Islamabad

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Background: Allergic rhinitis is a heterogeneous disease caused by IgE mediated nasal inflammation with high prevalence across the globe. In allergic diseases xenobiotics or allergens trigger the degranulation of mast cells and eosinophils. In response these cells release different types of substances leading to airway hyperactivity. Glutathione S transferase genes encode different enzymes (GSTT1, GSTM1) which are involved in the process of detoxification of these substances. GSTT1 and GSTM1 are polymorphic types having a null variant genotype and absence of corresponding enzyme can increase risk of disease in an individual but this is still controversial. The aim of this study was to investigate the role of null GSTT1 and GSTM1 in allergic rhinitis among population of Rawalpindi/Islamabad, Pakistan.**Methods:**IgE levels were analyzed in the serum of both patients and controls. Comparing means of log IgE of patients and controls showed a significant increase in the serum IgE levels of patients ($p < 0.05$). Genomic DNA from 129 patients and 122 controls was analyzed by polymerase chain reaction. **Results:** The frequency of both GSTT1 and GSTM1 null genotype in patients was higher as compared to controls ($p < 0.05$, χ=42.26). A significant association of GSTT1 and GSTM1 mutations was observed in males ($p < 0.05$, χ=36.21) but not in females patients stratified by gender. **Conclusion:** These results suggest that absence of GSTM1 and GSTT1 may alter the risk of allergic rhinitis in Pakistani population but further work is needed to be done on Pakistani population.

Keywords: GSTT, GSTM, Polymorphism, RFLP

2556O

Effect of Dioxin on proliferation and invasion of normal and endometriosis menstrual blood stromal stem cells

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Background: According to the current theories on the etiology of endometriosis, endometrial stem cells have been suggested to have a role in the pathogenesis of endometriosis. Although the exact etiology of endometriosis remains elusive, dioxin, as an environmental pollutant, has been linked with development of this disease. So Here, we investigated the potential impact of dioxin treatment on functional features of normal and endometriosis menstrual blood stromal stem cells (MenSCs). **Methods**: MenSCs were isolated from menstrual blood of normal and endometriosis individuals and characterized. The impact of dioxin on the capacity of MenSCs from normal or endometriosis donors to proliferate, invade into extracellular matrix and produce proinflammatory cytokines were assessed. Moreover, dioxin effect on IDO expression in both MenSC types was measured by Real-time PCR. **Results**: In the absence of dioxin, endometriosis MenSCs revealed significantly higher proliferation and invasion capacity compared to the normal ones. Dioxin treatment decreased endometriosis MenSCs proliferation and invasion capacity, but did not have any significant effects on those of the normal MenSCs. Additionally, secretion of IL-6 and IL-8 by endometriosis MenSCs was diminished upon dioxin treatment, while normal MenSCs showed enhanced production of these cytokines. Upon dioxin treatment, IDO expression did not show significant changes

between the two groups. **Conclusion**: This is the first study to show the differential effects of dioxin on normal and endometriosis MenSCs. Our results suggest that in non-endometriosis women dioxin exposure may be involved the onset of disease, while in endometriosis patients it could have beneficial impacts.

Keywords: Endometriosis, Dioxin, Menstrual blood stem cells, Proliferation, Invasion.

2640O

Effect of cadmium on glucose, lipid profile and oxi dative stress in streptozotocin-induced diabetic rats

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Background: Cadmium (Cd), highly toxic heavy metal, has been considered as a possible risk factor of diabetes and its complications. However, the underlying mechanisms of Cd-induced diabetes are not clear. The present study was designed to evaluate the effects of Cd on the oxidative system in diabetic rats. **Methods:** In this study, the rats were divided into the following groups of 8 animals each: control (C), diabetic (D) and Diabetic-exposed to Cd (1 mg/kg/bw) (D + Cd- exposed) groups. Diabetes was induced by streptozotocin (intraperitoneally (i.p.) injection) at a single dose of 60 mg/kg. Cd (i.p. injection) was administered from 3 days after streptozotocin (STZ) administration to the end of the study (4 weeks). After 4-weeks, blood was drawn to determine the changes of serum superoxide dismutase (SOD), catalase (CAT), glutathione-S-transfrase (GST) and glutathione peroxidase (GPx) activities as well as the levels of reduced glutathione (GSH), malondialdehyde (MDA) and lipid profile. **Results:** The results indicated that Cd exposure aggravated increased blood glucose (p<0.01), MDA (p<0.001), triglycerides (p<0.05), total cholesterol (p<0.05), LDL-C (p<0.01) as well as decreased GSH (p<0.05), HDL-C (p<0.01), levels and the activities of antioxidant enzymes in diabetic rats. **Conclusion:** These results suggest that Cd exposure deteriorates diabetic effect and its complications in STZ-diabetic model by induction of oxidative stress.

Keywords: cadmium, lipid profile, oxidative stress indices, diabetic rats

2792O

Cyanide level in smokers is correlates with imbalance of Th1 and Th2 cytokines

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Background: Cyanide has fatal effects, and cigarettes are the main cause of cyanide exposure in individuals who are not chemical industry workers. Moreover, immune system changes are caused by smoking and play important roles in ailments. With these facts in mind, this study measured and analyzed cyanide levels in smokers and non-smokers in saliva samples and its correlation with Th1 and Th2 cytokine production. **Methods:** Saliva samples were taken from 75 smokers and the same number of non-smokers. An ELISA test was performed according to

the manufacturer's instructions to measure IL-2, IL-4, IL-5, IL-12, IL-13, IFN-γ and TNF-α. Cyanide levels were measured using a spectrophotometric assay. **Results:** Cyanide level was significantly higher in saliva samples of smokers comparing with non-smokers (p=0.001). IL-4, IL-5 and IL-13 levels showed a significant increase in smokers (p=0.004), while there was slight decrease in the amount of IL-2, IFN- γ, IL-12 and TNF- α compared with non-smokers. **Discussion:** Our data shows there is a strong correlation between the amounts of body fluids cyanide and an imbalance of Th1 and Th2 cytokines with an increase in Th2 cytokines.

Poster Presentations:

2168P

Associations of flour inhalation in bakery workers with pulmonary function, respiratory symptoms, and sputum eosinophilia in Sanandaj, Kurdistan, Iran

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Background: Occupational flour inhalation has been a culprit in commence of several pulmonary maladies, such as asthma. We examined the relationship of occupational wheat flour inhalation in bakery and supermarket employees with pulmonary function, respiratory symptoms, and sputum eosinophilia in a cross-sectional study in, Kurdistan, Iran. **Methods:** 110 subjects from traditional bakeries and 137 subjects from supermarket employees were enrolled in the study. Flour exposure concentrations, respiratory signs, sputum analysis, and respiratory volumes and capacities were measured based on the standard methods. **Results:** Respirable concentration of flour in the bakery workers was two to four-fold of ACGIH's threshold limit value in which bread-bakers with 2.2 mg/m^3 experienced maximum exposures. The supermarket employees had not exposure to flour dust. The respiratory volumes in both bakery and supermarket employees were in the normal range. However, the median of voluminal percentage in bakery workers except forced vital capacity was reduced ($p< 0.001$). The mean respiratory volume of bread-bakers was reduced compared with supermarket employees ($p< 0.05$). In addition, we observed increased respiratory symptoms in the bakery workers, again more prevalent in the bread-bakers. There was a significant correlation between flour exposure concentration and sputum eosinophilia in which the percentage of eosinophilia in the bread-bakers was more than other bakery and supermarket employees. **Conclusion:** Albeit there were reductions in the respiratory volumes, results indicate no obstructive spirometric pattern. Noteworthy, sputum eosinophilia might be a suitable screening method to detect airway hyper-responsiveness in workers exposed to known as thmogens.

Keywords: Bakery, Hyper-responsiveness, Sputum eosinophilia, Flour

2540P

Role of triggers in asthma and autoimmunity

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Background: The activity of immune system against internal organ has different causes. For example the molecular mimicry by virus and trauma tend to laceration of organ which expose internal antigen to immune system. **Methods:** In this study the causes of asthma in a adulthood are investigated by opinion of the patients. **Results:** Of 92 patients 56 patients (60. 8 %) declared that the common cold as trigger of their asthma. For 17 (18.4%) patients consumption of bleaching, insecticide and other potent chemical substance were the trigger causes. For 6 patients (6.5%) post immigration for 4 patients (4.3%) post emotional stress. For 3 patients (3.2%) post pregnancy and delivery. For 2 patients (2.1%) post anesthesia and operation. For 2 patients (2.1%) post consumption of medications. For 2 patients (2.1%) post food and additive ingestion are the triggers of their asthma. Although above information are not completely reliable, but confirm this hypothesis that exposure to different material and situation can trigger immune system against internal organ and introduce allergy and autoimmunity.

Keywords: Autoimmunity, Triggers, Asthma

2823P

The Effect of chronic exposure to Cadmium on Serum Lipid, Lipoprotein and Oxidative Stress Indices in Male Rats

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Background: Cadmium (Cd) is an environmental toxic metal implicated in lipid abnormalities. Present study was designed to determine the possible association between chronic exposure to low Cd concentration and alterations in plasma lipid, lipoprotein levels and oxidative stress indices in rats. Twenty male albino rats assigned to 2 groups of 10 rats (test and control). Cd-exposed group obtained drinking water containing cadmium chloride ($CdCl_2$) in concentration of 2.0 mg Cd/L in drinking water for 3 months. At the end of experimental period, blood samples obtained to determine the changes of serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), reduced glutathione (GSH) and malondialdehyde (MDA). The results of the present study indicated that Cd administration significantly increased the serum levels of TC, TG, LDL-C and MDA with reduction in the HDL-C and GSH levels. In conclusion, this study indicated that chronic exposure to low Cd concentration can adversely affect lipid and lipoprotein profile via lipid peroxidation.

Keywords: Cadmium, Lipid, Lipoprotein, Oxidative Stress Indices

2949P

Isolation and molecular characterization of mercury resistant bacteria and PCR amplification of *bacillus cereus*merA gene from wastewater of Khowr-e-Musa, Iran

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Background: The development of industrial activities, population growth and interference of human being in the environment has caused increase of industrial, municipal and agricultural effluents in marine ecosystems. These wastewater contain contaminants such as agricultural toxins, nitrogen, phosphorous and heavy metal compounds. Heavy metals are one of the most important marine pollutants because of their stability and non-degradation features, entering the food chain and bioaccumulation in marine organisms. There are many ways for removal of heavy metal but recently biosorption phenomenon and the use of micro-organisms abilities have identified as appropriate and low cost method.This study was done to investigate of mercury resistant bacteria on the seashore wastewater of Khour-e-Musa in Mahshahr area, in the south west of Iran, which one of the greatest petrochemical chlor-alkali is located there. **Methods:** Thus, water samples were taken from wastewater of three station. Amount of total mercury in the samples was measured using cold vapor atomic absorption spectrophotometery. modern molecular approaches based on 16srRNA gene homology were used for identification. Mercury toxicity was measured via minimal inhibitory concentration method. **Results:** The isolated bacteria included *bacillus cereus,E.coli*and *staphylococcus aureus,* and resistance to mercuric chloride was at 400,450, and 75 ppm, respectively. The origin of resistant was determined by plasmid curing. We have characterized this gene of mer operon (merA) from *Bacillus cereus* of 1695 bp which is located on genomic DNA and 1695bp of merA gene was amplified by PCR Method. **Conclusion:** The results depicted that *E.coli, staphylococcus aureus, Bacillus cereus* were resistant and could grow on high concentration of mercury. Metal-resistant strains may also have application in remediation of metal-contaminated environments.

Keywords: Mercury Resistant Bacteria, Genomic DNA, meroperon, merA

3148P

The electromagnetic fields affect T-helper balance in rats

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Background: The biologic influences of Electro Magnetic Fields (EMFs) are being investigated for decades and the World Health Organization (WHO) established the International EMF Project, to determine the scientific evidence of possible health effects of EMFs. These fields have been classified as probable human carcinogen. Among all investigations, immunologic experiments, especially assessing cytokines, are rare. The present study undertakes to search for the T-helper (Th) balance outcome of exposure to these fields. **Methods:** Thirty Wistar rats were divided to three groups, one group included the control rats, another, rats for one month exposure, and the last group rats for 2 month exposure. An EMF in the frequency of 50 Hertz, 0.5 mili-Tesla was used, and the serum levels of cytokines of IL-4 (indicating Th2 activity)

and INFγ (indicating Th1 activity) were measured. **Results:** The results showed that after one month, the serum levels of IL-4 increased highly significantly (P value: 0.009), and the levels of INFγ decreased significantly (P value: 0.032). After 2 months these levels returned to the levels statistically similar to the control group (P value: 0.627 & 0.402 respectively for IL-4 & INFγ). **Conclusion:** in short periods, as much as one month, EMFs weigh the T-helper balance to the Th2 side in rats, however, in longer periods, as much as 2 months, the biologic homeostasis of rats causes tolerance to this influence of the EMFs.
Keywords: Electromagnetic fields, T-helper balance, Cytokine level, IFNγ, IL-4

Exercise Immunology & Aging

Oral Presentations:

1409O

Wrestlers' immune cells produce higher IL-6 and lower IL-12 and IL-13 in response to in vitro mitogen activation

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Background: Although recent investigations have shown chronic inflammation and inflammation-associated diseases might be ameliorated by exercise; little is known about the relation between exercise training and anti/proinflammatory cytokines. **Methods:** This cross sectional study was conducted to compare interleukin-4 (IL-4), IL-6, IL-10, IL-12, IL-13, IFN-γ levels in serum,and their production in whole blood (WB)cells and in peripheral blood mononuclear cells(PBMCs) cultures in response to mitogen lipopolysaccharide and phytohemagglutinin. Twelve elite wrestlers with three times per week exercise training history for more than 9.5 years, and thirteen healthy silent controls were recruited. To analysis the cytokine levels by enzyme linked immunosorbenttest (ELISA), the bloods were taken 24 hours after the last training session in the wrestlers. **Results:**Serum analysis for IL-4, IL-6, IL-10, IL-12, IL-13 and IFN-γ indicated no statistical difference in the two groups. Meanwhile, 48 h *in vitro* activation of WB and PBMCs by mitogens revealed that IL-6 production was elevated both in WB and PBMCs. Whereas, IL-12 and IL-13 were decreased in PBMCs and WB cells culture supernatant, respectively. **Conclusion:** It seems wrestling may enhance the capacity of immune system cells to release anti-inflammatory cytokines IL-6 and decrease this capacity in the production of proinflammatory cytokine IL-12 and IL-13.

Keywords: Cytokines, Interleukins, Athletes, Wrestling, Exercise

1910O

Evaluation the adjuvant activity of moderate short-term exercise training on antibody response against HSV-1 vaccine

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Background: Studies have shown that an acute exercise period before vaccination can improve antibody responses to the vaccine. In the present study effect of short term exercise training on antibody responsesagainst HSV-1 vaccine in female Balb/c mice is evaluated. **Methods:** 18 female Balb/c mice were prepared from Pasteur Institute of Iran and divided into 3 groups including; vaccine-exercise, vaccine and control groups. The vaccine-exercise group received vaccine immediately after the first session of exercise. Also, exercise was continued for 5 days in this group. Vaccine group injected with vaccine and control mice received saline buffer. Second and third S.C. immunization carried out with 2 weeks intervals. Two weeks after last immunization, bleeding carried out from mice and total antibody and isotopes were evaluated. **Results:** exercise improved antibody response especially IgM antibody.**Conclusion:** Short term exercise training could enhance antibody responses to vaccination. This kind of exercise training after vaccination should be explored further as a possible behavioral adjuvant to vaccination.

Keywords: HSV-1, Vaccination, Adjuvant, Antibody response, Short term Exercise training

2214O

The effect of ginger extract on biochemical and functional symptom of delayed onset muscle soreness

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Background: Inflammation and pain as a result of eccentric or unaccustomed activity called delayed onset muscle soreness and cause athletes to be far from exercise. Finding a safe and simple way to prevent or treat DOMS is one of the coaches and researches' problems. The purpose of this study was to survey the effect of ginger extract on biochemical and functional symptom of delayed onset muscle soreness. **Methods:** 36 subjects (age 22.02±2.48 yrs, height 159.3±5.67 cm, and weight 57.14±8) divided to 3 groups in random, including: ginger intake 1 hour before exercise (GIBE), ginger intake immediately after exercise (GIAE) and placebo (PL). Subjects consumed capsules contain 60mlg of ginger extract or placebo before and after exercise. The exercise protocol consisted of a 20 minute step test using a 46cm step. Blood samples were taken before, 1, 24 and 48 hour after exercise to assay CK and IL-6. Muscle pain, isometric strength and circumference of thigh muscle, and hip range of motion were recorded at mentioned time. For statistical analysis of data, ANOVA with repeated measure and Bonferroni post hoc was used ($p \leq 0.05$). **Results:** The results showed a significant reduction of pain in GIBE compare to GIAE after 24 and 48h of EE and GIAE compare to PL ($p > 0.05$). IL-6 changed significantly in GIBE compare to PL ($p > 0.05$) after 1, 24, and 48h after EE. The

other factors didn't change meaningfully. **Conclusion:** The finding of this study suggests that ginger extract may have atiinflammation and analgesic effect on DOMS.
Keywords: Delayed onset muscle soreness, eccentric exercise, ginger, IL-6, CK

3326O
Gene expression of Mn SOD enzymes in athlete's women: effect of a session intensive exercise

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Background: Exercise intensity and gender difference probably change the enzyme gene expression and enzyme activity. Mitochondria are supported by gene expression and antioxidant enzyme activity and Mn SOD is caused to safety in mitochondria of cells. But, mechanisms of gene expression in responding to exercise and effect of genders have not known in athlete's women. Therefore, the purpose of this research was investigation of the Mn SOD gene expression following to intensive exercise in athlete's women. **Methods:** A repeated measures design was used for this study. Thirteen athlete's women in the age range 22-25 years from urmia city were volunteered as subjects. Intensive exercise was included 20 minutes running on treadmill in the grade slope 6% and speed of 11 km/h. venous blood samples were taken in three stages, before exercise, immediately and 3 hours after exercise for Measured of Mn SOD gene expression and H2O2 activity. **Results:** The mRNA of Mn SOD and H2O2 activity 3 hours after exercise significant increased (P≤0/05). **Conclusion:** A session of intensive exercise increased the Mn SOD gene expression and free radicals in athlete's women.
Keywords: Mn SOD, Athlete's women, Intensive exercise

3050O
The comparison of effects of continues and sprint training on plasma Visfatin, IL-6 and TNFα in Healthy rats

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Background: Visfatin, which is alternatively as pre B-Cell is a newly characterized protein that is highly expressed in visceral adipose tissue .It has been linked to cytokine-like effects on insulin sensitivity and enzyme-mediated effects on pancreatic insulin secretion. Also, It has important role in insulin resistance and obesity and increased health risks. Exercise training has beneficial effects on adipose tissue, but there is less investigate on the relation ship between Visfatin, IL6 and TNFα to response types of training. For reason, we investigated the effects of continues and sprint training on Plasma Visfatin, TNFα and IL6 levels in healthy rats. **Methods**: 30 Wistar rat were randomly divided into three groups: Continues training(n=10), sprint training (n=10) and control (n=10).continues training was done for 8 weeks at 55% to 75% vo2max from 15 min to 50 min .sprint training was done for 8 weeks at 60% to 90% vo2max with 3(3*1) to 4(45*3) rep *1 min.The change of plasma IL6,TNFα and Visfatin levels were measured by ELISA analysis. Data were analysis with ANOVA and Tukey test. **Result**: Data analysis showed that sprint training led to significant decrease in

Visfatin(P=0.01) IL6(P=0.02) TNFα(p=0.019).Also this study showed that there is significant correlation between the changes between Factors. **Conclusion**: These results indicate that decreased levels of TNFα,IL6 and Visfatin correlated with intensity in comparison to duration . Therefore, sprint training with the reduction of these factors lead to preventing metabolic disease.

Key word: sprint training, continues training, Visfatin, IL6, TNFα

2538O

The effects of Taurine supplementation and intermittent exercises Taurine on Serum CD4/CD8 response in soccer players

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Background: The purpose of this study the effects of Taurine supplementation for two weeks and three sessions of 90 minutes of intense exercise on serum CD4/CD8 responses in well-trained soccer players. **Methods:** Therefore, Twenty-four football players under 24 years of league were selected and divided randomly to Taurine (TG=8), placebo (PG=8) and control (CG=8) groups. TG Daily supplements of 15 mg Taurine per kg of body weight and PG groups received the same amount of aspartame and both groups preformed three times a soccer specific exercise protocol. The CG group received no supplementation and follow-up was just your ordinary Program. Blood samples were taken in the sixth stage (48 h before Period, before and immediately after first & third exercise protocol and 48 h after the end of the Period) of the 5cc of the anterior forearm venous in sitting position. **Results:** Results of one-way ANOVA with repeated measures, significant differences (P=0/013) in serum showed a CD4/CD8. Tukey's test for the difference between the control group and the placebo (P=0/008) reported. It appears that the three 90-minute soccer specific protocol to enter stress on the immune system in soccer players such pressure may again be repeated during the competition season. On the other hand, results showed that Taurine supplementation before and during this period May be influenced by immune modulators. **Conclusion:** Thus, short-term use of Taurine supplementation during pressure-filled week of competition and training to be advised in trained soccer players.

Keywords: Taurine Supplementation, Soccer Specific Intermittent Exercise, Immune Response

3343O

A Comparison of the Acute Effects of Endurance, Resistance and Concurrent Exercise on plasma Interleukin-17 Concentrations in Active Young Men

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Background: Interleukin 17 (IL-17) is a new pro-inflammatory cytokine that have a critical role in inflammatory process and diseases. Alteration in IL-17 plasma level can be a useful biochemical marker to determine acute exercise-induced inflammation in skeletal muscles. Also, every type of exercises could lead to different immune responses in human body.

Therefore, the aim of this study was to comparison of the acute effects of endurance exercise (EE), resistance exercise (RE) and concurrent exercise (CE) on plasma IL-17 concentrations in active men. **Methods:** Twenty healthy young and active men (Age: 21.69±2.66; BMI: 21.92±1.89; Fat%: 14.49±3.05) voluntarily participated in this study and randomly were assigned into three groups: EE (n=7), RE (n=6) and CE (n=7). Exercise protocol consisted of 45 min running at 70-80% of maximum heart rate for EE group, circuit in 3 rounds, 8 stations at 80% 1RM in 45 min for RE and combination of both RE and EE (first RE then immediately EE with the same intensity and half of the volume of each programs) for CE group was performed. IL-17 plasma concentrations (Pre, immediate and 1 h post exercises) were measured by ELISA method. Data were analyzed using Repeated-Measures analysis of variance, One-Way ANOVA and Tukey post-hoc and Paired T tests ($p<0.05$). **Results:** Changes in the plasma levels of interleukin-17 was significantly different between groups immediately after exercise and 1 hour post exercise. **Conclusion:** Based on changes pattern of plasma levels of IL-17 in each group, CE can modify the effects of RE and EE.

Keywords: Interleukin-17, Endurance Exercise, Resistance Exercise, Concurrent Exercise, Active Men

2720O

Effects of nonlinear resistance and aerobic interval training on cytokines and insulin resistance in obese men

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Background: Regular exercise traininghas been shown to reducesystemicinflammation but there is limited research directly comparing different types oftraining. The purpose of this study was to compare the effects of nonlinear resistance training (NRT) and aerobic interval training (AIT) on serum interleukin-10 (IL-10), IL-20 and tumor necrosis factor α (TNF-α) levels, insulin resistance and aerobic capacity in middle-aged obese men. **Methods:** Sedentary volunteers were assigned to NRT (n = 12), AIT (n = 12) and (CON, n = 10) control groups. The experimental groups performed 3 weekly sessions for 12 weeks, whereas the CON grouped maintained a sedentary lifestyle. NRT consisted of 40-65 min of weight training at different intensities with flexible periodization. AIT consisted of running on a treadmill (4 sets of 4 min at 80-90% of maximal heart rate, with 3 min recovery intervals). **Results:** Serum IL-10, IL-20 and TNF-α levels did not change significantly in response to training (all, $P > 0.05$), but IL-10/TNF-α ratio increased significantly with AIT compared to CON (2.95 ± 0.84 vs. 2.52 ± 0.65,$P = 0.02$).After the training period, maximal oxygen uptake increasedsignificantly in AIT and NRT compared with CON (both, $P <0.001$, 46.7 ± 5.9, 45.1 ± 3.2 and 41.1 ± 4.7 ml/kg/min, respectively), and AIT greater than in NRT ($P = 0.001$). The2 exercise programs were equally effective at reducing insulin resistance index (both,$P <0.05$, AIT: 0.84 ± 0.34, NRT:0.84 ± 0.27, and CON: 1.62 ± 0.56) and insulin (both,$P <0.05$, AIT: 3.61 ± 1.48, NRT: 3.66 ± 0.92 and CON: 6.20 ± 2.64 µU/mL). **Conclusion**: The AIT seems to have better anti-inflammatory

effects (as indicated by the IL-10/TNF-α ratio) compared to NRT.
Keywords: Exercise training, IL-10/TNF-α ratio, Inflammation, Interleukin, Obesity

3031O
Endurance exercise training alters the HSP70, IL-6 and IL-1β responses in rat skeletal muscle to downhill running

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Background: Heat shock protein 70 (HSP70) seems involved in both adaptations to training and the muscular response to exercise-induced muscle injury. In this study we tested the hypothesis that muscular HSP70 levels are increased by endurance training and that this would alter the HSP70 and cytokine responses to downhill running (eccentric exercise) in rats. **Methods:** 12Wistar rats were randomly allocated to 8 weeks of treadmill endurance training (0° slope). Five of the trained and five sedentary rats were exposed to downhill running (-16°; 90 min). M. soleus was harvested 24 hours after the training period and 24 hours after the downhill running. All muscles were analysed for HSP70 mRNA and protein levels, and IL-6, IL-1β, and TNF-α. Serum was analysed for creatine kinase (CK). **Results:** The training period induced augmented intramuscular levels of HSP70 and IL-6, compared with sedentary control animals. HSP70 protein levels increased only in the sedentary rats after downhill running. Downhill running induced increased IL-6 levels in both trained and sedentary rats, while IL-1β increased only in trained rats. TNF-α levels were unaffected by both training and downhill running. **Conclusion:** Endurance training elevated the HSP70 levels in m. soleus, which might have preconditioned the muscles for the downhill running, as evaluated by CK and the HSP70 responses. Since IL-6 and IL-1β increased in trained rats only, and that these cytokines appeared to be separately regulated from the pro-inflammatory cytokine TNF-α, suggest that IL-6 and IL-1β are involved in the recovery and/or adaptation processes in the skeletal muscles after exercise.
Keywords: Eccentric exercise, Preconditioning, Heat shock proteins, Cytokines

3230O
The effect of gender differences in cytokine and hormone response to a bout of resistance exercise in warm and normal enviroment

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Background: Warm environment is an important source of physiological stress that cause malfunction of the body. Also men and women respond differently to an exercise. The aim of this study was the investigate effects of gender differences in cytokine and hormone response to resistance exercise in warm and normal environment. **Methods**: 14 students (men=7 and women=7) 24-29 years participated in this study. The exercise program includes 10 routine

movements with the resistive devices; 70% of 1RM, 3 sets, 10 reps, one minute rest between repetitions and a 5-minutes rest between each set. Exercise program was performed for two conditions, first in normal temperature environment (20° C) and then in warm temperature enviroment (35°C) in the next weeks. In both sessions, one hour before, immediately after and one hour after resistance exercise, blood samples obtained from each individual. Core temperture was measured for all condition. Serum concentraion of IL-6, IL-15, HS70, estrogen and cortisol were measured by elisa. ANOVA Repeated Measure to interpret the results and the Pearson correlation test to evaluate the association of variables was use to analyze the data. Alpha was set at $p<0.05$ for all analyses. **Results**: IL-6 levels after resistance exercise in a warm environment was different between the two groups, increased in males and decreased in females were observed. There were significant difference between men and women in levels of IL-15 (P=0.034), HSP70 (P=0.001), cortisol (P=0.004), testosterone (P=0.023) in the two environments, There were no significant differences between the initial value and estrogen response to resistance exercise in two environments. **Conclusion:** Heat stress causes additional pressure on the stress of resistance exercise on the athlete's body. Also, resistance exercise due to hormonal differences between men and women are created different responses between the two genders. In Summary, a bout resistance exercise in warm environment induce different response in levels of IL-6, IL-15, HSP70, cortisol, estrogen and testosterone in men than in women.

Keywords: Gender, Warm envinroment, Resistance exercise , Cytokine

2336P

Evaluation of two intensities of exercise training with differences in VO2max on some inflammatory biomarkers among soccer players

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Background: Exercise may change level of anti-inflammatory and pro-inflammatory cytokines among players. This study was aimed to assess the effects of two intensities of exercise training with differences in VO2max on some inflammatory biomarkers, includingIL-1β, TNF-α, hs-CRP, IL-6, sICAM-1, IL-10 and ratios of TNF-α/IL-10 and IL-6/IL-10. **Methods:** Twenty-four young male soccer players were participated. Aerobic capacity was measured using submaximal treadmill exercises **according** to the Bruce protocol. First protocol was a 30 -minute running at a speed of 65% of VO2max and second one was **six periodic repetitions** with **three minutes** at a speed of 85% of VO2max with a 90- second rest between each repetition. Blood samples were collected at the third protocol: baseline, immediately after the exercise and at rest. **Result:** Of the inflammatory biomarkers analyzed, significant changes were observed only in the first protocol. Plasma IL-1β decreased at rest from baseline and immediately after exercise (P = 0.008 and P = 0.013, respectively) in the first protocol. The same response pattern was observed for sICAM-1. The first protocol showed decreases in sICAM-1 at rest from baseline and post exercise (P = 0.013 and P = 0.038, respectively). **Conclusion:** Our data suggest that lower-intensity exercise (at 65% VO2max) has more anti-

inflammatory effects compared to higher one (at 85% VO2max). In addition, we observed a 24 hour-delayed change in reducing the IL-1β and sICAM-1 levels. It shows that lower-intensity exercise is more effective than higher one for reducing the inflammatory status.
Keywords: Soccer players, Inflammatory biomarkers, VO2max, Cytokines

2795O
Effect of Six weeks of Endurance Training on IL-12 Levels of Tumor Tissue in Female Mice with Breast Cancer

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Background: IL-12 has been shown to possess an angiogenesis-inhibiting effect, which is of great significance in cancer. Increases in local and systemic levels of IL-12 can contribute greatly to cancer treatment and tumor mass reduction. The aim of this study was to investigate the effect of a 6-week-long endurance training program on IL-12 tissue levels and tumor mass in breast tumor-bearing female BALB/c mice. **Methods:** Twenty female BALB/c mice (4-5 weeks of age) were randomly divided into identical groups: rest-tumor-rest (RTR) and rest-tumor-exercise (RTE). After induction of tumor, RTE group undertook endurance training at 55-70% VO_2max for 6 weeks. Tumor mass was measured on a weekly manner over the training period. At the end of training period, tumor tissue IL-12 concentrations were measured by ELISA. Data was analyzed using independent t-test and Pearson correlation coefficient test. **Results:** A significant difference (P=0.001) was found in IL-12 tissue levels between groups. Also, the amount of reduction in tumor mass in RTE group was significantly greater than that of RTR group (P=0.001). Moreover, a significant reverse correlation (r=-0.706; P=0.002) was found between IL-12 concentration and tumor mass, suggesting an anti-tumor role for IL-12. **Conclusion:** Tumor mass reduction in response to exercise training could to a great extent be dependent on cytokine modulation, which occurs through decreased production of pro-inflammatory and angiogenic cytokines such as IL-17 and IL-6 on the one hand, and increased production of anti-angiogenic cytokines, including IL-12, on the other hand. Thus, moderate-intensity endurance exercise could be used as an effective strategy in cancer treatment.
Keywords: Breast cancer, Endurance training, IL-12, Tumor tissue

2875O
Effect of combination resistance exercise and heat stress on cell production of TNF-α and IL-6 cytokines

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Back ground: Cortisol treatments have been demonstrated to suppress LPS-stimulated

cytokine production during inflammatory stress. Exercise and heat stress can exacerbate cortisol release. The aim of this study was to investigate effect of combination resistance exercise and heat stress on IL-6 and TNF-α production of mononuclear cells stimulated by LPS. **Methods:** Six active males (26.33±2 years; 86.05±6 kg) completed two (25 °C, NORMAL(N) or 35 °C, HEAT(H)) 60 minute resistance exercise session with 75% 1RM. Blood samples were collected three times (pre, immediately post and 1 hour post exercise) and analyzed for LPS-stimulated mononuclear cells cytokine production and circulating cortisol stress hormone. The amounts of IL-6 and TNF-α in the cell culture supernatant and plasma cortisol levels were measured by ELISA. **Results:** The Repeated-Measurements ANOVA showed that upon stimulation with LPS, cells IL-6 production respectively in H and N environment increased immediately (P_H=0.026; P_N=0.048) and decreased 1 h post-exercise and returned to basal levels (P_H=0.005; P_N=0.033) but TNF-α release was decreased immediately post-exercise only in H environment (P=0.877). The concentration of blood cortisol was unaffected up on N or H environment. **Conclusion:** These results demonstrate that exercise in heat environment had different effects on cytokines production. Exercise in heat environment increases amount of IL-6 cytokine produced from blood monocytes while TNF-α production decreases despite cortisol was stable that propose regulatory function of IL-6 on TNF-α release. These cytokine changes suggest application of combined exercises-heat method in some clinical therapies.

Keywords: Cortisol, IL-6, Heat stress, Resistance exercise and TNF-α

Poster Presentations:

1979P

Effect of Crocus sativus L. (saffron) ethanolic extract on oxidative stress and antioxidant status in the kidney of aged male rats

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Background: An imbalance between production of reactive oxygen species (ROS) and its elimination by antioxidant defense system are known to increase in kidney during aging, and may contribute to oxidative damage. The objective of this study was to observe the changes in activities of antioxidant enzymes (superoxide dismutase-SOD, glutathione peroxidase-GPx, catalase-CAT), lipid peroxidation and glutathione (GSH) levels occurring in kidney of 2, 10 and 20 months old rats, and to see whether these changes are restored to those of the two month old control levels rats after administration of saffron ethanolic extract. **Methods:**The aged rats (10 and 20 months) were given intraperitoneal injections of saffron (5, 10, 20 mg/kg/day) daily for 4 weeks. At the end of the experiment, animals were anesthetized with diethyl ether. The kidney samples were taken for biochemical analysis. **Results:** The results obtained in the present work revealed that normal aging was associated with a significant decrease in the activities of antioxidant enzymes, and GSH content with increase in lipid peroxidation level in kidney of aging rats. Saffron treatment ameliorated the oxidant/antioxidant balance.

Conclusion: Saffron could be a candidate to suppress the development of age-induced damage by protecting against oxidative stress and increasing antioxidant defenses in kidney.
Keywords: Saffron, Antioxidant, kidney, Aging, Rat

1742P

Comparison the effect of voluntary exercise with running wheels on BDNF levels in cortex and hippocampus of parkinsonianmalerats induced by 6-hydroxydopamine

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Background: Past researchsare showed training have positive effect on age disease such as Parkinson. Based on past researchs, the purpose of this study was investigation the the effect of pre-treatment of 12 week training on brain cortex and hippocampus BDNF level in rats that induced neurotoxic intraventricular injection of 6-hydroxydopamin. **Methods:** 32 rats were divided into four groups: healthy control, Parkinson-control, healthy training, Parkinson-training .training subjects trained with running wheels for 12 week. To induce Parkinson, 6-hydroxy dopamine (6-OHDA) was administered intracerebrovenricular (ICV) by a stereotaxic apparatus. BDNF level in the cortex and hippocampus were measured by ELISA. Data was analyzed with one-way analysis of variance (ANOVA). **Results:** 6-OHDA cause to decreasing cortex BDNF in Parkinson-control compared with healthy controls (P=0.02). Cortex BDNF level of Parkinson-training group were different with Parkinson control (P=0.03). Healthy control and Parkinson-training groups has not significant difference in level of cortex BDNF (P=0.640). 6-OHDA reduced BDNF protein level in the hippocampus of parkinsonian-control subjects (P=0.01). Hippocampus BDNF level of Parkinson-training group and Parkinson-control has not significant difference (P=0.98). **Conclusion:** Pre-treatment with voluntary training can prevent from reduceing of BDNF level, so can protect cortex neurons against lesion 6-OHDA toxicity. It can increase BDNF level of hippocampus but cannot protect hippocampus neurons against lesion 6-OHDA toxicity. It is possible because hippocampus is most vulnerable section of brain to oxidative stress, Probably more than other parts of brain affected by 6-OHDA toxicity and most has been destroyed.
Keywords: Voluntary training, 6-hydroxydopamine, BDNF, Cortex, Hippocampus

1939P

The effect of eight week aerobic exercise in the morning and evening on immune system of elite wrestlers

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Background: The aim of this research is to compare the effects of eight week of aerobic exercise (bruce protocol) in the morning and afternoon on the white blood cells of elite wrestlers.

Methods: The samples of this project include 32 wrestlers of Azad university club , in four group percapita ten groups experiment and Control, which they participated in bruce Test (aerobic exercise) in the morning and afternoon shifts. Blood Samples conducted on the sample before and right after the test session one and 24. The blood sample tested in the laboratory in order to determine the measure of safety factors existing in the blood (white

blood cells). Analysis of the samples accomplished with using the inferential statistics (Levin test, t test correlated and test F in p≤ 0/05 in software called spss-18. **Results:** Depending on the results derived from morning and evening groups that have done the aerobic exercises, there was a significant in the amount of white blood cells. This increase may due to use of independent variable (aerobic exercise and biological rhythm) on persons. **Conclusion:** It seem that the profile of human immune system's reaction to physical exercises needs further studies and evaluation of effective variables.

Keywords: Biological rhythm, bruce protocol, Leukocyte, white blood cell, elite wrestlers

3334P

Physical activity and Immunoglobulins: Sardasht-Iran Cohort Study

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Background: Sulfur mustard (SM) cause long term disorders in different organ of exposed people including lung, skin, eyes and immune system. Considering to the impact of physical activity on the immune responses and the various responses according to the severity and duration of activities in SM-exposed subjects, this study was performed to clear the association between physical activity and serum levels of immunoglobulins (Ig) including IgM, IgA, IgE, IgG as a part of Sardasht-Iran Cohort Study (SICS). **Methods:** In SICS, 372 male participants who were exposed to SM 20 years earlier were compared with 128 unexposed age-matched controls. At the time of study (2007), Physical activity was assessed by Global Physical Activity Questionnaire and at the same time, the sera were isolated, labeled and aliquots were kept frozen in -80°C. Serum levels of the immunoglobulins (Ig) including IgM, IgA, IgE, IgG were measured using quantitative Elisa method. **Results:** there were no significant association between physical activity and Igs level in control group. The higher level of IgM ($P<0.05$) and lower level of IgE ($P<0.05$) in SM-exposed subjects following heavy physical activity were seen. **Conclusions:** Regarding to the alteration of Ig levels by physical activity in SM-exposed population, physical activity programs may be a goal to achieve a regulation in some immune parameters.

Keywords: Physical activity, Immunoglobulin, Sulfur mustard

3065P

The comparative study of effects continues and interval training on HSP70 levels in healthy mice

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Background: Heat shock protein70 (HSP70)is a chaperone that maintains protein conformation

during heat stress. It has recently been observed that HSP70 may be released from cells in response to increased energy demand(e.g. exercise) and/or oxidative stress. they are know to convey protection against protein denaturation and a subsequent immediate stress .Also, this protein is a potent anti-cancer immune response .Since HSP70 levels should change in response to exercise training as types of stress ,we have investigate the effects of continues and interval training on Hsp70 levels in healthy mice. Continues training protocol was done for 6 weeks at 25% to 75% vo_2max and interval training protocol was done for 8 weeks at 20% to 55%vot_2 max between 1 until 10 interval rep *1 min. Blood samples were collected after protocol. The levels of Heat shock protein 70-kDa (Hsp70) was measured with ELISA method, and the resulting data was analyzed with SPSS 10 statistical software **Result**: Data analysis showed that the Hsp70 levels in interval group was decreased (P=0.459). but in continues group was increased (p>0/05). **Conclusion:** Based on the result of this research ; doing interval training lead to decrease Hsp70 levels and doing continues training give rise to increase Hsp70 levels. Therefore doing interval training can be effective in improving cancer treatment as supplement in addition other treatments.

Keywords: Interval training, Continue training, Hsp70

1727P

The effect of incremental exercise on Some Immune and Inflammatory Markers in pediatrics: the relationship between immune and inflammatory markers

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Background: There are many unanswered questions in associated with mechanism of exercise on inflammatory and immune markers of pediatrics cardiovascular diseases. The aim of this research was investigation Some of Immune and Inflammatory Markers following incremental exercise in pediatrics and the relationship between immune and inflammatory markers. **Methods:** This study was a semi-experimental research and thirty healthy non-active pediatrics boys with the age range of 8-10 years old volunteered to participate in the research after having expressed their consent through a consent form. Blood and salivary sample were collected in the two stages; pre of GXT (Graded exercise test) exercise test (grade: 5%, speed: 12 km/h, time: 20 minutes), immediately after exercise test. ELISA method was used for measurement of salivary immunoglobulin A, C-Reaction protein and fibrinogen serum levels, also paired t test and Pearson correlation coefficient methods used for statically analysis. **Results**: S-IgA, Fibrinogen and CRP serum concentrations were significant increased immediately after of exercise in exercise group ($p< 0.05$). A Significant relationship was observed between CRP and fibrinogen serum (p=0.012), CRP serum and S-IgA (p=0.022), Also fibrinogen serum and S-IgA (p=0.034) in exercise group. However, were reported no significant changes in the all stages of exercise in the control group. **Conclusion:** This study indicates that incremental exercise can increase S-IgA, C-reactive protein and fibrinogen levels in non-active pediatrics, So that incremental exercise may be increase cardiovascular risk factors in non-active Pediatrics.

Keywords: Immunoglobulin A, Fibrinogen, CRP, Pediatrics, Incremental exercise

1750P

Independent to selenium the majority of the old people has no detectable serum IL-10 limiting its application in evaluation of the cardiovascular disease.

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Background: Aging is a period of human life that the effect of risk factors for Cardiovascular Disease (CVD) reaches into maximum levels.Studies showed IL-10 has a balancer role in atherosclerosis.Anti-oxidant defense could be partner for IL-10 to diminish hazardous effect of atherosclerosis. In this study the serum levels of IL-10,lipid profile, selenium and total anti-oxidant capacity were assessed to investigate their association with CVD in elderly. **Methods**: serum prepared from258 old people (144 healthy and 114 people with heart diseases). IL-10 level was determined by ELISA. Selenium was measured by Atomic Absorption Spectrometry. Total plasma antioxidant activity was assessed by FRAP test. **Results:** Mean of IL-10 in patient and control groups was 3.33±2.38 and 9.48±7.92 pg/ml, $p<0.05$ if only the subjects with detectable IL-10 taken in to account.Because the most people from both groups had no detectable serum IL-10(91.2% and 90.3% for patient and control group respectively) this difference was not totally significant.There was no significant difference between two groups for serum selenium levels. However we found Positive correlation between selenium and LDL-cholesterol ($P< 0.01$). There was also a significant correlation between Frap test and Triglyceride ($P< 0.05$). Mean of lipid profile in both groups was equal. **Conclusion:** Despite the healthy old people produce significantly higher levels of IL-10, this cytokine is not detectable in majority of these individuals limiting its application in evaluation of CVD in these subjects. Also association between selenium and LDL-cholesterol makes this trace element as a possible risk factor for CVD in elderly.

Key word: Interleukin 10, Selenium, Lipid profile, Cardiovascular Disease

2765P

Inflammatory responses to high-intensity exercise in active women

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Background: Exercise intensity and duration influences on immune function. As a high intensity exercise may be threats immune system by changes in inflammatory markers like IL-6, TNF-α,C- reaction protein and stress hormones. **Methods**: 12 young healthy women (age 23.5±4.1) volunteered for participating in high-intensity exercise, including the Ellestadsport

protocol and were investigated for the presence of IL-6, TNF-α, CRP and cortisol hormone by ELISA. Blood samples were taken in a week before of exercise test, immediately and 1 hour after the end of the exercise. **Results and Conclusion**: The Paired t-test and the paired comparison data showed that the amounts of IL-6, TNF-α, CRP and cortisol hormone a week before of high intensity exercise and before exercise test were not significant (P≥ 0·05).The result showed that the concentrations of IL-6, TNF-α and cortisol hormone one week before beginning of exercise that were compared with before, immediately and 1 hour after high intensity exercise were increased (P≤ 0·05). But the levels of CRP before of high intensity exercise in comparison with1 hour after the exercise test was increased (P≤ 0·05). There was no significant difference in the other stages (P≥ 0·05). Data suggest that high-intensity exercise increased some inflammatory cytokines and cortisol hormone in active girls.

Keywords: IL-6, TNF-α, CRP, Cortisol hormone, High-intensity exercise, Active girls

1722P

Effects of aerobic exercise on per1 gene expression in middle-aged men: the role of per1 gene in cellular immune

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Background: Circadian ClockGenePer1 one of the most important genes at mammalian biological clock that expressed in supra chiasmatic nucleus (SCN) and peripheral tissues. And plays an important role in Immune cells Function and circadian rhythm. The aim of this study was effects of aerobic exercise on per1 gene expression in middle-aged men and the role of per1 gene in Immune system. **Methods:** Thirty middle-aged men with an age range of 40-50 years, randomly in the exercise (n=15) and control groups(n=15) were enrolled in this study. The exercise group performed aerobic exercise training (run on the treadmill) up to 65% intensity of training heart rate, 30 minutes in one session. Blood sampling were performed from the two groups before, immediately after and 12 hours. Real-time PCR method used for per1 gene expression. The research data were analyzed using Repeated-measures ANOVA ($p<0.05$). **Results:** per1geneexpression in the exercise group compared to the control group at the time of the afternoon showed a significant increase ($p<0.001$). Also, between two groups observed a significant difference inper1 gene expression in the morning hours ($p<0.025$).At all times of circadian per1 gene expression levels was significantly higher in the exercise group compared tothe control group ($p<0.001$). **Conclusion:** The investigation results show that moderate-intensity aerobic exercise causes the change inper1 gene expression of middle-aged men at different times of day and night. So that this Changesmay behelps tomaintain cellular circadian rhythm, and improve cellular immune Function.

Keywords: per1, cellular immune, Gene expiration, Physical activity

1743P

Pre-treatment effects of voluntary training on BDNF, SOD and MDA in the hippocampus of parkinsonian rats induced by 6-hydroxydopamine

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Background: Previous researchs are showed physical activity have positive effect on age disease such as Alzheimer and Parkinson. Based on past researchs, the purpose of this study was investigation the the effect of pre-treatment of 12 week voluntary training on BDNF,SOD and MDA in the hippocampus of rats induced by 6-hydroxy dopamine. **Methods:** 32 rats were divided into four groups randomly: healthy control, Parkinson-control, healthy training, Parkinson-training .training subjects trained with running wheels for 12 week. To induce Parkinson, 6-hydroxy dopamine (6-OHDA) (dissolved in saline) was administered intracerebrovenricular (ICV) by a stereotaxic apparatus. BDNF level in the hippocampus were measured by ELISA. To assesses the extent of MDA quantify Hadley and drapr method dual heating was used. Determination of SOD activity was evaluated based onkono method. Data was analyzed with one-way analysis of variance (ANOVA). **Results:** 6-OHDA reduced BDNF protein level in the hippocampus of parkinson-control subjects (P=0.01). Hippocampus BDNF level of Parkinson-training group and Parkinson-control has not significant difference (P=0.98). Voluntary wheel running significantly prevent decrease of SOD levels in Parkinson rats (P=0.001). but it can not prevent increase of MDA in parkinsonian rats. MDA level increase in Parkinson-training (P=0/005).**Conclusion:** It is possible because hippocampus is most vulnerable section of brain to oxidative stress, significantly affected by the oxidative stress induced by 6-OHDA. Appears to be a threshold level of exercise is needed to establish a protective effect against Parkinson disease.

Keywords: Voluntary exercise, Parkinson, BDNF, SOD, MDA

2069P

The effect of short term altitude exposure with recreational physical activity on IL-6 and TNF-α

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Background: Exposure to both altitude and hypoxia situations cause to impair homeostasis and cause some changes in body (Mazzeo, 2005). Residence in altitude and exposure to hypoxia are considered as preparation procedures for those who have to exercise in altitude. The aim of the present study was to determine the effect of short term altitude exposure with recreational physical activity on IL-6 and TNF-α. **Methods:** 12 healthy non-climber men participated in our study, which had no history of mountaineer and disease (such as cardiovascular, immune and hormonal diseases). The residence place was zard-kuh, located in the Zagros Mountains in the Chaharmahal and Bakhtiari Province of Iran with 4000 meters high above the sea level. The blood samples with a dose of 5cc were drown at the beginning and at the end of one week recreational physical activity. We used ELISA to measure bothIL-6 and TNF-αconcentrations. The significant level was $p<0.05$. **Results:** The results of study showed that one-week short

termresidence in altitude with recreational physical activity is followed by a changed pattern of the secretion levels of IL-6 and TNF-α (a decrease in IL-6 and an increase in TNF-α), although those changes were not significant ($p<0.05$). **Conclusion:** According to the results of present study, it seems that short term residence in high altitude or low intensity physical activity do not induce a significant change in secreting of IL-6 and TNF-α pro-inflammatory cytokines. So, more researches need to be done to evaluate the different time periods and workout intensity for demonstrating this hypothesis.

Keywords: altitude, immune system, physical activity

2024P

Physical activity and immunosenescence

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Background: Ageing is a complex process that has a negative impact on the development and function of the immune system. These age-related defects range from defects in the haematopoietic bone marrow to defects in peripheral lymphocyte migration, maturation and function. The gradual deterioration of the immune system brought about by the natural process of aging is termed immunosenescence. This age-associated immune deficiency involves both the individual's capacity to respond to infections and the development of long-term immune memory, especially by vaccination. Immunosenescence is considered a major factor contributing to the increased frequency of morbidity and mortality among the elderly. This type of immune deficiency involves alterations at the cellular and molecular levels that affect both innate and adaptive immunity, leading not only to increased incidences of infectious disease morbidity and mortality but also to heightened rates of other immune disorders such as autoimmunity, cancer, and inflammatory conditions. Current empirical evidence suggests physical activity may be an effective and easy strategy to implement for counteracting immunosenescence. Long-term, moderate physical activity interventions in geriatric populations is associated with reduction in infectious disease risk, increased rates of vaccine efficacy, and improvements in both physical and psychosocial aspects of daily living. Exercise is also therapeutic when pharmacological treatment is unavailable, ineffective, or inappropriate. Exercise impacts multiple aspects of immune response including T cell phenotype and proliferation, antibody response to vaccination, and cytokine production. The use of physical activity programs by the healthcare community can help improve the health of geriatric populations.

Keywords: Exercise, Immunosenescence

1933P

Different effects of 10 weeks of Concurrent, strength and endurance training on some immune parameters in inactive male

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Background: The present study investigated the effects of 10 weeks of resistance, endurance and Concurrent training (combination of resistance and endurance training) selected on TNFα and

IgA. **Methods**: The subjects included 28 male physical education students that were randomly classified to the three group (n = 10), resistance training (n = 9) and a parallel exercise group (n = 9). Exercise program was three times a week for 10 weeks. For measurement of TNF-α and IgA by kits with The method ELISA was used. For this study used descriptive statistics and t- dependent. **Results**: Results were analyzed by Tuky-post hoc test. Results showed that aerobic training caused a significantly change in TNF-α and IgA Serum of subjects. But results in resistance training didn't show that a significantly change in the percentage level of TNF-α and IgA. Also Results in parallel exercise group showed no difference in the before and after training. **Conclusion**: According to these results, we can say that the effect of resistance training on changes in some immunity components was important, and this type of training is enhanced acquired immune.

Keywords: Immune factors, Endurance training, Strength training, Concurrent training

3034P

Influence of carbohydrate ingestion on the immune response following acute resistance exercise

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Background: Despite the numerous studies controversial results exist in carbohydrate ingestion on the immune responses to acute resistance exercise. The aim of this study was to determining the effects of carbohydrate ingestion (CHO) on the total leukocyte, neutrophil, and monocyte concentrations response following acute resistance exercise in football players. **Methods:** Seventeen male football players completed a randomized, double-blind protocol with exercise separated by 7 days. The exercise consisted of two warm-up sets of 10 repetitions of that exercise at 45% and 55% of 1-RM and four sets of 10 repetitions at 65% of 1-RM with rest periods of 1 min were used between sets of exercises. Subjects consumed 1.0 g ·kg body mass^{-1} CHO or an equal volume of placebo (PLC) 10 min prior to and 10 min following acute resistance exercise. Blood samples were obtained and the levels of serum cortisol, lactate, total leukocyte, neutrophil, and monocyte were determined at rest, immediately post exercise, and at 1.5 h of recovery. **Results:** Acute resistance exercise program led to significantly increases in plasma cortisol, lactate, total leukocyte, neutrophil, and monocyte concentrations compared to baseline ($p < 0.05$); these increases did not differ significantly between the CHO and PLC groups. The Lymphocytes values returned to normal by 1.5 h post exercise ($p <0 .05$). However, the lymphocytes values were lower in the CHO group compared to the PLC group, and this difference was statistically significant ($p <0 .05$). **Conclusion:** These data support a possible effect of carbohydrate supplementation on lymphocytes concentrations following acute resistance exercise.

Keywords: Cortisol, Immune function, Weight training

2894P

Comparison the effect of two types of exercise in two different intensities on sensitivity inflammatory marker measure predictor for cardiovascular disease (hs-CRP) in athletes and non-athletes

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Background: Inflammatory markers such as C-reactive protein (CRP) and inflammatory cytokines contribute to cardiovascular disease. The purpose of this study was to evaluate two different exercise's intensities in CRP inflammatory index in athletes and non-athletes. **Methods:** In this study, two groups of athletes (10 cases) and non-athletes(10 cases) participated voluntarily. Each group exercise program included two maximums (30 minutes 65 percent of the intensity (VO2max) and 6 minutes repeat 3 with the 1.5 minute rest among each repetition intensity(85 percent VO2max) on a treadmill. Blood samples were collected before, immediately and 24 hours after each activity and hs-CRP levels were measured using ELISA method. Data was analyzed using the statistical method of paired T groups and independent T for differences between groups in the level of $P \leq 0.5$. **Results:** No change has been seen after practice sub-maximal in the amount of CRP. Also in comparison of groups there was no significant difference between the two groups in sub-maximal exercise. Maximal exercise caused significantly increasing CRP significantly in non- athletes after 24 hours. Furthermore, there was no significant difference between athletes groups. In comparison of the groups, significant difference in amount of the CRP, 24 horses after exercise have been seen. **Conclusion:** The results showed significant difference after exercise between CRP response in athletes and non-athletes groups. It is possible that maximum level of CRP is resulted of low physical activates and low level of fitness in non-athletes, which may lead to Cardiovascular disease.

Keywords: Athletes, CRP, ELISA, Cardiovascular disease

Humoral Immunodefficiency

Oral Presentations:

2868O

Inflamatory bowel disease in 47 CVID patients in allergy and immunology department of Rasool E Akram hospital in Tehran

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Bacground: CVID is the most common clinical primary antibody deficiency, with prevalence of 1n 25000 to 50000 people. CVID has been reported to be the most common symptomatic PID in Iran. CVID is a heterogeneous disease with infectious, autoimmune and autoinflamatory features. GI manifestations of CVID are infectious or noninfectious. IBD is a common noninfectious GI complication of CVID with a prevalence of 3 to 32 percent of patients in different CVID populations. The pathogenesis of CVID colitis differs from classical IBD in normal population .CMV collitis predisposes the patients to IBD. **Methods:** We report IBD cases in 47 CVID patients in Rasool E Akram hospital in Tehran. Patients have been diagnosed as CVID with the PAGID-ESID diagnostic criteria in our department or referred from other clinics for follow up and treatment. Diagnosis of IBD has been made by an expert gastroenterologist with the means of endoscopy and biopsy. We had 9 IBD cases (19 %) among 47 CVID patients. Mean age of the onset of CVID symptoms in these cases was 15 years. One case has been died because of severe hepatic failure and two cases has develop malignancy in the course of disease.

Conclusion: IBD and other granulomatos complications of CVID are common and clinicians should be aware of them. This predisposes patients to malignancy and poorer prognosis as many investigators say.

2853O

Update on Classification of Predominantly Antibody Deficiency

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Primary antibody deficiencies (PAD) are the most common types of primary immunodeficiency

diseases, ranging from a severe reduction of all serum immunoglobulin classes and absent number of B cells to a selective antibody deficiency with normal serum immunoglobulin. Recurrent infections, chronic inflammation, autoimmunity, and cancers are the main manifestations of the affected patients. Recent advances in understanding of the genetics of B cell development have led to identification of the genes involved in PAD. Several gene mutations have been identified in association with defects in early B-cell development, including *BTK*, *IGA*, *IGB*, λ*5*, μ heavy chain, *BLNK*, *PIK3R1*, and the E47 transcription factor, which lead to low number of B cells and agammaglobulinemia. Indeed a number of genes that play a key role in class-switch recombination (CSR) and somatic hypermutation (SHM) are *CD40L*, *CD40*, *IKBKG*, *AID*, and *UNG*, which their mutations lead to low serum levels of IgG, IgA, and IgE in association with normal or increased IgM levels. And finally, terminal stages of B cell development are controlled by *TACI*, *BAFF-R*, *TWEAK*, *MSH5*, *CD19*, *CD20*, *CD21*, and *CD81*, which lead to hypogammaglobulinemia. As differential diagnosis among subgroups of PAD is important, a clear algorithmic approach to a patient with hypogammaglobulinemia is needed.

3195O

Evaluation of humoral immunity in patients with organic academia

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Background: Organic acidemias are a group of metabolic diseases caused by abnormal aminoacid catabolism, leading to accumulation of Organic acids in the body. Some of these patients seem to be prone to infections and suffer from recurrent infections and hospitalizations. We evaluated humoral immunity in some patients with organic academia. **Methods:** In 31 patients with different organic acidemias, humoral immunity was screened for deficiencies. For this purpose, serum Ig M, IgG, IgA, IgE, Isohemagglutinin titer, and antibody response to vaccines (diphtheria and tetanus) were measured. The sampling was done when the patients were not in acidotic phase of their disease. **Results:** Among patients, 11 had maple syrup urine disease, 10 methylmalonic academia, 5 isovaleric academia, 4 glutaric academia type I, and 1 propionic academia. In screening of humoral immunity, 2 patients (6.45%) had low serum IgM for their age. Serum isohemagglutinin titers were less that 1/8 in two other patients. Serum IgG, IgA, and IgE were in normal range for all the patients, but two patients had low anti diphtheria and anti tetanus antibodies (IgG) (less than 0.1 IU/mL). There was no significant relationship between any of these deficiencies and the type of the acidemias. **Conclusion:** The initial screening of humoral immunity in non-acidotic phase of organic academia shows a probable defect in some patients, which should be evaluated more by advanced tests.

2380O

Alterations in CD19+ B lymphocytes and relating to total body surface area (TBSA) following thermal burn injury in Iranian patients.

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Background: Thermal burns injury is associated with dysfunction of host defense system. **Methods:** The present study used monoclonal antibodies and flowcytometric immunofluorescence analyses to investigate changes in number and phenotype of CD19+ B lymphocytes and correlation between this cells level and total body surface area (TBSA) thermal burn with 30- >70 % at third and seventh day postburn of 67 adult patients . They were divided into three groups according to the TBSA%. Group 1: (n=29), 30-50% TBSA, Group2: (n=23), 50-70% TBSA and Group3: (n=15) 70% and more TBSA. **Results:** Compared to normal controls , patients and a significant (P<o.o5) increase of CD19+ B cells number up to 3 and 7 days postburn. In patients with TBSA 30% - 70% and more at three days after burn injury, mean percentage of CD19 + B lymphocytes gradually were significantly (P<o.o1) increased when compared level of cells in each group of burn size respectively. The mean percentage of CD19+ B cells appeared to be slightly higher 7 day postburn with injuries affecting 30-50% of their TBSA when compared with third day postburn. Whereas, patients with greater burn size of (50-70 % and more) TBSA % as a groups , showed insignificant reduction in CD19+ B cells at 7 days postburn. **Conclusion:** TBSA % can reflect postburn lymphocytes activation.

Keywords: Thermal Burns, CD19+ Marker, Flow cytometry, TBSA %, Peripheral blood

2989O

Clinical and immunological features of common variable immunodeficiency in Isfahan

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Background: Common Variable Immune Deficiency (CVID) is the common primary immunodeficiency of clinical significance. The aim of study was to evaluate the clinical and immunological characteristics of patients by CVID in Isfahan. **Methods:** Base on retrospective study, we collected data from 25 patients from the Isfahan University of Medical Sciences between 1376 and 1392. The patients were diagnosed according to the Immunodeficiency Disease criteria. **Results:** The age at onset was between 1 to37 years and diagnosis was between 2 to 39 years. The median levels of IgG, IgM, and IgA were 96, 10, and 2 mg/dL, respectively. The percentage of CD19+ B cells was 8.35%, CD20+ 8, CD3+ 80.88,CD4+ 28.72,CD8+ 38.88,CD16+CD56+ 11.08. Sinusitis (79%), pneumonia (85%) and acute otitis media (40%) were the most common manifestations. In addition, Bronchiectasis was showed about 25% and Autoimmunity) Thrombocytopenia, RA)were 33%. Allergic symptoms were present in 80% of patients. All the patients received Intravenous immunoglobulin (IVIG) as a fundamental part of the treatment at a mean dose of 500 mg/kg. **Conclusion:** CVID patients present with a range of infections, immunological dysfunctions like autoimmunity, allergy and malignancy and different component of hummoral and cellular immunity defects could be involved.

Keywords: CVID, Immunodeficiancy, Autoiimunity, Thrombocytopenia

Poster Presentations:

2279P

Salivary immunoglobulin a response to incremental exercise in non- active pediatrics

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Background: Immunoglobulin A is one of the most important antibodies and anti-pathogenic and first line of defense known in the immune system that plays great role in deal with the topical infections in pediatrics. The aim of this research was investigation salivary immunoglobulin A (S-IgA) response to incremental exercise in non- active pediatrics. **Methods:** thirty healthy non-active pediatrics boys with the age range of 8-10 years old volunteered to participate in the research after having expressed their consent through a consent form. Salivary sample were collected in the three stages; pre of GXT (Graded exercise test), immediately after and 1h after Graded exercise test (GXT). ELISA method was used for measurement of (S-IgA), also used independent t test and one-way ANOVA methods for statistically analysis. **Results**: S-IgA levels immediately after exercise showed a significant increase in the exercise group compared with the control group and baseline condition ($p< 0.05$). A significant increase showed in S-IgA levels 1 h after exercise in exercise group compared with baseline conditions and the control group ($p< 0.05$). **Conclusion:** The incremental exercisecausesresponse increase S-IgA levels innon- active pediatrics. So that it can lead to immune disturbance and increase susceptibility to infectious diseases in pediatrics.

Keywords: Immune system, Pediatrics, Exercise

1865P

Griscelli syndrome (Partial albinism with immunodeficiency): Report of a case

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Background: Partial albinism with immunodeficiency (Griscelli syndrome) is an uncommon disorder characterized by pigmentary dilution and variable immunodeficiency. Features include a silvery-gray sheen to the hair, large clumped melanosomes in hair shafts, and prominent Mature melanosomes in cutaneous melanocytes with sparse pigmentation of adjacent keratinocytes. Immunologic abnormalities most often include impaired natural killer cell activity, absent delayed-type hypersensitivity, and impaired responses to mitogens. **Case report:** A 2 years old girl that who presented at 4 month with lack of strength to hold head steady while sitting and silvery hair. The diagnosis of Griscelli syndrome was suggested by the Mashhad immunology service at 1year of age after long period of fever and confirmed by microscopic hair examination. Complete blood cell count at year1 of life revealed a white blood cell count of 7500 cells/mm3 hemoglobin 11.2 g/dl Hematocrit 34.4% platelets of 271 ´1000/ dL and an absolute neutrophil count 2700. tuberculine test was 12mm total IgG level was 234mg/dl IgM level was 84mg/dl and IgA level was 42mg/dl IgE level was 67IU/ml tetanus Ab

and diphtheria Ab level was in normal range and hemagutinin Anti B level was 1/8 the result of NBT test was 70%. karyotype was normal(46XX) . Axial brain CT scan had no abnormality. Abdominal sonography was normal except two small nephrolithias and no hepatosplenomegaly was ditected. metabolic disorders study was normal and Immunophenotyping flow cytometry was normal for CD3, CD4 ,CD8, CD19, CD16+56. Pathologic examination of hair: Small and large clumps of melanin in irregular polarized light microscopic examination of hair is normal. Morphological finding suggestive for Griscelli syndrome (Type I) or Elejalde disease.

2440P

Not every elevated IgM, is hyper IgM syndrome –Report of an instructive case and review of literature

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Case description: A Three years old boy with high IgMlevel and theoriginal diagnosis of hyper IgM syndrome were referred to our center for monthly IVIG therapy. He had experienced respiratory infections and failure to thrive from 1 year old. His parents were first cousins and he was the only child. We noticed his oral feeding is very slow and his gait subtly ataxic suspected of ataxia telangiectasia. Therefore we measured alpha fetoprotein as a screening test that was high. Other laboratory tests confirmed this diagnosis His gait deteriorated slowly progressive and there was not obvious telangiectasia until age 5.

Discussion: when we encounter high levels of IgM during our investigation for definitive diagnosis of immunodeficiency disorders first we should rule out the possible causes of elevated IgM such as congenital rubella syndrome, waldenstrom·sgamopathy, ataxia telangiectasia and…

Ataxia telangiectasiasometimes is misleading diagnosis. Occasional patients may not exhibit overt ataxia in early years and some may develop telangiectasia in later years if at all.In this time period they may be misdiagnosed as hyper IgM syndrome because of elevated IgMdue to monomeric inefficacious IgM in these patients. We need to measure alpha fetoprotein in any unexplained ataxia to rule out or confirm this diagnosis.

Keywords: hyper IgM syndrome, Ataxia telangiectasia, differential diagnosis

3057P

Case report of CVID patient with GLILD (Granulomatosis lymphocytic interstitial lung disease) follow by entropathy complication

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Background: Common variable immunodeficiency (CVID) is the most common and clinically most important severe primary antibody deficiency. This diagnostic category represents a heterogeneous group of disorders. Patients can now be categorized into 4 distinct clinical phenotypes included infections only, autoimmune, lymphoproliferation and enteropathy. We report a boy with allclinical complication that uncommon for CVID patient. In November 2011 a 5 year oldboy with consanguineous parents and known CVID diagnosed in 2009admitted.

He had been treated with intravenous immunoglobulin replacement therapy monthly. He had previously been diagnosed as sinusitis which was treated with long term antibiotics. He was admitted to our unit complaining of productive cough fever and progressive dyspnea for 5 months. Chest radiography had bilateral pulmonary patchy infiltration, and high resolution computed tomography (HRCT) Showed inter lobular septal thickening and ground glass opacity, there are multiple ill-defined parenchymal nodule. Bronchoscopic lavage did not identify any bacteria, mycobacteria, virus or fungi on cultures. Histological examination showed interstitial fibrosis and mild lymphocytic infiltration. A diagnosis of GLILD (Granulomatosis lymphocytic interstitial lung disease) was made and he was treated with prednisolone and cyclosporine. The patient's symptoms improved and there was resolution of crackles and reduction in the extent of nodularity and ground glass opacifications on the HRCT scan. In summer 2012 the patient complain of diarrhea. Workup for infectious cause was negative. With diagnosis of entropathy, treatment with prednisolone continued. **Conclusion:** Delay indiagnosis and pre-existing structural lung disease with impaired lung structure or function are risk factors for further progression of lung disease, and, this may occur, despite adequate levels of immunoglobulin replacement and the reduction in the occurrence of pneumonia.

2873P

Viral infections in 47 CVID patients in allergy and immunology department of Rasool E Akram hospital in Tehran

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Background: CVID is a heterogeneous primary immune deficiency with infectious, autoimmune and autoinflamatory features. It is most common symptomatic PID in Iran, with prevalence of 1 in 25000 to 50000 people. CVID has been divided into some phenotypes to produce more homogenized subpopulations. CVID is not a pure Ab deficiency .and because of both abnormalities in Tcell and innate immunity in combination with B cell dysfunction these patients are predisposed to viral and opportunistic infections.

Method: prevalence of viral infections is reported in 47 CVID patients registered in Rasool E Akram hospital in Tehran. Patients have been diagnosed as CVID with the PAGID-ESID diagnostic criteria in our department or referred from other clinics for follow up and treatment. Diagnosis of viral germs has been made by clinical signs, pathological significances and in some cases by PCR.

Cases: 9 patients (19%) had problems with viral infections. Infections occurred befor diagnosis of CVID in some cases or after that. Four patients (8.5 %) had problems with wart. Sever mucocutaneus HSV infection has occurred in 3 (6 %), recurrent zona in one (2 %) and CMV infection as colitis or pneumonitis in 3(6 %) patients. Sever progressive lethal CNS infection with JC virus occurred in one patient.

Conclusion: evidences show that CVID is not a pure B cell defect, and we should be aware of opportunistic and viral infections that in some cases may be fatal.

3157P

The patient CVID with multiple granulomatosis

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Background: CVID is a clinically and immunologically heterogeneous disorder characterized by hypogammaglobulinemia, recurrent bacterial infection,, and an increased incidence of malignant and autoimmune disease. The underlying pathogenesis is due to a failure of terminal B-lymphocyte differentiation, resulting in immature B-cells that are unable to transform into antibody-secreting plasma cells. Thus the hallmark of this disorder is a decrease in serum immunoglobulin levels, with IgG and IgA being more severely affected than IgM incidence of malignant and autoimmune disease. CVID has an estimated prevalence of about 1:50,000. The typical patient is between 20 and 40, and males and females are equally affected. About 20% of patients are diagnosed in childhood. Departement of Allergy and Immunology,Hazrat-e rasool akram Haspital,Iran university of medical science have 47 patients with cvid. Case:This paper describes a case report of a 15-year-old girl, who was admitted 6times during the previous 2 years with recurrent diarrhea, herpetic gengivoestomatite, pneumonia, FTT & seizure, the beginning of these manifestations was since she was 9 years old. In physical examination hepatosplenomegaly was detected. Laboratory evaluation showed decreased concentrations of two types of immunoglobulins: IgA=2g/L (L), IgG=450 g/L(L) [IgG-1 =87 (L), IgG-2=11(L), IgG-4=2(L)] Anti-D= 0.01 & Anti-T= 0.02Multiple & ALP was 469. Lymphocytes immune phenotypisation revealed decreased CD19=3 & CD20=4.5. granulomas, were diagnosed in lung ,liver & head during evaluations.The etiopathogenesis of CVID is not fully known. The main treatment consists of life-long immunoglobulin substitution in intravenous or subcutaneous form. **Conclusion:** CVID Common variable immune deficiency is primarily a disorder of B-cell differentiation; in some cases T-lymphocyte dysfunction may be seen as well. Patients display decreased antibody synthesis and a propensity for recurrent sinopulmonary disease. Non-caseating granulomas have been reported in 8%-20% of individuals with CVID. A syndrome similar to sarcoidosis can affect patients with CVID. It is characterized by noninfectious cutaneous granulomas, with underlying visceral granulomas of the lungs, liver, spleen, or conjunctiva in most patients. These cutaneous granulomas are nonspecific in patients with CVID and can appear as follows: Maculopapular rash and Infiltrated erythematous papules, plaques, excoriated papules, and ulcers. Granulomas, were in skin, lung, liver & head and another organ. The etiopathogenesis of CVID is not fully known. We describe a case of in a patient CVID with granulomas in lung, liver & head that treatment consists of life-long immunoglobulin substitution in intravenous. The patient cvid with granulomas is poor prognosis.

3258P

Immunological changes inPediatric patients withβ-thalassemia major

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Background: Beta-thalassemia major is one of major health problem in our country. Many

studies have confirmed the fact that, these patients have an increased susceptibility to infection diseases. In this study, we have assessed the humoral immune system in 110 thalassemic patients(age 6–16 years) by measuring their serum concentration of immunoglobulin IgG, IgM, IgA, IgE, Ferretin, C3 and C4 in order to find out a responsible immune defect. **Methods**: 110 β-thalassemia major patients were enrolled randomly from referrals to Jahrom Motahari clinic of thalassemia. The same number of case controls with matched age and sex were selected from healthy people without any history of recent or recurrent infections. Serum IgG, IgM, IgA, C3 and C4 levels were assessed using Single Radial Immunodiffusion (SRID). IgE and ferritin were assessed by ELISA. **Results**: Serum level of IgG, IgM and IgA were significantly higher ($P<0.01$) and those of C3 and C4 were significantly lower ($P<0.01$) in thalassemic patients than the controls. Considering the result of analytic tests, it was revealed that, thalassemia patients show much more increase in serum immunoglobulin levels as they get older. Splenectomized patients had higher serum IgG, IgA and IgE levels than non-splenectomized patients but had no difference in serum ferretin, IgM, C3 and C4. Serum ferretin level had no correlation with the changes of humoral immunity. **Conclusion**: These results can be due to the continuous exposure to antigens, repeated infections, chronic liver disease and splenectomy but not iron overload. The only probable cause of humoral immune deficiency found in these patients is a defect in serum complement levels.

Keywords: Thalassemia Major, IgG, IgA, IgM, IgE, C3, C4

Immunodermatology

Oral Presentations:

3191O

Association of serum IL18 and IL18bp levels and Pruritus in sulfur mustard exposed

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Background: Pruritus can be defined as an unpleasant cutaneous sensation that according our previous study, in the sulfur mustard (SM) exposed patient was seen significantly more often than in the control group.IL-18 is an important mediator involved in chronic inflammatory conditions such as cutaneous lupus erythematous, psoriasis and chronic eczema. The aim of this study was to evaluate the association of IL-18 and IL-18bp serum levels with Pruritus disorder in a SM exposed population 20 years after SM exposure. **Methods**: This study is a part of Sardasht – Iran cohort study (SICS) which is designed to evaluate long term complications of SM exposure. All participants were visited by clinicians and their skin was examined by specialists. The serum levels of cytokines were assessed by ELISA assay. **Results**: According to our results, the serum level of IL-18bp in the exposed group with Pruritus disorder was significantly higher than the control group. Furthermore a significant elevation in IL-18bp levels was found in the exposed subjects with Pruritus disorder compared to those exposed participants without Pruritus disorder. While, the serum levels of IL-18, in the exposed group with Pruritus disorder does not show a significant difference compared to those control and exposed participants without Pruritus disorder. **Conclusion**: The relationship between the IL18 and IL18bp and SM induced skin complications require further study, but it may be said that the imbalance betweentheIL18 and IL18bpcanat least partly to be effective in the skin complications caused by mustard gas.

Keywords: Sulfur mustard, itching, IL18, IL18bp, Skin

2712O

Hemorrhagic bullous lesions in a 4−year−old girl with Henoch−Schoenleinpurpura

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Background: Henoch−Schonleinpurpura (HSP) is an Immunoglobulin A mediated vasculitis and mainly affects skin, joints, gastrointestinaltract and kidneys. Skin lesions usually present as erythematous maculopapular, petechiae and purpura and often involve lower extremities and buttocks. Dermatological complications are rarely seen in HSP. Herein, we described a

4−year−oldgirl with typical skin lesions of HSP and hemorrhagic bullous lesions in her thighs. **Methods:** A 4−year−old girl was referred to Besat Hospital affiliated to Hamadan University of Medical Sciences because of arthralgia, abdominal pain and hemorrhagic bollous lesions in inner sides of both thighs especially in the right side. **Results:** Physical examination on admission revealed a body temperature of 37.3°C, pulse rate of 90 bpm., respiratory rate of 24/min and blood pressure of 100/60 mmHg. There were multiplepurpuric lesions in lower extremities, varying in size from 2 mm to 7 mm in diameter. The bullous hemorrhagic lesions were seen in inner sides of both thighs .The right knee was swollen and painful. The examination of the abdomen revealed no abnormality. The remainder of physical examination was normal. The color of urine was normal. Past medical history was unremarkable. Laboratory examinations were normal. Biopsy specimen of skin showed leukocytoclasis which helped to confirm the diagnosis of HSP. The signs and symptoms resolved with supportive therapy .The bullous lesions began to resolve within a few days. There have been no complications of HSP during 6 months of follow−up. **Conclusion:** Bullous hemorrhagic lesions in HSP are seen in children, rarely. They do not effect on the course of the disease. The presence of bullae may lead to diagnostic confusion.

Keywords: Bullous, child, Henoch−Schonleinpurpura

1854O

Common allergens in patients with skin allergic diseases in Bushehr: based on skin prick testreactivity

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Background: Skin allergic disease such as atopic dermatitis (eczema) and urticaria are prevalent in general population. Identification of prevalent allergens in each area has a very important role in the diagnosis and treatment of skin allergic disease. The aim of this study was to identify common allergens in patients with skin allergies in Bushehr. **Methods:** Skin prick test with common allergens including food and aeroallergens was performed to the patients that referred to allergy clinic of Bushehr university hospital with diagnosis of a skin allergic disease. **Results:** Among 837 patients referred to allergy clinic, 91 patients had eczema and 143 had urticaria. The prevalence of both eczema and urticaria in female was higher than male, significantly (P=0.001). The most common food allergens in patients with eczema were almond (56.6%), walnut (47.7%) and soybean (46.1%) Also, common aeroallergens in the patients were house dust mite (HDM) (63.7%), Russian tistle (57.7%) and Alternariaalternara (51.6%). In addition, in patients with urticaria, almond (58%), walnut (53.1%) and tomato (48.2%) were the most common food allergens. Meanwhile, the common aeroallergens in the patients were HDM (66.4%), Russian tistle (52.4%) and date palm (51%). **Conclusion:** Our findings indicated that almond and walnut are important food allergen in eczema and urticaria. SPT reactivity to HDM and weeds as aeroallergen were also seen.

Keywords: Atopic dermatitis, Urticaria, Skin Prick Test, Allergen

2082O

Down-regulated expression of Toll-like receptor 2 and 4 mRNA in peripheral blood monocytes from patients with vitiligo

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Background: Vitiligo is a rather common disease characterized by depigmentation of skin and mucosae due to the loss of melanocytes, most likely as a consequence of autoimmune phenomena. Recent studies have been shown that toll like receptors (TLR) involved in some inflammatory skin diseases such as psoriasis and atopic dermatitis. However, there is a paucity of data examining the expression of TLRs in vitiligo. Thus, in the present study, we examined TLR2 and TLR4 mRNA expression in monocytes of active vitiligo patients. **Methods:** We investigated the mRNA expression of TLR2 and TLR4 in freshly isolated monocytes of thirty one patients with newly vitiligo and thirty one healthy controls by Quantitative Realtime PCR. **Results:** Expression of TLR2 and TLR4 mRNA was significantly lower in vitiligo patients than in healthy controls ($p < 0.001$ and $P = 0.011$, respectively). TLR2 mRNA expression in the monocytes was positively correlated with the TLR4 mRNA in the patients and the control group ($rs = 0.619$, $p<0.001$ and $rs = 0.615$, $p = 0.003$, respectively). **Conclusion:** Our data show that active vitiligo is associated with a decreased expression of TLR2 and TLR4 mRNA. Down-regulation of TLR2 and TLR4 may be important in vitiligo pathogenesis.

Keywords: TLR2, TLR4, Vitiligo, Gene expression

2729O

Genetic variation in L-selectin might prone the individuals to atopic dermatitis

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Background: Atopic dermatitis (AD) is a common chronic or relapsing inflammatory skin disease, that often precedes asthma and allergic disorders. Chronic inflammation is the main pathogonemic feature of AD. Selectin adhesion molecules participate in the interaction between leukocytes and the endothelium, as well as in inflammatory cell recruitment. Genetic factors have an important influence on the risk of developing atopic disease. The aim of this study was to evaluate the association between genetic variants of L-selectin and susceptibility of AD. **Methods:** This case-control study recruited 122 patients with AD; aged 8.92±5.07 years old and 151 age, sex, and ethnic background matched healthy controls. Genomic DNA was isolated, and amplification of L-selectin 206 Phe/Leu polymorphic region was performed by PCR incorporating sequence-specific primers (PCR-SSCP) to distinguish the genotypes. **Results**: The frequency of the 206 Phe/Leu polymorphism was significantly more prominent in AD patients compared to the controls (27.9% 15.2%, p=0.003). Logestic regression analysis when fixed for covariates sex, age, self and familial histories of atopy revealed that the presence of Leu/Leu genotype increased the disease risk up to 2.75 (95% CI; 1.4-5.4). **Conclusion**: The higher frequency of L-selectin 206 Leu genetic variant in patients with AD than in control individuals suggests that the F206L polymorphism could make individuals more vulnerable to atopic dermatitis.

3216O

Down-regulated expression of Myd88 in peripheral blood monocytes from patients with vitiligo

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Background: Myeloid differentiation primary response gene 88 (Myd88) is a key adaptor protein in toll like receptors (TLR) signaling pathway. Recent studies have been shown that TLR signaling pathway via Myd88 involved in some inflammatory skin diseases such as psoriasis and atopic dermatitis. Vitiligo is a kind of dermal disease characterized by depigmentation of skin and mucosae due to the loss of melanocytes, most likely as a consequence of autoimmune phenomena. However, there is a paucity of data examining the expression of Myd88 in vitiligo. Thus, in the present study, we examined Myd88 expression in monocytes of active vitiligo patients. **Methods:** We investigated the expression of Myd88 in freshly isolated monocytes of thirty one patients with newly vitiligo and thirty one healthy controls by western blotting. **Results:** Expression of Myd88 protein was significantly lower in vitiligo patients than in healthy controls ($p< 0.01$). **Conclusions**: For the first time, our data show that active vitiligo is associated with a decreased expression of Myd88. Down-regulation of Myd88 may play an important role in vitiligo pathogenesis.

Keyw Words: Vitiligo, Monocyte, Myd88, Western blotting

2252O

Autohemotherapy in chronic urticaria:what could be the autoreactive factors and curative mechanisms?

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A subset of patients with chronic urticaria (CU) may have an autoimmune basis for their condition which is shown by a positive autologous serum skin test (ASST). So there could be some histamine releasing factors in their serum. Intramuscular application of autologous whole blood or serum, reportedly has a curative effect on CU patients. In this study we suggest some potential histamine releasing factors and the potential curative mechanisms of autohemotherapy.

Poster Presentations:

3262P

A cross−sectional hospital based study of clinical and immunological profile of systemic lupus erythematosus patients from emam Khomeini hostipal in Saghez, Kurdisestan, Iran

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Background: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder, the expression of which is greatly influenced by the combined effect of genetic, environmental, demographic and geographical factors. Various studies regarding clinical and immunological profile of SLE patients in India has been reported from a different region of India, especially from the urban area. We performed this study to understand the clinical and immunological profile of the SLE patients refered Saghez Emam Khomeini Hostipital. **Methods:** .All patients' records from 2012 to 2013 available with hospital having a discharge diagnosis of SLE and fulfilling the revised American College of Rheumatology criteria (1997) for SLE were analyzed regarding clinical and immunological profile. **Results:** We found 56 SLE patients out of 32.157 patients admitted in medicine department from 2012 to 2013 in the hospital record and included in the analysis. Nearly, 84% patients were female and 76% patients were under the age of 40 years. Common features present in these patients were immunological (91.6%), mucocutaneous (83.9%), hematological (72.4%) and renal (69.0%). Malar rash was the most common clinical feature presented in 71.3% patients followed by photosensitivity (63.2%) and oral ulcers (34.8%). Lymphopenia was the most common hematological abnormality present in 48.3%. Involvement of neurological, cardiovascular and respiratory system was found to be less common. Anti−nuclear antibodies were found to be positive in nearly97% patients. **Conclusion:** Analysis of clinical profile of hospitalized SLE patients shows that the disease is more common in female patients, especially during the child bearing age group. The present study shows high frequency of mucocutaneous, hematological and renal manifestation in these patients.

Keywords: Clinical and immunological profile, Systemic lupus erythematosus, Saghez

2888P

Topical application of Triamcinolone acetonide formulated in Volon® A Haftsalbe and Triadent® ointments for treatment of ulcers caused by Leishmania major

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Background: Triamcinolone acetonide is a synthetic corticosteroid used to treat various skin

conditions, relieve the discomfort of mouth sores and in nasal spray form, to treat allergic rhinitis. It is a more potent derivative of triamcinolone, and is about 8 times as potent as prednisone. In this study we attempt to evaluate the antileishmanial effects of the Volon® A Haftsalbe (from Germany) and triadent® (produced in Iran) in treatment of Cutaneous Leishmaniasis. **Methods**: For evaluation of the effects of these drugs, promastigotes of Leishmania major injected to the base of tails of forty BALB/c mice and after appearing the lesions, treatment began according to the below:10 mice treated with Glucantime® , 10 mice treated with Volon® A Haftsalbe , 10 mice treated with triadent® and 10 mice were reserved as positive control. Treatment was done twice a day, for 4 weeks. The diameter of the lesions was measured weekly post infection with calipers in two Dimensions, and the mean were determined. **Results:** data showed that the period of healing of Volon® A Haftsalbe group was similar to Glucantime® group but interestingly Triadent® could not decrease the progress of infection. **Conclusion**: Cutaneous leishmaniasis is an increasingly prevalent disease causing ulcerative lesions. Triamcinolone acetonide is used to treat the itching, redness, dryness, crusting, scaling, inflammation, and discomfort of various skin conditions. It is also used to relieve the discomfort of mouth sores.Topical treatment with Volon® A Haftsalbe offers few adverse effects, better compliance, reduced costs and is feasible for a rural setting.

Keywords: Volon® A Haftsalbe, Triadent®, Leishmania major, Treatment.

3190P

Evaluation of relationship between the serum levels of nitric oxide and sulfur mustard induced pigmentation disorders: Sardasht-Iran Cohort Study

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Background: Excessive melanin production and accumulation are characteristics of a large number of skin diseases, including post-inflammatory hyper pigmentation. According to our pervious study, in the sulfur mustard (SM) exposed population; pigmentation disorder was seen significantly more often than in the control group. Nitric oxide (NO) is a reactive endogenous molecule with multiple functions. NO plays a key role in physiologic as well as pathophysiologic processes, including inflammation, melanogenesis stimulation and skin cancer. The aim of this study was to evaluate the association of NO serum levels with pigmentation disorders in a SM exposed population 20 years after SM exposure. **Methods:** This study is a part of Sardasht – Iran cohort study (SICS) which is designed to evaluate long term complications of SM exposure. All participants were visited by clinicians and their skin was examined by specialists. The serum levels of cytokines were assessed by ELISA assay. **Results:** The serum level of NO in the exposed group with pigmentation disorder s was significantly higher than the control group. Furthermore a significant elevation in NO levels was found in the exposed subjects with pigmentation disorder compared to those exposed participants without pigmentation disorder. **Conclusion**: According to previous results, the serum level of NO was not significantly different between the exposed and control groups. Thus, our results indicate that elevated serum levels of NO may be associated with progression of pigmentation or other disorder in the SM exposed subjects.

Keywords: Sulfur mustard, pigmentation, Nitric oxide, Skin

3142P

Staphylococcus and Streptococcus are producing Allergic wounds

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Background: Staphylococci are a common type of bacteria that live on the skin and mucous membranes (eg. in nose) of humans. Staphylococcus aureus (S. aureus) is the most important of these bacteria in human diseases. About 15-40 per cent of healthy humans are carriers of S. aureus, that is, they have the bacteria on their skin without any active infection or disease (colonisation). Most staph. infections occur in normal individuals but underlying illness and certain skin diseases increase the risk of infection. Streptococci are bacteria that are commonly found harmlessly living in the human respiratory, gut and genitourinary systems. Several species are capable of causing disease in humans, including skin diseases. Skin diseases due to direct infection with streptococcus include: Impetigo , Ecthyma , Cellulitis , Erysipelas and Necrotising fasciitis . Ecthyma is a skin infection characterised by crusted sores beneath which ulcers form. It is a deep form of impetigo as the same bacteria causing the infection are involved but ecthyma causes deeper erosions of the skin. Cellulitis is caused by bacterial infection. It can occur by itself, or complicate an underlying skin condition or wound. **Methods:** Total of 51 clinical Staphylococcus aureus and Streptococcus pyogen isolates from wound for evaluated. **Results:** The results showed that 33.6% and 16.4 % of wound were infected by St. pyogenes and S.aureus respectively. **Conclusion**: Streptococcus pyogenes and/or Staphylococcus aureus are the bacteria responsible for impetico ecthyma , sever atopic dermatitis and cellulitis. The most common infecting organisms are Streptococcus pyogenes (two thirds of cases) and Staphylococcus aureus (one third).

Keywords: Staphylococcus, Streptococcus, Allergic wound

Immunogenetics

Oral Presentations:

3316O

Clonotype analysis of CD4+, CD8+ and regulatory T cells isolated from breast cancer draining lymph nodes based on the T cell receptor Vβ usage

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Background: Tumor draining lymph nodes (TDLNS) host the anti-tumor lymphocyte subsets. The aim of this study was to analyze the clonotype of CD4+, CD8+ and regulatory T cells isolated from breast cancer draining lymph nodes based on the T cell receptor Vβ (TRBV) usage. **Methods:** CD4+, CD8+, and regulatory T cells were isolated from TDLNs of 14 breast cancer patients and 4 non-malignant controls by using antibody-coated beads and magnetic-activated cell sorting (MACS). The purity of the cells was verified by flow cytometry. 28 specific primer pairs were used to amplify all functional alleles of 25 TRBV families, along with Cβ region primers as internal control. Fragment analysis and Gene Scan method was performed on ABI-310 genetic analyzer to qualify and quantify the usage of different TRBV families and to determine the clonality of different cell subsets. **Results:** Spectratype analyses revealed disperse oligoclonalities in several TRBV families among three investigated subsets with more restricted usage amongst CD8+ T cells. The uniform oligoclonal pattern was observed in TRBV18 in BC patients, but not in the controls. CD4+ T clones expressing TRBV7.2, 14, 18 and 29 observed to be significantly higher among patients than controls. Frequency of different TRBV usage by CD8+ and Treg subsets was not observed to be different between patients and controls. **Conclusion:**The oligoclonal pattern in the TRBV18 seems to be specific to breast cancer patients. Isolation of different clones with TRBV18 usage, subsequent analysis of total TCR sequence and investigation of the target antigens may provide new clues in breast cancer immunology and immunotherapy.

Keywords: Breast cancer, Lymph nodes, TCR, Clonotype

2696O

Association of polymorphisms in Interleukin-16 gene and susceptibility to chronic Hepatitis B infection

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Background: Host genetic background is known as an important factor in patients' susceptibility to infectious diseases such as viral hepatitis. The aim of this study was to determine the effect of genetic polymorphisms of Interleukin-16 (IL-16) cytokine on susceptibility of hepatitis B virus (HBV) infected patients to develop chronic HBV infection. **Methods:** Study population was consisted of two groups each including 100 individuals. Chronic Hepatitis B (HBV) infected patients and HBV clearance individuals with HBcAb positive and HBsAg negative tests. Genotyping was conducted using PCR followed by enzymatic digestion and RFLP (Restriction Fragment Length Polymorphism) analysis. We genotyped three single nucleotide polymorphisms (SNPs) in the Il-16 gene (rs11556218 T>G, rs4778889 T>C and rs4072111 C>T) to test for relationship between variation at these loci and patients' susceptibility to development of chronic HBV infection. **Results:** Analyzing the genotyping data revealed that genotype distribution of rs11556218 polymorphism was significantly different between two study groups. The results showed that G allele and GG genotype of this polymorphism are in association with the higher susceptibility to chronic HBV infection development. **Conclusions:** Our results showed that Il-16 gene polymorphisms are highly associated with increasing the risk of HBV. These findings have leaded us to conclude that Interleukin16 must be considered as an important immune regulatory cytokine in development of chronic HBV infection.

Keywords: Genetic Predisposition to Disease, Chronic Hepatitis B, Interleukin-16.

2941O

Determination of IL-23 Receptor Gene Polymorphism in Iranian Patients with Ankylosing Spondylitis

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Background: The result of recent genome wide association studies revealed that in addition to *HLA-B27*, few non-*HLA* genes are associated with susceptibility to ankylosing spondylitis (AS) in Caucasian populations. According to these studies *IL-23R*is one of these genes that is associated with AS. In this study we evaluated five important single nucleotide polymorphisms (SNPs) of *IL-23R* gene with the susceptibility to AS and also its effects on the severity of the disease in *HLA-B27* positive and negative patients and several subtypes of

HLA-B27. **Methods**: The study population consists of 294AS patients and 352 age, sex, and ethnicity matched healthy controls. All patients were examined by rheumatologist and met modified New York criteria for the disease. Five SNPs (rs1004819, rs11209032, rs1495965, rs11465804, and rs1004819) of the IL-23R gene were genotyped using Real-Time PCR TaqMan genotyping method. **Results:** We found that none of the selected SNPs alone were associated with susceptibility of AS and also HLA-B27 and its subtypes. Also there was no association between these five polymorphisms and BASDAI, BASFI, and BASMI indices. Two haplotypes ACGAT and ACGAG were found to be associated with heritability of AS. Also two significant protective diplotypes (D1, ; and D2,) were discovered. **Conclusion:** This study supported our previous findings regarding the differences between genetic patterns of AS disease among Iranian patients compared with other parts of the world.

Keywords: ankylosing spondylitis, HLA-B27, IL23R, SNP

2230O

Genetic variation of human platelet alloantigens (HPA-1 to -6, -9 and -15) in a healthy Iranian population

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Background: Human platelet alloantigens (HPA) play a major role in the occurrence of platelet alloimmunization and are important in studies of population diversity. HPA may also be considered as histocompatibility antigens in transplantation as well as predisposing markers for cardiac disease. The aim of this study was to investigate the distribution of HPA -1 to -6, -9 and -15 biallelic polymorphisms in a healthy Iranian population. **Methods:** Samples of DNA from 100 healthy and unrelated Iranians were isolated from peripheral blood leucocytes and genotyped by a lab-designed polymerase chain reaction-sequence-specific primers (PCR-SSP) technique. The frequencies of HPA-1a/1b, -2a/2b, -3a/3b, -4a/4b, -5a/5b, -6a/6b, -9a/9b, and -15a/15b alleles and genotypes were determined by direct counting and compared to other populations. **Results:** The HPA allele frequency was 88.5 and 11.5% for HPA-1a and -1b, 89 and 11% for HPA-2a and -2b, 57 and 43% for HPA-3a and -3b, 100 and 0% for HPA-4a and -4b, 90.5 and 9.5% for HPA-5a and -5b, 100 and 0% for HPA-6a and -6b, 100 and 0% for HPA-9a and -9b, and 43.5 and 56.5% for HPA-15a and -15b, respectively. Homozygosity for variant (b) alleles (bb genotypes) was observed in HPA-2 (1%), HPA-3 (22%), HPA-5 (4%), and HPA-15 (33%) loci in our population. The genetic distribution of HPA in Iranians was comparable to distributions reported in the European and other Caucasian populations. Whereas, dissimilarity in frequencies of certain HPA variants was found between our population and a number of African and Asian ethnic groups. **Conclusion:** The results of this study would be useful as a control group for potential clinical researches involving HPA genetic variation in Iranians.

Keywords: Genotyping, Human platelet alloantigens (HPA), Iranians, PCR-SSP

2294O

Assessment of CXCL12 (SDF-1a) Polymorphisms and its serum level in post transfusion occult HBV-infected patients in southeastern Iran

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Background: Occult hepatitis B infection (OBI) is defined as a form of hepatitis in which, despite absence of detectable HBs Ag, HBV-DNA is present in peripheral blood of patients. The main aim of this study was to determine an association between polymorphisms inþ801 of CXCL12 (SDF-1a) and its serum level in OBI patients. **Methods**: In this experimental study, plasma samples of 3700 blood donors were tested for HBs Ag and anti-HBc by ELISA. The HBsAg/anti-HBc samples were selected and screened for HBV-DNA by PCR. HBV-DNA positive samples assigned as OBI cases and PCR-RFLP techniques were performed to examine the CXCL12 (SDF-1a) polymorphisms. The serum level of CXCL12 (SDF-1a) was also analyzed by ELISA. **Results**: Of 3700 blood samples, 352 (9.5%) were HBsAg/anti-HBc and HBV-DNA was detected in 57/352 (16.1%) of HBsAg/anti-HBc samples. our results showed a significant difference in genotypes and alleles ofþ801 region of CXCL12 (SDF-1a). However, the serum level of CXCL12 (SDF-1a) was decreased in OBI patients but was not significant. Our results also showed that the alleles of þ801 region of CXCL12 (SDF-1a) were also not associated with serum level of the chemokine. **Conclusions**: The polymorphisms in þ801 region of CXCL12 (SDF-1a) are possibly related to OBI.

Keywords: Occult hepatitis B infection, CXCL12 (SDF-1a), Polymorphism

2512O

Genetic analysis of selected BRCA1 and BRCA2 exons in Iranian patients with breast carcinoma

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Background: Breast cancer is the most common cancer in women. Germ line mutations in BRCA1 and BRCA2, as early-onset breast cancer susceptibility genes, increase the risk of breast cancer up to 87%. It is suggested that each population and ethnic group have its own mutations. The aim of present study was to investigate the mutations in BRCA1 and BRCA2 genes in breast cancer patients from southern Iran. **Methods:** 70 breast cancer patients with family history and 70 age-matched normal women as control group were enrolled in the present study. DNA was extracted from peripheral blood cells and exons 2, 15, 16 and 20 of BRCA1, as well as exon 11E of BRCA2 were amplified using specific primers. PCR products were sequenced with ABI 310 DNA-sequencer. Exon 16 of BRCA1 and exon 11E of BRCA2 were screened with DHPLC prior to DNA sequencing. Chromatograms and electropherograms were analyzed with Navigator TM 2.2.0 and DNA Sequence Analysis 5.1, respectively. **Results:** In total three mutations were detected in the population. (c.[4837A>G]) polymorphism was observed in exon 16 of BRCA1 with a significant different frequencies between patients and controls. (c.[4956G>A]) single nucleotide polymorphism was observed

in exon 16 of BRCA1, again with different frequencies between two groups. A novel mutation (c.[5961G>T]) mutation in its heterozygote form was found in exon 11E of BRCA2 only in the patients. **Conclusion:** Present data suggest that southern Iranian population may have its own specific germ line mutations in BRCA1/2. Further studies are required to define the incidence of the observed mutations, as well as their functional and clinical significance.
Keywords: BRCA1, BRCA2, Breast Cancer, Southern Iran

3306O
MicroRNA-155 induces apoptosis in human T-cell leukemia Jurkat cells via targets caspase transcripts

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Background: Caspase play crucial roles in induction of apoptosis. Previous studies suggested that micro-RNAs (miRNAs) are also candidate molecules in the regulation of apoptotic pathways previous studies demonstrated that miRNA-155 (miR-155) displays both apoptotic and anti-apoptotic functions in various cell lines. Therefore, the aim of this study was to examine the effects of miR-155 on the survival of Jurkat cells (a tumor T lymphocyte cell lines) and its effects on the mRNA levels of caspases transcripts. **Methods:** Jurkat cells were transfected with miR-155, as well as a scrambled sequence and PBS, as controls, using Lipofectamine 2000 commercial kit. The expressions of caspases transcripts were and quantities against beta-actin and GAPDH (as housekeeping genes) using Real-Time PCR technique. **Results:** The results identified that the mRNA levels of caspase-2 and 10 were significantly increased following miR-155 transfection, while, the expression of caspase-8 was decreased. **Conclusion:** Based on our results, it may be concluded that miR-155 can lead to apoptosis in Jurkat cells via up regulation of caspase-2 and 10 mRNAs. Thus, it seems that miR-155 induces apoptosis via extrinsic pathway.
Keywords: MiR-155, Caspase family, Jurkat cell

2592O
Il-17 gene polymorphisms in patients with basal cell carcinoma of the skin

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Background: Basal Cell Carcinoma (BCC) is the most common type of skin cancer. The aim of this study was to investigate the impact of IL17 gene polymorphisms (rs763780 in IL-17F and rs2275913 in IL-17A gene) on susceptibility to BCC. **Methods:** This case-control study

was carried out on 400 subjects assigned to two groups of BCC patients with average age of 60.94±13.94 years (N=200) and healthy control group with average age of 60.93±13.98 years (N=200). Genomic DNA was extracted using modified salting-out method. Genotypes were determined using PCR-RFLP methods. **Results:** Comparing the results between patient and control group showed that at rs763780 position in IL-17F, the frequency of G allele in BCC patients (8.5%) was lower than that of the control group (11%). However, the difference was not significant (P=0.28). The frequency of homozygous mutant genotype was 1% in the control group; this genotype was not observed in the BCC patients. The frequency of A allele at rs2275913 position in IL-17A was 31.5% and 30.5% in patients and controls, respectively, indicating no significant difference between groups (P=0.82). The frequency of homozygous mutant genotype AA was 12.5% in BCC patients and 9.5% in control group indicating small non-significant difference between two groups (P=0.42). No association was found between investigated polymorphisms and clinic pathological characteristics of the patients including size of lesion, number of lesions, sunlight exposed lesions and etc. (p>0.05). **Conclusion:** Our findings do not verify the association of rs763780 and rs2275913 gene polymorphisms in IL-17 gene with susceptibility to BCC.

Keywords: Basal Cell Carcinoma, Interkulein-17, single nucleotide polymorphism

2872O

The assessment of IL-1 gene family polymorphisms in Iranian children affected by febrile seizure

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Background: Febrile seizures (FS) are the most common childhood neurological pathologies which occur in 2–3% of children between 6 months and 6 years of age. FS could be the first sign of epilepsy. A number of genetic mutations contribute to FS manifestation. Previous studies demonstrated that genetic polymorphism of some pro-inflammatory cytokines such as IL-1 is clinically important since they might associate with higher or lower cytokine activity. The aim of this study was to evaluate genetic polymorphism related to FS patients IL-1 (as a well-known fever inducer), IL-1R and IL-1RA comparing healthy controls. **Methods:** DNA samples of 90 febrile seizure patients and 140 healthy controls were collected. The following SNPs were assessed by polymerase chain reaction with sequence-specific primers (PCR-SSP): IL-1A rs1800587, IL-1B rs16944 and rs1143634, IL-1R1 rs2234650 and IL-1RN rs315952. **Results:** Among all the evaluated SNPs, interleukin-1 receptor antagonist (Mspa-I 11100 C/T) (rs1143634) showed significant differences between groups. Allele T was significantly increased in FS patients (89% vs 77.1%, p=0.003). The frequency of the T/T genotype was significantly higher in the patient group (80.5% vs 57.1%, p < 0.001) with an odds ratio of 3.1 (95% CI: 1.5-6.3), while the C/T genotype was significantly lower in the patients with

FS (p=0.001). Furthermore, in IL-1B rs1143634, the frequency of the C/T genotype was significantly lower in the patient group comparing controls. **Conclusion:** the results indicate that the IL-1RA (Mspa-I 11100) T/T genotype may contributes to the development of FS and it could be a useful marker for predicting susceptibility to the disease.

Keywords: Febrile Seizure, IL-1 gene family, SNP.

2455O

Ectopic Stable Expressing the cDNA Coding for Human CD34 in NIH-3T3 Cell Line

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Background: Hematopoietic stem cells (HSCs) are widely used in the treatment of hematopoietic disorders. One of the most important markers for identification and isolation of HSCs is CD34, a surface transmembrane glycoprotein expressed on early hematopoietic stem and progenitor cells. The aim of study was to clone and stably express human CD34 molecule in mouse NIH-3T3 cell line. **Methods:** After confirming surface expression of CD34 on KG1a cell line, total RNA was extracted then cDNA was synthesized. CD34 cDNA was amplified by Pfu DNA polymerase and then ligated to pGEM-T easy cloning vector. Ligation mixture was transformed in DH5α competent bacteria and single positive clone was finalized by sequencing and comparing to reference sequence.CD34 cDNA was sub-cloned to pCMV6-Neo expression vector by double digestion using KpnI and HidIII enzymes and obtained construct was transformed in NIH-3T3 cells. Cells were grown in the presence of increasing concentrations of G418 and stable expression of surface CD34 was shown by flow cytometry after 4 weeks. **Results:** 1176 bp amplicon of human CD34 was obtained and full nucleotide sequence was validated by alignment to reference sequence in NCBI database. Surface flow cytometric analysis performed on cells growing in cell culture medium containing G418 confirmed stable expression of human CD34 in NIH-3T3 mouse fibroblast cell line. **Conclusions:** Human CD34 cDNA amplified from KG1a cell line was completely matched to CD34 expressed by HSCs and stably CD34-expressing NIH-3T3 cells would be valuable in forthcoming experiments to produce monoclonal antibodies used in diagnostic and research areas.

Keywords: CD34, Cloning, HSCs, KG1a, Stable Expression

1752O

The SDF-1α3'A genetic variation is correlated with susceptibility of asthma in Iranian patients

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Background: Chemokine/receptor axis is a predominant actor of clinical disorders. They are key factors of pathogenesis of almost all clinical situations including asthma. Correspondingly, CXCL12 is involved in the immune responses. Therefore, this study was designed to explore the association between gene polymorphism at position +801 of CXCL12, known as SDF-1α3'A, and susceptibility to asthma in Iranian patients. **Methods:** In this experimental study, samples were taken from 162 asthma patients and 189 healthy controls on EDTA. DNA was extracted and analyzed for CXCL12 polymorphisms using PCR-RLFP. The demographic information was also collected in parallel with the experimental part of the study by a questionnaire which was designed specifically for this study. **Results:** Our results indicated a significant difference ($P < 0.0001$) between the A/A, A/G, and G/G genotypes and A and G alleles of polymorphisms at position +801 of CXCL12. We also showed an elevated level of CXCL12 circulating level in Iranian asthma patients. **Conclusion:** Our findings suggest that SDF-1α3'A (CXCL12) polymorphism plays a role in pathogenesis of asthma. It can also be concluded that circulatory level of CXCL12 presumably can be used as one of the pivotal biological markers in diagnosis of asthm.

Keywords: SDF-1α3'A, Polymorphism, Asthma

1550O

Spleen tyrosine kinase's genetic polymorphism among allergic asthma patients in Pakistani population

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Background: Asthma is a complex syndrome with many clinical phenotypes in both adults and children. Its major characteristics include airway obstruction and swelling, bronchial hyper responsiveness which leads to coughing, wheezing, chest tightness and shortness of breath. With the advancement of studies related to allergic mechanisms, it has come to our knowledge that Spleen Tyrosine kinase gene has been found to have important role in regulation of inflammatory responses. It is involved in FcεRI mediated signaling events and mast cells activation. This study is aimed to detect the genetic polymorphism of SKY gene in allergic asthma patients and healthy controls. Association of gene polymorphism was determined with various demographic features. **Methods:** A candidate gene study of 170 allergic asthma patients and 170 healthy control individuals were carried out. Blood samples of asthma patients were collected from National Institute of health, Pakistan. DNA was extracted and Polymerase Chain reaction was performed on16 exons of SYK gene to detect polymorphism of these exons. Amplified DNA sequences were analyzed by single stranded polymorphism and selected variants were sent for sequencing. **Results:** Sequencing results confirmed polymorphism in exon no 14 of SYK gene. Mutation identified was synonymous (G>C).This mutations was

significantly present in non-smokers ($p<0.05$) and asthmatic patients with no family history. **Conclusion:** SYK's single nucleotide polymorphism was found in these patients, which was found to be associated with environmental factors.

Keywords: Spleen Tyrosine Kinase, Allergic asthma, SNP, PCR

1638O

Evaluation of relationship between gingival expression of microRNA-146a with periodontal diseases

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Background: MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression by binding to complementary target mRNAs and either promoting their decay or inhibiting their translation .microRNA (miRNA)-mediated RNA interference has been identified as a novel mechanism that regulates protein expression at the translational level . MicroRNAs (miRNAs) have been demonstrated to play an important role in regulation of the immuno-inflammatory response; however, the function of miRNA146-a in periodontal inflammation has not been investigated. The objective of this study was to explore the properties of miRNA146-a in periodontal inflammation by comparing miRNA expression level of inflamed and healthy gingival tissues. **Methods:** 10 tissue samples have been obtained from healthy subjects and 40 tissue samples from two groups patients that had aggressive and chronic periodontitis. after homogenization and RNA extraction we converted it to cDNA and the expression level of MicroRNA146-a in this two groups of patients compared with healthy subjects by real-time PCR. **Results:** MicroRNA146-a predominantly expressed in periodontitis tissues (almost 28.17 fold in aggressive priodontaitis patients and 40.45 fold in chronic priodontitis patients related to healthy subjects). undergoing inflammation enhanced expression of MicroRNA146-a has anti- inflammatory effects. MicroRNA146-a existing in the periodontitis tissue had direct correlation with CAL (clinical attachment lost) that is a significant sign for diagnose the stage of disease ($P<0.05$). **Conclusion:** MicroRNA146-a would be involved in inflammation probably via its antagonist effects with other proinflammatory cytokine. Our current findings expand our understanding of the biological function of MicroRNA146-a and argue that MicroRNA146-a may act on decrease of inflammation.

Keywords: MicroRNA146-a, Periodontitis, Gingival, Periodontal Disease, Inflammation

1941O

Polymorphisms in the CTLA-4 (-318 C/T) and PTPN22 C1858T genes on leprosy risk

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Background: Leprosy is a human chronic granulomatous infectious disease caused by

Mycobacterium leprae. Several types of study support a role for host genetics in susceptibility to leprosy. The PTPN22 gene encodes an intracellular lymphoid-specific phosphatase (Lyp) that has been shown to play a negative regulatory role in T-cell activation. CTLA-4 behaves as a negative regulator of activation and effector function of T cells. In the present study, for the first time in the world, we examined polymorphism in the CTLA-4 (-318 C/T) and PTPN22 C1858T (R620W) genes with respect to leprosy in a case-control study in the Azeri population of Northwest Iran. **Methods:** One hundred and fifty-three treated leprosy patients and 188 healthy and ethnic matched controls were included in this study. Tetra-primer amplification refractory mutation system–polymerase chain reaction (T-ARMS–PCR) and restricted fragment length polymorphism (RFLP) methods were used to type CTLA-4 (-318 C/T) and PTPN22 C1858T polymorphisms. **Results:** There was a significant difference in the distribution of the genotypes and allele frequencies of CTLA-4 (-318 C/T) polymorphism between Leprosy patients and controls (P=0.047, and P=0.006; respectively). However, there was no significant difference in the distribution of the genotypes and allele frequencies of PTPN22 C1858T polymorphism between Leprosy patients and controls (P=641, and 0.645; respectively). **Conclusion:** Our findings suggest a role of CTLA-4 (-318 C/T) but not PTPN22 C1858T polymorphisms in susceptibility to leprosy in the Azeri population of Northwest Iran.
Keywords: Leprosy, CTLA-4, PTPN22, T-ARMS–PCR

2074O

Association of CXCL8 gene expression with CXCR2 transcripts in patients with multiple sclerosis

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Background: CXCL8 and its receptors (CXCR1 and CXCR2) play important roles in CNS development, neuronal survival, modulation of excitability, and neuroimmune response. The aim of this study is to evaluate gene expression of CXCL8 and CXCR1/CXCR2 in peripheral blood cells (PBCs) of patients with relapsing remitting (RR) form of multiple sclerosis (MS). **Methods:** We explored the mRNA expression of CXCL8 and its receptors in PBCs of 49 RR-MS patients in remitting status and 60 healthy controls by quantitative Real- Time PCR. **Results:** Median expression of CXCL8 mRNA in peripheral blood of MS patients decreased more than 3-fold compared to control group ($p< 0.001$), while there were not significant differences in CXCR1 and CXCR2 gene expression between MS patients and healthy subjects ($p= 0.159$ and $p= 0.248$, respectively).CXCL8 mRNA expression in PBCs was positively correlated with the expression of CXCR2 transcripts in patients and controls ($rs= 0.435$, $p= 0.002$ and $rs= 0.292$, $p= 0.029$, respectively).To assess whether CXCL8 positive results are associated with CXCR2 positive results, a chi-squared test was done that showed that such an association existed only among the MS patients ($p= 0.013$). There was a significant negative

correlation of CXCR2 expression with EDSS (rs= - 0.432, p= 0.004). Also, patients with low EDSS (below the mean value) displayed a significantly higher CXCR2 expression (p= 0.011). **Conclusion:** It appears that decreased expression of CXCL8 may lead to a raised risk of MS.
Keywords: CXCL8, CXCR1, CXCR2, Multiple sclerosis, Gene expression

1675O

Genetic variants of Toll-like receptor 4 gene as a risk factor for migraine

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Background: The pathogenesis of migraine involves many immune-mediated mechanisms in the vascular endothelium. TLR4 is a signaling receptor of innate immunity which plays a role in various neuropathologies related to neuron inflammation. This study aimed to investigate whether is a contribution of TLR4 Asp299Gly mutation in a Caucasian population with migraine. **Methods:** A total of 170 migraine patients (130 females, mean age 33.24 ± 10.57 years) and 170 age, sex, and ethnicity matched healthy controls (118 females, mean age of 31.11 ± 9.83 years) were recruited. Genotyping was carried out using the tetra- primer amplification refractory mutation system (ARMS)-PCR. **Results:** The frequency of Gly allele was higher in migraine patients than the controls (15% vs. 4.7%; p<0.0001). Interestingly, the distribution of heterozygous Asp299Gly genotype was statistically differed between migraineurs and controls (25.3% vs. 8.2%, p=0.00002, OR; 3.87, 95% CI; 2.02-7.4). Multivariate logistic regression analysis indicated that Gly allele in affected female migraineurs is an independent factor associated with increased risk of migraine (OR; 3.2, 95% CI; 1.23-8.24, p=0.01). **Conclusion:** Our results seem to highlight the role of TLR4 polymorphism as a genetic risk factor for migraine. However, further studies in different population/ethnic background are required to elucidate the precise role of TLR4 Asp299Gly mutation in susceptibility to migraine.
Keywords: Migraine, Toll-like receptor, Genetic polymorphism, Female, Inflammation

1935O

Association between functional R381Q variant (rs11209026) in IL-23 receptor gene and recurrent spontaneous abortion (RSA)

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Background: Recurrent spontaneous abortion (RSA) is defined as three or more consecutive abortions before the 20th week of gestation. There is increasing evidence to support an immunological mechanism for the occurrence of RSA. The purpose of our study was to investigate the association between a functional single nucleotide polymorphism (SNP) in the interleukin-23 receptor gene (IL-23R; rs11209026, 1142 G wild-type→A reduced function, Arg381Gln, R381Q) in patients with RSA. **Methods:** This is a case-control study. We recruited 200 patients with RSA (case group) using established diagnostic criteria and 200, normal individuals (control group) at the fertility and infertility center in Yazd city and Isfahan city during the period from 2012 to 2013 . By PCR-RFLP method we screened the R381Q

variant in IL23R in patients and controls, and we performed an association analysis between R381Q variant and RSA. The data was analyzed by SPSS16 software using Chi-square test. **Results:** Our result indicated there is a significant relationship between case group and control group due to R381Q existence (P value:0.01,Odds ratio:0.25).**Conclusion:** The frequency of protective SNP in IL-23 receptor (R381Q) in patients with recurrent spontaneous abortion is less than in the controls.

Keywords: Recurrent spontaneous abortion, R381Q

1835O

Analysisof IL-9 and IL-9R genetic polymorphisms as risk factors for allergic rhinitis in Iranian males

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Background: The development of allergic rhinitis entails a complex interaction between genetic predispositionand environmental exposure to different factors that allergens are the most important. Responding molecules are; chemokine's and their receptors, interleukins and their receptors, eosinophil peroxidase and leukotriene's, among others. The interleukin-9 receptor gene (IL-9R), belonging to the hematopoietic receptor super family, is located on the pseudoautosomal region of X and Y chromosomes. It is expressed on T cells, macrophages, mast cells, eosinophils, and neutrophils Upon IL-9 binding to IL-9R, JAK-STAT signaling pathway is activated. **Methods:** DNA was extracted using standard phenol-chloroform method. The screening of mentioned polymorphisms was performed using PCR-RFLP procedure. A case-control association study was performed (rhinitis group; n=139 and control group; n=61). Chi-square test was performed to compare proportions of subjects with different clinical features among subjects with different genotypes. (All statistical analyses were performed using SPSS). **Results:** There was significant association betweenIL-9 (rs 2069885), IL-9R (rs 731476) and allergic rhinitis($P<0.0001$), ($P=0.002$) in Chaharmahal va Bakhtiari province's males. **Conclusion:** Our data indicated that the IL-9 and IL-9R may play an important role in the inflammatory response and promoting allergic rhinitis and in Iranian males.

Keywords: IL-9, IL-9R, Allergic rhinitis

2111O

Relationship between FAS/FASL genes polymorphisms and pre-eclampsia disease

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Background: The Fas/FasL system play an important role in maintains of tissue homeostasis in the pregnancy and reproductive organs to keep adapted immune tolerance. The activated T lymphocytes that recognize paternal antigens undergo to apoptosis through expressing Fas interact with the FasL expressed on trophoblast.A critical factor for normal pregnancy is ratio of activity TH1 cells to TH2 cells. In normal pregnancy the TH1 subtype, responsible for produced inflammatory cytokines, reduced and TH2 subtype increased to prohibit of inflammation. But in pre-eclampsia shown that the TH1 cells were increased and subsequently the inflammation

and trophoblast destruction accrued. Suggested that Polymorphism in promoter region of the Fas/ FasL genes canreduce the expression the Fas/Fasl and probably responsible for TH1 increasing. We examined Fas -670A/G, -1377G/A, and Fasl -844C/T polymorphism in pregnant women with pre-eclampsia compared with pregnant healthy group. **Methods:** DNA from 153 pregnant women with pre-eclampsia and 140 healthy pregnant women were genotyped through PCR-RFLP. Fisher's exact test was used to compare the distribution of each individual polymorphism. The genotype distributions were tested for Hardy-Weinberg equilibrium. **Results:** Fas -1377 AA, AG and GG genotypes was observed in 2.68%, 18.30% and 79.08% respectively of patient group opposed to 0.00%, 27.14% and 72.85% of control group(P_V=0.037).Similarly Fas -670 AA, AG and GG genotypes was observed in 37.90%, 41.80% and 20.30% respectively of patients opposed to 33.60%,50.70% and 15.70%of control group(P_V=0.291).There are not significant statically difference between FasL-844 genotype between groups(P_V=0.690).**Conclusion:** These results suggested that Fas-1377AA genotype is associated with pre-eclampsia.

Keywords: FAS/FASL, Pre-eclampsia, Polymorphism

Poster Presentations:

1768P

Vitamin D receptor TaqI polymorphism in children with urolithiasis in Kerman

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Background: Polymorphisms in the Vitamin D Receptor (VDR) gene have recently been reported to be associated with calcium metabolism disorders such as urolithiasis. We undertook this study to examine the association between VDR TaqI gene polymorphism in children with urolithiasis in Kerman. **Methods:** We investigated the VDR TaqI polymorphism in 169 individuals including 81 children with urolithiasis and 88 healthy children. DNA was isolated from peripheral blood and genotyping was performed with PCR-based methods. **Results:** The frequency of low producing TaqI TT genotype was significantly higher in controls than children with urolithiasis (chi square=7.736, p=0.021). **Conclusion:** VDR genotype determination may provide a tool to identify the children who are at a risk for urolithiasis. Investigation between TaqI TT genotype and the strength of the family history is our goal for further studies.

Keywords: Urolithiasis, VDR gene polymorphisms, TaqI genotype, children

2141P

HLA-DRB allele frequencies in Iranian patients with autoimmune hepatitis

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Background: Autoimmune hepatitis (AIH) is an organ-specific autoimmune disease characterized by chronic inflammation of the liver. Genetic susceptibility to AIH is linked mainly to human leukocyte antigen (HLA)-class II genes and in particular to HLA-DRB1 locus. Different HLA-DRB1 alleles have been associated to AIH in studied populations. In the present study the frequency of HLA-DRB alleles in Iranian AIH patients has been compared to matched healthy individuals. **Methods:** Fifty four AIH patients and 100 unrelated subjects with no medical history of autoimmunity and cancer were included in the study. DNA was extracted from peripheral blood leukocytes and low-resolution HLA-DRB1/3/4/5 typing was performed using polymerase chain reaction-sequence-specific primers (PCR-SSP) technique. Allele frequencies in patient and control groups were determined by direct counting and compared with Chi-squared test. **Results:** HLA-DRB1 alleles including DRB1*13 (15.7% vs. 9.5%, P=0.065) and DRB1*03 (14.8 vs. 8.6, P=0.059) were found with higher frequency in AIH patients compared to controls. In contrast, significant decreased frequency of DRB1*11 allele (11.1% vs. 25.0%, P=0.002, Odds ratio=0.38) was revealed in patient group. Regarding HLA-DRB3/4/5 allele frequencies, AIH patients and controls had similar distributions. **Conclusion:** It is concluded that HLA-DRB1*13 and HLA-DRB1*03 may be susceptibility alleles for the occurrence of autoimmune hepatitis in our population; While, HLA-DRB*11is suggested as a protective gene against this disease. However, a larger cohort of patients is needed to confirm these results.

Keywords: Autoimmune hepatitis, HLA-DRB1, Susceptibility alleles

1888P

Thr399Ile polymorphism in Toll-like receptor 4 gene may be a predictive factor for severity of ulcerative colitis

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Background: Ulcerative colitis (UC) is a multi-factorial disease with different causes that affects the lining of the large intestine. Toll like receptor 4 (TLR-4) has several gene polymorphisms such as Asp299Gly and theThr399Ile which are reported to be more important than the others. In the current study we tried to determine the frequency of the above mentioned gene polymorphisms in the UC patients. We also scrutinized the association between severity of ulcerative colitis according to endoscopic view of colon and Asp299Gly and theThr399Ile gene polymorphisms. **Methods:** 85 UC patients who underwent endoscopic investigation enrolled in our study. DNA was extracted and PCR-RFLP technique was employed to determine Asp299Gly and Thr399Ile polymorphisms in TLR-4 gene. **Results:** The frequencies of mutant

alleles for Asp299Gly and theThr399Ile were 11.8% and 13% respectively. Based on the endoscopic views, there was a significant association between Thr399Ile gene polymorphism and severity of disease. Frequency of mutant alleles based on severity is as fallows; mild: 90.9%, moderate: 9.1%, severe: 0.0%, (χ^2 *p*.value: 0.019, OR: .012). **Conclusion:** Our results showed a significant association between Thr399Ile and severity of UC disease. In patients carrying theThr399Ile gene polymorphism, milder degree of disease severity was shown in the endoscopic view, which may indicate protective role of this genotype for patients with UC. According to our knowledge, this is a novel finding which has not been reported elsewhere.

Keywords: Toll-like receptor 4 gene, Asp299Gly, Thr399Ile, Ulcerative colitis

1774P

Interleukin-28B variation increased deterioration of H.pylori in gastric cancer

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Background: Gastric cancer is one of the most common gastrointestinal cancers in the world. Gastric cancer is the most common cancer in north of Iran. IL-28B, interferon-lambda family memberandactivatingpathwayJAK/STATEmaybe involvedin the development ofgastric cancer. This study aimed to assess the association between IL-28B- rs12979860 polymorphism and potential susceptibility to gastric cancer. **Methods:** In this case-control study, 212 gastric cancer patients, 116 samples (54.7%) males and 96 samples (45.3%) females with mean age of 62.03±12.61 years, and 173 healthy volunteers with age, sex and geographical area matched with same patients were recruited. All originating were from Mazandaran province. DNA was extracted and amplified for IL-28B gene SNP (rs-12979860) by Tetra-ARMS-PCR using the Eppendorf thermocycler. **Results:** In the present study, the frequencies of the IL-28B genotypes (rs-12979860) in patients were as follows: C/C, 41%; C/T, 37.7% and T/T, 21.2%. Frequencies of the IL-28B genotypes in healthy control were in healthy control as follows: C/C, 41.6%; C/T, 41% and T/T, 17.3%. The frequencies of the IL-28B genotypes(TT+CT) and CC in patients with Helicobacterpylori compared with healthy controls were as follows: (72% VS. 28%) and (54% VS. 46%). **Conclusion:** There are no significant differences in subjects carrying the rs-12979860 CC responder genotype compared with those with the CT or TT genotype in gastric cancer or healthy controls. There was not a significant difference in polymorphism of IL-28B between patients and controls. There are significant differences in Helicobacter pylori infected individuals carrying the rs12979860 T allele compared with the CC genotype in gastric cancer(p=0.009).

Keywords: IL-28B (rs12979860), IFN-λ_3,Gastic cancer, Polymorphism

1826P

Investigating the association between polymorphism of IL-17 and migraine

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Background: The treatment of children and adolescents who suffer from migraine headaches must be individually tailored, flexible, and balanced with a blend of bio-behavioral measures, agents for acute treatment and, if needed, daily preventive medicines. **Methods:** In this case-control study, 164 Migraine patients and 168 healthy volunteers with age, sex and geographical area matched with same patients were recruited. Genomic DNA was extracted and genotypes of IL-17 polymorphism were assessed through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The association of genotypes or allele with disease was analyzed by logistic regression model and odd ratio was obtained at confidence level of 0.05. **Results:** Although the homozygous AA genotype frequencies were higher in migraine patients than in controls, but the difference was not statistically significant (p=0.24) and genotype frequencies (GG) at position G197 promoter IL-17A was not significantly different between control group and patients with migraine(4.44% VS. 6.38%).**Conclusion:** Our findings indicated that IL-17F polymorphism alleles in the promoter of IL-17F gene is not significant difference between Migraine disease and disease severity.

Keywords: IL-17, Polymorphism, Migraine

1943P

CTLA-4 (-318 C/T) and PTPN22 C1858T gene polymorphisms is not associated with type 1 diabetes in Azerbaijan, northwest Iran

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Background: Type 1 diabetes (T1D) is a complex trait caused by T-cell mediated autoimmune destruction of islet beta cells in the pancreas, resulting from the interaction between genetic and environmental factors. Like other autoimmune disorders, the possible role of specific cytotoxic T lymphocyte antigen-4 (CTLA-4) and Protein tyrosine phosphatase, non-receptor type 22 (PTPN22)gene polymorphisms in predisposing to T1D has been hypothesized, but it remains controversial. **Methods:** CTLA-4 promoter (-318C/T) and PTPN22 C1858T (R620W) polymorphisms have been analysed in 153 Iranian patients with T1D and in 188 unrelated matched healthy controls bytetra-primer amplification refractory mutation system–polymerase chain reaction (T-ARMS–PCR) and restricted fragment length polymorphism (RFLP) methods. **Results:** There was no significant difference in the distribution of the genotypes or allele frequencies of CTLA-4 (-318 C/T) and PTPN22 C1858T polymorphism between T1D patients and controls (P= 0.627, or P= 0.893 and P=0.840, or P= 0.842; respectively). **Conclusion:** In summary, the CTLA-4 (-318C/T) and PTPN22 C1858T (R620W) is not

relevant in susceptibility to T1D in the Azeri population of Northwest Iran.
Keywords: Type 1 diabetes, CTLA-4, PTPN22, T-ARMS–PCR

2183P
Toll-like receptor 2 Arg753Gln polymorphism is associated with susceptibility to pulmonary tuberculosis in the Lur population of Iran

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Background: Toll-like receptors (TLRs) are the pattern recognition receptors playing major role in the innate immunity. The TLRs polymorphisms often have association with the susceptibility to different infectious diseases. In this study, we investigate whether 677 C/T TLR2 (Arg677Trp), 753G/A TLR2 (Arg753Gln), 299 A/G TLR4 (Asp299Gly) and 399 C/T TLR4 (Thr399Ile) polymorphisms have association with the susceptibility to pulmonary tuberculosis in the Lur population of Iran. **Methods:** The genotyping of TLR2 and TLR4 polymorphisms accomplished by using polymerase chain reaction-restriction fragment length polymorphism in 50 pulmonary tuberculosis patients and 50 healthy control individuals of Lur population of Iran. **Results:** GG genotype frequency of the 753 G/A TLR2 (Arg753Gln) polymorphism decreased significantly in the patient group compared with the control group (58% in the patient group vs. 84% in the control group, P=0.004 ،OR=0.263،CI=0.103-0.675). G allele frequency of the 753 G/A TLR2 (Arg753Gln) polymorphism decreased significantly in the patient group compared with the control group (71% in the patient group vs. 89% in the control group, P=0.001 ،OR=0.303،CI=0.141-0.648).**Conclusion:** Our results suggest that 753 G/A TLR2 (Arg753Gln) polymorphism may play role in the susceptibility to the pulmonary tuberculosis in the Lur population of Iran.
Keywords: TLR, Polymorphism, Tuberculosis, Lur population

2182P
Association between tumor necrosis factor -308G/A polymorphism and pulmonary tuberculosis in Lur population of Iran

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Background: Tumor necrosis factor-α (TNFα) is a type of cytokines implicating in the innate immunity primarily formed against a pathogen. TNFα genetic polymorphisms are considerable in the immune response efficacy against major infections. In this study we investigate whether -238G/A TNFα and -308G/A TNFα polymorphisms have association with the susceptibility to pulmonary tuberculosis (TB) in the Lur population. **Methods:** TNF polymorphisms genotyping was performed by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) system in 50 pulmonary TB patients and 50 healthy controls of Lur population

of Iran.**Results:** The allele and genotype frequency of -238G/A TNFα has significant difference between the pulmonary TB patients and the healthy controls. Only, in TNFα -308G/A polymorphism, a significantly decreased frequency of genotype GG was observed among patients as compared to controls (62% in the patient group vs. 94% in the control group, P=0.0001, OR=0.104, CI=0.028-0.382).In the TNFα -308G/A polymorphism a significantly decreased frequency of G allele was considered among the patient group compared with the control group (81% in the patient group vs. 97% in the control group, P=0.0001, OR=0.132, CI=0.038-0.462). **Conclusion:** Our findings suggest that -308G/A TNFα polymorphism may play role in the susceptibility to pulmonary TB in the Lur population of Iran.

Keywords: TNF, Polymorphism, Tuberculosis, Lur population.

1940P

Association of the IL-4R single-nucleotide polymorphism I50V with recurrent Spontaneous abortion (RSA)

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Background: Recurrent spontaneous abortion (RSA) is defined as three or more consecutive abortions before the 20th week of gestation. There is increasing evidence to support an immunological mechanism for the occurrence of RSA. The purpose of our study was to examine whether single-nucleotide polymorphisms (SNPs) of the interleukin-4 receptor gene IL-4R influence susceptibility to, recurrent spontaneous abortion. **Methods:** This is a case-control study .We recruited 200 patients with RSA(case group) using established diagnostic criteria and 200, normal individuals (control group) at the fertility and infertility center in Yazd city and Isfahan city during the period from 2012 to 2013 . We screened the I50V variant in IL-4R in patients and controls by PCR-RFLF method, and we performed an association analysis between I50V variant and RSA. The data was analyzed by spss 16 software using Chi-square test. **Results:** No differences in the genotype and allele frequencies of the I50V SNPs were identified Between patients with RSA and healthy controls. **Conclusion:** The frequency of SNP in IL-4 receptor (I50V) in patients with recurrent spontaneous abortion is not differed significantly compared with the control group. and Analysis of IL-4R SNP haplotypes or complex alleles suggested no dominant protection in patients with RSA.

Keywords: Recurrent spontaneous abortion, IL-4R Single-Nucleotide Polymorphism, I50V

1993P

Survey on scavenger receptor B1 c.1119 C>T (rs5888) single nucleotide polymorphism in normal Iranian population

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Background: Scavenger Receptor-B1 (SR-B1) is glycoprotein involved in the recognition of polyanionic structures of either endogenous (e.g. oxidized or acetylated LDL) or exogenous

(e.g. bacterial lipopolysaccharides). This molecule expressed by macrophages and dendritic cells and certain endothelial cells and are involved in immune responses and lipid metabolism this study shows c.1119 C>T polymorphism of SR-B1 in Iranian normal subjects. **Methods:** RFLP method using Hae III restriction enzyme was designed to determine SR-B1, c.1119 C>T (rs5888). DNA extraction was performed using salting out method on 36 Iranian normal subjects. PCR was done on DNA samples using specific primers and the products were affected by HaeIII for 16 hrs and evaluated by electrophoresis and ethidum bromide staining. **Results:** Evaluation of the results indicated that distribiution of c.1119 CT genotype was 86.1% (31) and TT genotype 13.8 (5) and c.1119 C allele was 43.06 (31) and T allele was 56.94% (41) in our study population. **Conclusion:** our results showed that c.1119 TT genotype and c.1119 T allele are the more frequent scavenger receptor variants in Iranian subjects. With regards to broad functions of scavenger receptor in modulation of innate immunity, its polymorphism could be helpful to determine next steps of innate immune responses in Iranian subjects.

Keywords: Scavenger receptor, Polymorphism, c.1119 C>T, Iranian Subjects

2047P

The association of -330 interleukin-2 gene polymorphism with its plasma concentration in Iranian multiple sclerosis patients

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Background: Multiple sclerosis (MS) is a chronic neuroinflammatory demyelinating disease of the central nervous system. The cytokine genes are involved in autoimmune diseases such as MS. In this study, we report the influence of -330 interleukin-2 (IL-2) gene polymorphism on its plasma levels in a group of Iranian MS patients. **Methods:** In this study 100 MS patients and 100 ethnically, age and sex matched healthy controls were selected from medical genetics department of Sarem women hospital. Blood samples of all individuals were collected in EDTA tubes. The restriction fragment length polymorphism PCR (RFLP) method was applied to determine various alleles and genotypes in these individuals. Plasma concentration of IL-2 was measured in all the samples using human IL-2 kit. **Results:** The frequency of -330 T/T IL-2 genotype was higher in MS patients compared to normal individuals. Accordingly, the plasma levels of IL-2 was significantly higher group ($P<0.0001$) in patients when compared to the control group.

Conclusion: In conclusion, in case of MS patients the -330 T/T IL-2 genotype is associated with higher plasma levels of IL-2.

Keywords: IL2, Polymorphism, Plasma IL-2, Multiple sclerosis

2048P

Correlation of interleukin-2 and uric acid plasma concentration with multiple sclerosis in Iranian patients

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Background: Multiple sclerosis (MS) is an inflammatory and autoimmune disease which immune system can have important role on its pathogenesis. As uric acid (UA) is considered

as a endogenous peroxynitrite-scavenger, it may effect on MS. In this study, concentration of interleukin-2 (IL-2) and UA in plasma samples of MS patients were assessed. **Methods:** One hundred Iranian MS patients (mean± SD age of 32.95 ±6.51 years, range of 20-48 years) from medical genetics department of Sarem Women hospital were selected. Besides, one hundred ethnically, age and sex matched healthy individuals (mean± SD age of 29.8±7.8 years, range of 20-50 years) without personal or family backgrounds of autoimmune disorders were enrolled as a control group. IL-2 plasma concentration was calculated by Human IL-2 kit of eBioscience Company. Plasma level of UA was detected using Uric Acid Assay Kit of Abcam Company. **Results:** The Plasma concentration of IL-2 was significantly higher in MS patients than controls (p: 8.435×10-7). Also plasma concentrations of uric acid were significantly lower in MS group in comparison with control subjects (p: 1.548×10-30). The patients had around 2.2 times lower UA plasma concentration than controls. **Conclusion:** Our studies suggested that measurement of plasma IL-2 and UA concentrations may provide an objective marker of disease in patients with MS. In addition, it seems that studies with larger sample size are required to bring about more authentic results.

Keywords: Interleukin-2, Uric acid, Plasma concentration, Multiple sclerosis

2049P

The association of -308 TNF-α polymorphism and multiple sclerosis in Iranian patients

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Background: MS is a chronic neuroimmune disorder of the central CNS. MS belong to the large group of multifactorial and multigenic disease. The TNF-α gene encodes proinflammatory cytokine that takes an ambiguous part in the development of various disorders especially autoimmune disease such as MS. In this study, we investigated the association of -308A/G TNF-α polymorphism and multiple sclerosis in Iranian patients. **Methods:** One hundred MS patients and one hundred ethnically, age and sex matched healthy control individuals were selected. -308 TNF-α polymorphism was analyzed based on polymerase chain reaction with sequence-specific primers (developed during the 13IHWC and supplied by Heidelberg University (Heidelberg, Germany). **Results:** The frequency of the G allele and G/G genotype at the -308 TNFα position was significantly higher in MS patients in compare to control subjects (P: 0.032, OR: 1.685, 95%CI: 1.046–2.715, P: 0.041; OR: 1.933, 95% CI: 1.095–3.411, respectively). **Conclusion:** It indicated that G allele and G/G genotype had susceptibility effect on MS among Iranian patients. But more studies with large sample size and specially investigation of different TNF-α alleles in relation to other genes and haplotypes are needed to explain exact effect of TNF-α polymorphisms in the MS.

Keywords: Multiple Sclerosis, TNF-α, Polymorphism

1932P

RAGE polymorphism and multiple sclerosis

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Background: Multiple sclerosis (MS) is a demyelinating disease of central nervous system which has different clinical manifestations. The receptor for advanced glycation end products (RAGE) is a cell surface molecule that is involved in the pathogenesis of inflammatory and autoimmune diseases. A functional polymorphism within the V-type immunoglobulin domain of RAGE gene, p.82G>S (c.557G>A), has been shown to affect ligand binding affinity and thus may affect susceptibility to MS. In this study, we investigated the effect of this polymorphism of RAGE on susceptibility of MS in Iranian population. **Methods:** The RAGE p.82G>S polymorphism was genotyped in 158 patients with MS and 156 healthy controls using polymerase chain reaction-restriction fragment length polymorphism. **Results:** No differences were observed between the patients with MS and the controls, concerning the frequencies of G82S genotypes of the RAGE ($P > 0.05$).**Conclusion:** Results of this study suggested that the mentioned functional polymorphism is not likely to cause susceptibility to MS. Based on our knowledge, this is the first study to investigate the association of RAGE p.82G>S with MS in Iranian population.

Keywords: Multiple sclerosis, RAGE, Polymorphism

1402P

Molecular Analysis of Interleukin 17-A Gene Polymorphisms in Multiple Sclerosis Patients

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Background: Multiple sclerosis (MS) is a complex autoimmune disease that damages central nervous system. Both environmental and genetics factors are involved in the initiation and pathogenesis of this disorder. Cytokines such as IL-1, IL-6, IL-21, IL-17, TNF-α and TGF-β have important role in immunopathology of MS. There is evidences regarding the existence of polymorphism in this autoimmune disease and some SNPs have been known as markers. **Methods:** We analyzed the IL-17A gene sequence for two exons (2 and 3) in 40 patients and compared the results with 40 normal subjects. **Results:** There were some SNPs in the structural part of IL-17A gene. No significant difference associated with the exon 2 of IL-17A gene was observed between our MS patients and control group but our findings suggested that there was a significant difference in IL-17A exon 3 polymorphisms between the two study groups.

Keywords: Multiple Sclerosis, Polymorphism, IL-17A, Autoimmunity

1868P

Association of programmed cell death-1 gene polymorphism with delayed graft function in kidney allograft recipients

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Background: The genetic variations of costimulatory molecules can affect the extent of T cell activity during T-cell mediated immunity especially in transplant patients. This study aimed to investigate the association of programmed cell death-1 (PD-1) and PD-1 Ligand gene polymorphisms with clinical outcome of renal transplantation. **Methods:** A total of 122 patients who underwent kidney transplantation between 2007 and 2012 were included in this retrospective study. Patients were classified into two groups based on the occurrence of biopsy-proved acute rejection (BPAR) or having stable graft function (SGF) during meanly five-year follow up. Four single nucleotide polymorphisms in PD-1 and PD-1L genes were determined by PCRRFLP method in both groups of patients as well as in 208 healthy control subjects. **Results:** The frequencies of PD-1.3 (+7146 G/A), PD-1.9 (+7625 C/T), PD-L1 (8923 A/C) and PD-L1(+6777 C/G) genotypes and alleles were not statistically significant between both groups of patients. In compare with healthy controls, PD-1.9 (+7625 C/T) genotype and T allele were significantly more frequent in all patients (P=0.001 and P<0.0001 respectively) and in those group with SGF (P=0.001 and P=0.001 respectively). Overall, 27 of 122 recipients experienced delayed graft function (DGF) and a higher frequency of PD-1.9 (+7625 C/T) genotype and T allele was observed in this group versus those without DGF (P=0.04 and P=0.05 respectively). Similarly, a significant high frequency of this genotype was found among BPAR group of patients with DGF (P=0.05). **Conclusion:** Our results indicate that potentially functional genetic variation in PD-1 can influence the outcome of renal transplantation.

Keywords: PD-1, Gene polymorphism, Kidney allograft

1837P

Possible role of human leukocyte antigen-G in gestational diabetes

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Background: Diabetes can develop up to 10% of pregnant women who have not previously disease. This condition which usually begins in the second half of the pregnancy is called gestational diabetes mellitus (GDM). The cause of GDM is unknown. Human leukocyte antigen-G (HLA-G) expression protect the fetus from immune attack. In this study, we evaluated HLA-G molecule in GDM patients. **Methods:** In this case-control study, serum samples collected from 24 pregnant women with gestational diabetes in comparison with 30 matched normal pregnant women. We analyzed HLA-G protein using a validated sandwich ELISA(enzyme linked immunosorbent assay). **Results:** HLA-G levels in pregnant women with gestational diabetes were significantly lower than controls ($p<0.05$). **Conclusion:** Our results indicate that HLA-G levels in serum from women with gestational diabetes are lower than controls. This suggests that determination of circulating HLA-G protein concentration may be useful as an early predictor for the development of GDM.

Keyword: HLA-G, Gestational diabetes, ELISA

1952P

Expression of CK19 marker in different breast cancer cell lines

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Background: Cytokeratin19 (CK19), a part of the cytoskeleton of epithelial cells is abundantly expressed in the majority of epithelial tumor cells and is used as a tumor marker in cancer. CK19 is a sensitive and specific marker for breast carcinomas, which has been described in 98.2% of breast adenocarcinomas. The results of several studies suggest that CK19 could be useful in the diagnosis of breast carcinoma. On the other hand, cell lines provide indispensable tools in many aspects of laboratory research, particularly as *in vitro* models for cancer research. **Methods:** In this regards, Human breast cancer cell lines (MCF7, MDA-MB-231, T47D, SKBR3) and cervical cancer cell line (HeLa) as a negative control were cultured in RPMI 1640 (supplemented with fetal bovine serum (FBS) and penicillin-streptomycin antibiotic). Proteins were extracted from cell lines (1×10^6 cells) and were assessed in 12% Sodium dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE). CK19 protein expression was detected by immunoblot technique using mouse monoclonal anti-human CK19. **Results:** In this study, CK19 (40 kDa) expression was confirmed in breast cancer cell lines by immunoblot analysis. However, CK19 expression in T47D, MCF7 and SKBR3 cell Lines was shown while MDA-MB-231 cell line was not expressed CK19 by Enhanced chemiluminescence (ECL) method. **Conclusion:** CK19 expression in cancer cell lines could be assessed by ECL immunoblotting. On the other hand, CK19 as a molecular human breast cancer marker could differently express in cancerous cells. In conclusion, different CK19 expression in breast cancer cell lines should be more considered in cancer research.

Keywords: Human Breast cancer, CK19, Cell Lines, Immunoblot

1988P

Genetic association of the IL-7 gene polymorphism with multiple sclerosis susceptibility in an Isfahan population

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Background: Multiple sclerosis (MS) is an inflammatory neurodegenerative disease in which the insulating membrane of central nervous system (CNS) is damaged. The etiology of MS remains mysterious. A Genome-Wide Association Studies (GWAS) recognized genetic single nucleotide polymorphisms (SNP) linked with MS predisposition among which immunologically related genes are considerably over signified. From these, various association studies of polymorphic immune-associated genes described for MS. The purpose of this study

is to explore the association of rs1520333 C/T polymorphism in the IL-7 gene genes variant with the risk of MS in Isfahan population. **Methods:** In this case control study, 110 cases with MS and 110 controls were contributed. DNA was extracted from blood samples and to amplify fragment of interest contain rs1520333 SNP, polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method was implemented for genotyping of the DNA samples with specific primers for restriction enzyme (MwoI). SPSS for Windows software (version 18.0; SPSS, Chicago, IL) was used for statistical analysis. **Results:** We demonstrated the important association between C allele (OR =1.6614, CI =1.12 – 2.47, P= 0.0124) and CC genotype (OR = 7.45, 95% CI = 2.13 - 25.97, P 0.0016) of the rs1520333 SNP for susceptibility to MS after adjustment for age, and gender. Odds ratio adjusted for age, sex, and BMI have displayed similar outcomes. **Conclusion:** These results indicate that the rs1520333 SNP is a significant susceptibility gene variant for development of MS in the Iranian population. Nevertheless, functional studies are required to completely elucidate how this SNP contribute to MS pathogenesis.

Keywords: Multiple sclerosis, GWAS, IL-7 gene, Polymorphism

1716P

The G-308A promoter variant of the tumor necrosis nactor-alpha geneis associated with migraine without aura

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Background: Migraine is considered to be a polygenic multifactorial disease with various environmental and genetic etiologies. Tumor necrosis factor-alpha (TNF-α), a potent immunomodulator and pro-inflammatory cytokine, has been implicated in many pathological processes in brain. The hypothesis of this study was that migraine without aura (MWA) might be associated with TNF-α (-308) polymorphism, resulting in increased TNF-α production. **Methods:** Genotyping was performed on DNA extracted from peripheral leukocytes by PCR–SSP method in 221 patients with WMA and 183 healthy control subjects from Iranian population. **Results:** The results showed that the frequency of -308 A variant allele was higher in MWA compared to control group (40.6% versus 22.3%, OR 3.73, 95% CI 2.4-5.82, P<0.0001). TNF-a GA heterozygous genotype, high producer, was significantly more prevalent in patients with MWA than controls (74% versus 44.7%, P<0.0001) whilst low producer GG homozygous genotype was lesser in patients compared to controls (22.4% versus 55.3%, P<0.0001). **Conclusion:** The logistic regression analysis showed a significant association for TNF-a (-308A) allele carriage females with MWA at reproductive ages (OR 2.56; 95% CI, 1.57-4.16, P<0.0001) when compared with their matched control subjects.

Keywords: Factor-alpha, G-308A, Migraine, Tumor Necrosis

1931P

NRAMP1 gene polymorphisms and host susceptibility to cutaneousleishmaniasis

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Background: Association between polymorphisms in the natural resistance associated macrophage protein gene 1 (Nramp1) and susceptibility to Cutaneous leishmaniasis (CL) has been demonstrated worldwide. However the results have been inconsistent. This study aimed to determine the association of NRAMP1variantsand susceptibility to CL by compare the frequency of NRAMP1 gene polymorphisms among leishmaniasis patients and control group in Isfahan province of Iran. **Methods:** The single nucleotide polymorphisms (SNPs) of NRAMP1 gene at 577-18G/A, A318Vand D543N were analyzed by PCR-RFLP. Two test groups consisted of patients with CL (n=100) from Skin Diseases and Leishmaniasis Research Center in Isfahan and healthy controls (n=54) during 2012. The frequencies of variants were estimated by Chi square test and descriptive statistics. **Results:** The genotype pattern of 577-18G/A loci was identical in healthy controls and patients and all of them had G/G genotype. However genotype patterns between two groups were different for A318V and D543N loci. For A318V, C/C genotype was detected in patients group but all possible genotypes (C/C, T/T and C/T) were represented in healthy controls. The G/A genotype for third polymorphism D543N was seen in patients group while persons in control group had G/G genotype. **Conclusion:** This study showed that there were significant differences in the genotype frequencies for A318V and D543N polymorphisms between patients and control groups (p= 0.005 and 0 respectively). Our results indicated that genetic variation of NRAMP1 might be associated with the susceptibility to CL. These data may be used for detection of sensitive persons and prevention of CLin endemic areas of Isfahan province.

Keywords: Cutaneous leishmaniasis, Nramp1 gene, PCR-RFLP

3345P

CCR4 C1014T and CCL22 C16A genetic polymorphisms in the Iranian patients with colorectal adenocarcinoma

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Background: C-C motif chemokine 22 (CCL22) C16A genetic variation (rs4359426) and C-C chemokine receptor type 4 (CCR4) C1014T variations (rs2228428) have been suggested to affect the expression level of the associated proteins. **Methods:** In order to investigate the plausible association of these polymorphisms with colorectal cancer, 165 patients with colorectal adenocarcinoma (age 54.4±13.4) and 150 age-sex matched healthy individuals (age 52.4±12.0) were enrolled. Genotyping was performed by PCR-RFLP methods. **Results:** indicated the frequency of 16A allele in CCL22gene to be 31/330(9.4%) and 33/300(11%) in patients and controls, respectively (P=0.59). The frequencies of CC, CA, and AA genotypes at this locus were not significantly different between patients and controls (135/165;81.8%,

29/165;17.6%, 1/165;0.6% in the patients and 121/150;80.1%, 25/150;16.6% and 4/150;2.6% in the control group, P= 0.34). At the locus 1014 in CCR4, T allele was observed with the frequency of 107/330 (32.4%) and 83/300 (27.7%) among patients and controls, respectively (P=0.22). Analyses indicated no significant differences in the frequencies of CC, CT and TT genotypes at this locus between patients and controls (77/165; 46.7%, 69/165;41.8% and 19/165;11.5%; versus 83/150;55.0%, 51/150;33.8% and 16/150;10.6%, respectively, P= 0.29). The presence of individual genotypes observed not to be significantly associated with clinicopathological characteristics of the disease, including tumor size, tumor grade and LN involvement (all with P>0.05). **Conclusion:** These findings collectively suggest that CCR4 C1014T and CCL22C16A genetic variations are neither associated with the risk, nor with the prognostic factors of colorectal cancer in Iranian population.

Keywords: Chemokine, CCL22, CCR4, Colorectal cancer, Iranian population

1969P

SNP (rs3804100) of TLR2 gene is associated withtype 1 diabetes (T1D) and asthmain the Egyptian population

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Background: To investigate the association of asthmatic Type one diabetes patients in Egypt - with polymorphism TLR2 gene involved as a members of Toll-like receptors (TLRs) and could illustrate a molecular linkage of the microbial infections and immune-mediated diseases. a strong evidence suggests that TLR2 gene may contribute to the initiation and progression of Asthma and T1D . **Methods:** The study included 124 (Asthmatic T1D) patients categorized into two equal groups (female and male). In addition, 124 healthy individuals were selected to serve as a control group. Genotyping was carried out via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** We genotyped SNP (rs3804100) of TLR2 gene in asthmatic T1D patients. Strongest predictors were rs3804100 SNP (TLR2) ($P_{Genotype}$=0.014; $X^2_{Genotype}$=8.53), (P_{Allele}=0.003; X^2_{Allele} =11.52, OR=0.536).Moreover, this association was stratified by gender to male patients for (rs3804100) (*P*=0.0007, X^2 =14.38). **Conclusion:** The SNP (rs3804100) in TLR2 gene is considerably associated with asthmatic T1D, implying that rs3804100 SNP in TLR2 gene may intercede pathogenesis by changing TLR2 function possibly in Immune system. These findings suggest a possible diagnostic/ prognostic relevance of the TLR2 genotype.

Keywords: T1D , Asthma, TLR2, SNP, Egyptian

2010P

Association between Ghrelin gene (Leu72Met) polymorphism and Ghrelin serum levels with coronary artery diseases

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Background: Research shows that Ghrelin gene polymorphism has some association with coronary artery diseases (CAD). Due to genetic differences among nations and the high prevalence of CAD, we conducted this study to examine the possible association between the polymorphism of Ghrelin gene Leu72Met and CAD. **Methods:** This case-control study was undertaken with patients who were referred to referral heart center , in 2011 with chest pain or a positive exercise test. Patients with risk factors for heart disease or who were surgery candidates that underwent angiography and echocardiography were also included. DNA extractions were performed using a modified salting out method and the Ghrelin region was amplified using PCR. The presence of the Leu72Met polymorphism and the serum levels of Ghrelin were determined using the restriction fragment length polymorphism (RFLP) method and an Enzyme-Linked Immunosorbent Assay (ELISA) , respectively. **Results:** The results indicated that in CAD patients, the incidence of heart failure was significantly different between groups with genotypes CC or AA+CA (P=0.041). Mean serum level of Ghrelin in the CAD group was significantly higher than in the control group ($p<0.0001$). Additionally, there was a significant relationship between the distribution of Ghrelin genotypes and serum levels of Ghrelin in both the CAD and control groups ($p<0.0001$). **Conclusion:** This study indicates that there was a significant association between heart failure in CAD patients and presence of the polymorphism, as well as an increase in serum levels of Ghrelin associated with genotype distribution such that Ghrelin levels have an inverse relationship with the frequency of the CC genotype.

Keywords: Ghrelin, polymorphism, coronary artery diseases, heart failure

3215P

Quantitative analysis of vascular endothelial growth factors in peripheral blood mononuclear cells of patients with acute myeloid leukemia

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Background: The crucial role of angiogenesis in the pathophysiology of acute myeloid leukemia (AML) it has been proposed. One of the key regulators of angiogenesis is the vascular endothelial growth factor (VEGF). Among VEGF family, it has been observed that VEGF-A and VEGF-C are expressed by AML cells and mediated leukemic cell proliferation, survival and resistance to chemotherapy. Emerging evidence however, suggests that elevated levels of VEGF, or a proangiogenic phenotype, may impede, rather than promote, early tumor development and progression. As the significance of VEGF-A and VEGF-C levels in the pathogenesis of AML has not been clarified well, the aim of this study is to evaluate gene expression of these growth factors in peripheral blood mononuclear cells of Iranian patients

with AML. **Methods:** We investigated the mRNA expression of VEGF-A and VEGF-C in peripheral blood mononuclear cells of fifty-eight patients with newly diagnosed AML and sixty-nine healthy controls by Quantitative Real time PCR. **Results:** Expression of VEGF-C mRNA was significantly lower in AML patients than in healthy controls (p= 0.002). However, there was no significant defiance in expression of VEGF-A mRNA of AML patients compared to control group (P=0.325). **Conclusion:** In conclusion, our data showed that AML is associated with a decreased expression of VEGF-C mRNA. It seems that VEGF had a tumor inhibitory role and high levels of VEGF-C inhibit the growth and progression of AML leukemic cells through recruitment of tumor inhibitory monocytic cells.

Keywords: Acute Myeloid Leukemia, Gene expression, VEGF-A, VEGF-C, Angiogenesis

3106P

Myxovirus resistance A (MxA) gene polymorphisms and treatment response in hepatitis C patients

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Background: Myxovirus resistance A (MxA) gene is an interferon stimulating gene which code the interferon-induced human protein located in the cytoplasm of induced cells. MxA gene product are known to inhibit the replication of many negative- sense single stranded RNA viruses, also double stranded RNA viruses and the reverse transcribing DNA virus hepatitis B. SNPs of MxA gene as a genetic host factor influence on the outcome response to IFN –α in chronic HCV patients. On the other hand, expression of antiviral MxA was increased by Ribavirin. Intracellular antiviral proteins, such as 2-5 oligoadenylatesynthetase, dsRNA –activated protein kinas and MxA induced by IFN and play an important role in defense against HCV infection. MxA protein expression as a sensitive marker for HCV replication could be a predictor factor of SVR in HCV patients with G4 who treated with IFN-α2 and Ribavirin. Rs2071430 MxA gene polymorphism is located at position -88 in the gene's promoter region. GG genotype is associated with lower activity of MxA promoter, versus TT genotype with protection to hepatitis C virus infection. SNPs are located on -88 and -123 of MxA gene promoter which associated with susceptibility to hepatitis C virus infection and also treatment response to IFN-alpha. -88 (G/T) and -123 (C/A). IFN-α induced gene enhanced by Ribavirin which included antiviral mediated MxA. The researches indicate, Ribavirin leading to a novel MxA mediated immune-modulator mechanisms which is IFN –α antiviral activity against HCV infection.

3036P

Association of inflammatory bowel disease with familial Mediterranean fever patients in North West of Iran

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Background: Inflammatory bowel disease (IBD) with ulcerative colitis (UC) and Crohn's disease (CD) as the most common forms is an inflammation of the gastrointestinal tract. Familial Mediterranean fever (FMF) is another auto-inflammatory disease as well.We investigated MEFV mutations and prevalence of FMF disease among the North West of Iran patients with IBD and their relationship with the disease severity. Since there are similarities between FMF and IBD, the responsible gene for FMF (MEFV) has been introduced as modifier gene for IBD. **Methods:** This study conducted on 32 patients with ulcerative colitis (UC) and 9 with Crohn's disease (CD). All patients were screened for 6 common MEFV mutations.DNA was extracted from patients, then will be examined mutations in patients with RFLP-PCR and then confirmed the results with direct sequencing methods. **Result and Conclusion:** The aim of this study was to investigate the possible correlation between UC and MEFV gene alterations in North West of Iran. Demographic, clinical and laboratory characteristics of the patients will evaluate as well as the parameters of disease severity.We expect that disease-causing MEFV mutations and FMF disease rate will be increased among our patients with IBD in North West of Iran.

Keywords: Inflammatory bowel disease, ulcerative colitis

2021P

HLA-DRB1 gene variation in southwestern Iranian patients with oral lichen planus

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Background: Oral lichen planus (OLP) is a premalignant chronic inflammatory mucosal disorder. It is a multifactorial disease and in addition to genetic background, infections, stress, drug reactions are suggested as risk factors. Since inheritance of certain HLA alleles may bind and present specific epitopes of self antigens or microbial antigens and induction of inflammation that eventually leads to tissue damage in OLP, genetic analysis of DRB1 was done in this study. **Methods:** HLA-DRB1 typing was done by PCR-SSP method in 40 southwestern Iranian patients with erosive OLP (36 women and 14 men) and mean age of 45±13.3 years. The results were compared with 72 healthy individuals from the same ethnic group and 816 Iranians from different parts of the country. **Results:** No difference was observed in the frequency of DRB1 alleles between patients and controls. **Conclusion:** Analysis of other HLA loci would be helpful to determine if there is any relation between certain HLA alleles and tendency to this disease.

Keywords: Erosive oral lichen planus, HLA-DRB1 gene variation

2186P

Association of the polyomavirus BK infection with CTLA4 genetic polymorphisms in kidney transplant patients

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Background: Polyomavirus BK is one of the most important viral causes of nephropathy

post kidney transplantation. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is expressed on CD4+CD25+ regulatory T cell, and is believed to play a key role in controlling regulatory T cell activation and may contribute to the development of transplant tolerance. Therefore in this study the association between CTLA-4 gene polymorphisms with polyomavirus BK infection was evaluatedin kidney transplant patients. **Methods:** In this cross sectional study EDTA-treated blood samples were collected from 60 kidney transplant patients experienced or not the acute rejection episodes, between years: 2006-2010. The prevalence of polyomavirus BK infection was evaluated by an in-house-nested PCR method. The genetic polymorphisms of CTLA4 (1722 T/C and 1661 A/G) gene were also analyzed by PCR-RFLP protocols. **Results:** The 21 of 60(35%) transplant patients experienced acute rejection and rest of them (39 of 60, 65%) were not experience acute rejection. Polyomavirus BK infection was detected totally in 26 of 60(35.1%) kidney transplant patients. Also polyomavirus BK infection was found in 8 of 21(38.1%) of acute rejected patients. The AA genotype of the CTLA-4 (-1661 A/G) gene was significantly higher frequent in polyomavirus BK infected patients experienced acute rejection. Significant higher frequency of A allele of the CTLA-4(-1661 A/G) gene was found in transplant patients experienced acute rejection. Significant higher frequency of TT genotype and also the T allele of the CTLA-4 (-1722 T/C) gene was also found in polyomavirus BK not-infected patients experienced acute rejection. **Conclusion:** Determination of the higher frequency of AA genotype of the CTLA-4 (-1661 A/G) in polyomavirus BK infected kidney transplant patients experienced acute rejection present that CTLA4 (1661 A/G) genetic polymorphism associate with acute rejection and also with polyomavirus BK infection post kidney transplantation need to confirm in further completed studies.

Keywords: Kidney Transplant, Viral infection, Co-stimulatory Molecule, CTLA-4

2942P

The association between RNase L R462Q *polymorphism* and prostate cancer

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Background: RNase L is a cytoplasmic enzyme of the innate immune system that destroys RNA viruses and also plays an important role in the apoptosis of different cells. The association of RNase L gene polymorphisms with susceptibility to prostate cancer in different populations has been reported. In this study, we investigated the association of RNase L GR462QA polymorphism and prostatic cancer in Sanandaj, Iran. **Methods:** *This* study enrolled61 menwith pathologic confirmationof prostate cancer and101healthy men as control group. The serum PSA levels of the control group were measured using ELISA kits.For men in the prostate cancer group,the genomic DNA was extracted from tissue embedded in paraffin blocks.About 5 ml of whole bloodwas taken from healthy volunteers andthen DNAwas extracted from them.The polymorphism of RNase L GR462QA was identified using ARMS-PCR. **Results:** The mean ages of the patients and control group were 70.12±13.37 and 71.05±9.26 years, respectively. The mean serum PSA level was 1.52 ng/ml, which is normal. The frequencies of GG and GA genotypes in the two groups were not different significantly ($p>0.05$). The frequency of AA genotype in patients was 18.03% while in the control group was 5.94% ($p= 0.02$, OR= 4.71, CI: 1.46-15.2). **Conclusion:** The results of this study indicate that the AA

genotype polymorphism in RNase L R462Q is associated with prostate cancer.
Keywords: RNase L, R462Q, Polymorphism, Prostate cancer.

3233P
Association between Interleukin 27 gene polymorphism and risk of type1 diabetes in the Azerbaijan, Northwest Iran

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Background: Interleukin 27 (IL-27) is a newly discovered cytokine consists of two subunits, the Epstein-Barr virus-induced gene3 (EBI3) and p28. It can promote both anti- and pro-inflammatory immune responses, also Th1 differentiation. IL-27 has been linked to the activation of CD8+ T cells and promotion humoral responses. Therefore, it has been proposed that IL-27 plays a potential role in autoimmune diabetes. However, data regarding to the role of IL-27 in autoimmune diabetes are scarce. Thus, the aim of this study was to investigate p28 gene -964 A>G and 2905T>G polymorphisms in Type 1 diabetes mellitus (T1D) compared to healthy control group. **Methods:** DNA was extracted from blood samples of 110 T1D patients and 302 sex, age and ethnically matched healthy controls. Flanking region of -964 and 2905 position of the IL-27 p28 subunit encompassing 468 bp and 120 bp nucleotides were amplified by PCR and analyzed by restriction fragment length polymorphism (PCR- RFLP). **Results:** IL-27 2905 T>G was not polymorphic in the study populations. However the significant differences were found in allele and genotype frequencies of IL-27 -964 A>G polymorphism between type 1 diabetes and controls (p=0.007 and p=0.028; respectively). This difference was the result of a higher incidence of the IL-27 AA genotype in type 1 diabetes compared to healthy control group. In addition significant differences were not found in IL-27 -964A>G polymorphisms and sex or onset age of type 1 diabetes. **Conclusion**: these data indicate that carriers of IL-27 -964 A allele may have increased susceptibility to type1diabetes.
Keywords: interleukin-27 gene, single nucleotide polymorphism, PCR-RFLP

3046P
Association of eNOSG894T polymorphism with pro-oxidant-antioxidant balance in Thromboangiitis Obliterans

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Background: Geographical distribution of Thromboangiitis Obliterans (TAO) may imply any particular genetic background for this disease. High oxidative stress has been reported in TAO. In addition, It has been suggested that the SNP in endothelial nitric oxide synthase eNOS (G894T) may affect the response of vascular endothelium to increase oxidative stress. The aim of this study was to investigate the association between "T" allele and pro-oxidant-antioxidant balance (PAB) in TAO. **Methods:** Polymerase chain reaction and restriction fragment analysis was done to detect the presence of G894T variant of the eNOS gene in 31 TAO patients. The oxidative stress status in TAO patients was also determined, through direct assessment of PAB assay. **Results:** The frequency of the eNOS GG, GT, and TT genotypes was found to be 54.9, 45.1, and 0 per cent, respectively. The mean of PAB value for GG and GT groups was 63.9±11 and 81.6±12, respectively. A significant difference between the PAB value of GG and GG genotypes in TAO patients was observed (p=0.03). **Conclusion:** The 'T' allele variant was more common in Caucasians (34.5%) than in African-Americans (15.5%) or Asians(8.6%). The higher frequency of "T" allele was also observed among TAO patients. The increased PAB value in the TAO might be due to impairment of the oxidative and anti oxidative pathways; and the effect of cigarette smoke on oxidative stress might be exaggerated in TAO and may lead to inflammatory and thrombotic events. Further studies for evaluating antioxidant therapies on the outcome of TAO, in particular GT genotype, are recommended.
Keywords: Buerger's disease, Thromboangiitis Obliterans, Oxidative stress, polymorphism, eNOSG894T

2710P

Various pattern of CC chemokine expression in term and pre-term neonates along with their respected mothers

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Background: The pathology of pre-term delivery is mostly unknown but is a frequent disorder, worldwide. Previous studies revealed the involvement of chemokines in Pre-term infancy. This project aimed to detect the circulating levels of CCL2, CCL5 and CCL11 in term and pre-term delivered neonates and their respected mothers. **Methods:** Cord and peripheral blood samples were collected from 53 pre-term and 53 term neonates along with their mothers. Serum levels of CCL2, CCL5 and CCL11 were measured by ELISA and their mRNA levels were detected by real time PCR. The demographic parameters were also collected by a questionnaire. **Results:** Present results indicated elevated levels of CCL2 in mothers with pre-term delivery and their respected neonates. Although our results demonstrated that CCL5 was elevated in mothers with pre-term pregnancy but this chemokine was undetectable in their corresponding neonates, however, it was detected in term neonates. We also observed decreased of CCL11

in mothers with pre-term neonates, however, this chemokine was inversely increased in pre-term neonates in compare to term neonates. **Conclusion:** our results are indicative for the fact that chemokine in cord and mothers obtained here could possibly applied as marker for rapid detection of pre-term either due to inflammatory responses or other leading causes observed before delivery. These type of accurate laboratory based examination of early pre-term delivery is also valuable for optimal monitoring of the most favorable pregnancy outcome and to minimize related risks in mother and fetus.

Keywords: Pre-term, Term, Delivery, Neonate, Chemokine, CCL2, CCL5, CCL11

3119P

Interleukin 4 single nucleotide polymorphisms in Febrile Seizures

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Background: Interleukin-4 **(**IL-4) plays a critical role in forming the nature of immune responses. As of its importance in inhibiting the production of pro inflammatory cytokines by monocytes and activated T cells, the IL-4 gene polymorphisms were investigated in a group of patients with febrile seizure (FS), **Methods:** Ninety patients with febrile seizure were enrolled in this study and compared with 140 controls. The allele and genotype frequency of 3 single nucleotide polymorphisms (SNPs) within the IL-4 gene were determined. **Results:** The frequency of the IL-4 -590/C allele in the patient group was significantly higher than in the control group ($p = 0.0001$). The most frequent genotypes in patients with febrile seizure were IL-4 (-33) CC ($p = 0.01$), IL-4 (-1098) GT ($p = 0.046$), IL-4 (-590) CC ($p = 0.0001$) and IL-4(-33) TT ($p = 0.02$). The frequency of the following genotypes was significantly lower in patients compared to controls: IL-4 (-590) TC ($p = 0.0001$) and IL-4 (-33) TC ($p = 0.001$). The most frequent IL-4 haplotypes in the patient group, which were significantly higher than in the control group, were TCC ($p = 0$), TCT ($p = 0.02$), and GTC ($p = 0.02$) haplotypes. In contrast, the frequencies of the following haplotypes in the patient group were significantly lower than the controls: GCC ($p = 0.01$), TTT ($p = 0.007$), and TTC ($p = 0.0006$). **Conclusions:** Certain alleles, genotypes, and haplotypes in IL-4 gene were overrepresented in patients with febrile seizure, which possibly could predispose individuals to this disease.

Keywords: Febrile seizure, Gene polymorphisms, Interleukin-4, Interleukin-4 receptor alpha

2975P

Association between RAD50 Single Nucleotide Polymorphism and Common Variable Immunodeficiency

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Background: Common variable immunodeficiency (CVID) is the most prevalent symptomatic primary immunodeficiency, comprises a heterogeneous group of predominantly antibody deficiency resulting from a block in terminal differentiation of B cells to plasma cells of the different isotypes. Some studies proposed defective DNA mismatch repair system along with defective class-switch recombination and somatic hyper mutation as basis for CVID. This study aimed to evaluate association of a recently reported SNP in *RAD50* with CVID. **Methods:** Thirty nine patients with CVID and thirty four healthy volunteers were enrolled in this study. Real-Time PCR allelic discrimination TaqMan genotyping assay was used to find allele frequencies of *Rs2237060* in all groups. **Results:** The alleles of *Rs2237060* were found with similar frequencies among the patients and healthy controls, though AA genotype was seen with higher frequency in patients with CVID. **Conclusions:** As no association was found between the previously reported genetic variant in *RAD50* and susceptibility to CVID, it could be suggested that identification of a SNP in association with primary immunodeficiencies should be tests in different areas to confirm the results.

Keywords: Immunodeficiency**,** Polymorphism**,** Common variable immunodeficiency (CVID)

2519P

Association of rs3761547 Single Nucleotide Polymorphism of $FOXP_3$ gene with Behçet's disease in Azeri Population of Iran

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Background: Behçet's disease (BD) is a multisystemic inflammatory disorder, which results in recurrent oral and genital ulcers. Nowadays, genetic association studies are employed to explore the contribution of genes or genomic regions to a particular disease. $FOXP_3$ is an

important gene required for the development and function of regulatory T cells in the immune system. SNPs in the FOXP3 gene contribute to susceptibility to some autoimmune disorders. The aim of this study was to investigate the correlation between $FOXP_3$ rs3761547 SNP and BD. **Methods:** PCR -RFLP by *MunI* enzyme was employed to genotype $FOXP_3$ rs3761547 SNP. A sample population of 100 (50 Behçet's patient and 50 control) from Azerbaijan-Sharghi, Iran was considered for this purpose. SPSS 19 software was then utilized for DATA analysis. **Results:** The frequencies of AA, AG and GG genotypes corresponding to rs3761547 SNP were 90%, 6% and 4% in the BD patients and 71%, 12% and 17% in the controls, respectively. The frequencies of AA, AG and GG genotypes corresponding to rs3761547 SNP were 90%, 6% and 4% in the BD patients and 71%, 12% and 17% in the controls, respectively. Moreover, the frequencies of A and G alleles were 93% and 7% in BD patients and 77% and 23% in controls. ***Conclusions*:** This study was the first to analyse the distribution and association of the rs3761547 SNP with BD in the Iranian population. According to results, $FOXP_3$ rs3761547 SNP was not associated with BD. Results of the current study could be completed by further studies with other SNPs or populations to discover the possible relationships.
Keywords: Behçet's disease, FOXP3, rs3761547 Single Nucleotide Polymorphism (SNP), Iran

2921P

Establishment of a recombinant HEK cell line over expressing TIM-3 protein

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Background: TIM-3 includes an amino-terminal immunoglobulin variable domain followed by a mucin domain, a transmembrane domain and a cytoplasmic tail. It was first identified as a receptor specifically expressed by T helper type 1 cells that binds ligands, including galectin-9 and exposed, cell-surface phosphatidylserine. When galectin-9 bound to Tim-3, generates an inhibitory signal that results in the apoptosis of T helper type 1 cells. The utilize of Tim-3 protein recombinant can in a broad diversity of cellular and biological implementation of immune responses be useful. **Methods:** pcDNA3.1 vector contains Tim-3 and selection marker (hygromycine) was purchased from genecopia. The restriction enzymes and primers were designed using Gene Runner software. Plasmids were transformed into the E.Coli Top10 strain to proliferate. Bacteria were cultured in LB-Broth medium (Merck) containing 100 microgram/ml ampiciline and cultured in L.B agar. All clones and subclones were confirmed by colony PCR. The positive product of colony PCR were culture in LB-Broth medium to extract plasmid pcDNA3.1 -Tim-3 and digested with Eco31 to created linear form and this product used for transfection in HEK-293 cell line. Transfection was done by calcium-phosphate protocol and treated with 150 mg hygromycine antibiotic. After two weeks the rate of transfection and expression of tim-3 was detected by FACS calibure. **Results:** There was 88% stable cell line transfection in tim-3 expression with anti- Tim-3 PE. And so the PCR was shown it. **Conclusion:** Using FACS analysis the target protein TIM-3 expression in the stable transfectants cultured was detected. These results demonstrated that the stably transfected cell lines that express high titer of recombinant protein can be simply obtained by using selective growth medium and we can use these for further studies or clinical trial studies.
Keywords: Recombinant HEK, TIM-3 protein

2817P

TGF-β, IL10, and IL4 allele and haplotype ferequencies in Iranian patients with chronic rhinosinusitis

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Background: chronic rhinosinusitis is a common disease characterized by inflammation of paranasal sinuses mucosa and is a major health problem. Different mechanisms have been proposed to explain the etiology of CRS, including cytokine single nucleotide polymorphisms. **Methods:** In this case control study, 85 confirmed CRS patients aged16) to 52 years old) and 215 healthy subjects, age and sex matched as control group were enrolled. Genomic DNA was extracted using salting out method. Cytokine gene polymorphism including, allele, genotype and haplotype of TGF-β (codon 10 &25), IL4 (-33, -590, -1089) and IL10-1082 ,-819 ,-592)) were determined by PCR-SSP method. **Results:** The results of this study showed that IL-4 C allele (P=0.00001, OR=3.25) and TGFβ codon 10 C allele (P=0/0005, OR=1/79) and IL-10 -1098 GG, -590 CC and -33 CC genotype (P<0.00001, OR=0.23, P=0.00001, OR=127.86 and P=0.00001, OR=1.82 respectively) were considered as predisposing factors in CRS patients. **Conclusion:** The results of this study with other studies on cytokine gene polymorphisms and CRS, indicating the important role of cytokine gene polymorphisms in the etiology of the disease.

Keywords: Chronic rhinosinusitis, CRS, Gene polymorphisms, Cytokine, SNP

2768P

Association of the SNP: rs10954213 in IRF5 gene With Recurrent Spontaneous Abortion(RSA)

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Background: Recurrent spontaneous abortion (RSA) is defined as three or more consecutive abortions before the 20th week of gestation. There is increasing evidence to support an immunological mechanism for the occurrence of RSA. The purpose of our study was to examine whether single-nucleotide polymorphisms (SNPs) in IRF5 influence susceptibility to, recurrent spontaneous abortion. **Methods**: This is a case-control study .We recruited 200 patients with RSA(case group) using established diagnostic criteria and 200, normal individuals (control group) at the fertility and infertility center in Yazd city and Isfahan city during the period from 2012 to 2013 . We screened the SNP: rs10954213 in IRF5 in patients and controls by Real time method, and we performed an association analysis between SNP: rs10954213 in IRF5 variant and RSA. The data was analyzed by SPSS 16 software using Chi-square test. **Results:** significant differences in the genotype and allele frequencies of the SNP: rs10954213 in IRF5 were identified between patients with RSA and healthy controls. **Conclusion**: The frequency of SNP: rs10954213 in IRF5 in patients with recurrent spontaneous abortion is differed significantly compared with the control group.

Keywords: Recurrent spontaneous abortion, IRF5, SNP: rs10954213

2471P

Relation of Choline dehydrogenase gene polymorphism with reduced mobility or lack of sperm motility

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Background: Annually, about 60 to 80 million people worldwide suffer from infertility; Inability of a couple to conceive after one year of intercourse without contraception referred Infertility, and Ten to fifteen percent of infertile couples can be seen. The causes of infertility can be about male or female or both. Approximately 40% of infertile related to the men, 40% women and 10% is about of both them. About 10% of couples, infertility is unclear. Today, using supporting reproductive techniques ARTs to treat infertility is on the rise. Normally, the testes produce sperm and after male ejaculate, it comes out of him. If there has been a problem about sperm movement ability, Chance of fertilization is considered as low and men will be infertile. In this experiment the oxidation of choline metabolite betaine is the most important part of our consideration. Chromosomal location of this gene is 3p21.1, which is studied in polymorphism rs 6445606. Guanine will mutate to adenine, which causes the Low sperm mobility or lack of mobility. **Methods:** In this project, according to specialist, the men who had been diagnosed with idiopathic infertility, collecting sperm (semen) of 75 patients with 75 healthy subjects with high mobility and low mobility of the male population in Rasht. DNA was extracted using the GPP solution sets of samples were taken from patients and healthy males and the quality of the extracted DNA were analyzed using 0.8% agarose gel. **Results:** Primers Preparation of single nucleotide polymorphism rs6445606 and proper PCR primers for detecting studied polymorphisms samples is being conducted. Observed PCR products on 2% agarose gel and learn to understand the impact of polymorphisms on susceptibility to idiopathic infertile men with idiopathic infertility, data analysis and statistical analysis using software (Medcalc version 12) for Meaning or the lack of results is under review. **Conclusion:** May be the role of the genetic composition in association with Polymorphism will be investigated. Although future results may change with population genetic Treasury. The results of this research are not conclusive and need further studies to confirm the performance of this polymorphism. In addition, this polymorphism plays a significant role in many diseases such as certain relationships between some cancers have been observed.

Keywords: Choline dehydrogenase (CHDH), polymorphism, low sperm motility, PCR

2399P

Production of Stable human CD31-expressing Mouse Fibroblast NIH-3T3 Cell Line

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Background: PECAM-1 or CD31 is a glycoprotein expressed on endothelial cells and bone marrow precursor cells. It plays some important roles in angiogenesis, maintenance and integration of the cytoskeleton, and direction of leukocytes to the site of inflammation. We aimed in this study to clone the cDNA coding for human CD31 from KG1a cell line and stably express in NIH-3T3 mouse cell line. **Methods:** KG1a cells were cultured and surface expression of CD31 protein was confirmed by flow cytometry. Then, total RNA was extracted and cDNA was synthesized. Specific band amplified by Pfu DNA polymerase was ligated to pGEM-T easy vector. DH5α competent bacteria were transformed by heat shock method and plasmid from a single positive colony was extracted. After sequencing and confirming the complete sequence, CD31 cDNA was sub-cloned in pCMV6-Neo expression vector and produced construct was transformed in NIH-3T3 cells. Stable expression was obtained by continued growing of cells in presence of G418 antibiotic. **Results:** 2235 bp band size for human CD31 was seen and correct nucleotide sequence was confirmed by full alignment of the amplicon to reference sequence in NCBI database. Stable expression of human CD31 on NIH-3T3 cells, was confirmed by surface flow cytometry after 4 weeks screening of transfected cells. **Conclusions:** Because of mouse origin of NIH-3T3 cell line, prepared stably CD31-expressing NIH-3T3 cells could be helpful to produce mouse monoclonal antibodies used in research and diagnosis.

Keywords: CD31, Angiogenesis, Cloning, Stable expression, PCR.

2398P

Stable Expressing the Human CD19 Full-length cDNA in Mouse Fibroblast Cell Line

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Background: CD19 is a pan-B cell marker and appealing target for therapeutic monoclonal antibody approaches to treat some autoimmune and malignant diseases. The aim of this study was to produce a mouse cell line stably expresses human CD19 molecule to prepare an appropriate immunogen as first step in antibody production during future experiments. **Methods:** Total RNA was extracted from Raji cells in which expression of CD19 was confirmed by flow cytometry. Then, cDNA was synthesized and used for CD19 gene amplification by PCR using Pfu DNA polymerase. PCR product was ligated to pGEM-T easy vector, ligation mixture was transformed to DH5α competent bacteria and colonies were screened using blue/white selection method. Selected single positive colony was confirmed by sequencing. Then, CD19 coding sequence was sub-cloned into pCMV6-Neo expression vector by double digestion using KpnI and HindIII enzymes. CD19-pCMV6-Neo recombinant vector was subsequently transfected to NIH-3T3 mouse fibroblast cell line using Jet-PEI transfection reagent. After that, stably-transfected cells were selected by increasing concentrations of G418 antibiotic. After 4 weeks, stable surface expression of CD19 on transfected NIH-3T3 cells was confirmed

by flow cytometry. **Results:** Amplification of CD19 cDNA gave rise to 1701 bp amplicon confirmed by alignment to reference sequence in NCBI database. Flow cytometry analysis indicated that CD19 molecule was successfully expressed on NIH-3T3 cells. **Conclusion:** As NIH-3T3 is mouse fibroblast cell line, stable cells expressing human CD19 molecule can be used as proper immunogen for production of monoclonal antibodies, extensively used in diagnostic, therapeutic and research areas.

Keywords: CD19, Cloning, Stable Expression, NIH-3T3, Immunotherapy.

2453P

Iranian Patients with the Phenotype of Hyper Immunoglobulin E Syndrome: A Long Term Follow up of 51 Patients

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Background: Hyper IgE syndrome (HIES) is one of the known primary immunodeficiency presented by recurrent cutaneous and lung infections, eczema, and connective tissue and skeletal abnormalities as well as elevated serum IgE. **Methods:** We investigate for recognized aspects of the clinical phenotype and discuss recent genetic and immunologic findings in 51 cases (25 males, 26 females) with HIES who were followed for a total of 240 patient years. **Results:** We documented the molecular, cellular, and clinical features of 17 patients with heterozygous *STAT3* mutations and 1 patient with *DOCK8* mutation. As a result of our survey we found that SCORAD >51 is a good screening test for HIES, however for finding of STAT3 deficient patients, screening NIH score needs to be consider with other parameters especially pneumatocele. **Conclusion:** Specific molecular study of *STAT3* and *DOCK8* mutations in patients with HIES clinical manifestations helps physician to definitively characterize the disease and tounderstand more about the mechanism of eczema, IgE regulation, infection susceptibility, coronary artery disease, scoliosis, and bronchiectasis as well as provide strategy into therapeutic modalities.

Keywords: Hyper IgE syndrome, Pneumatocele, STAT3, DOCK8

2242P

Response to therapy in patients infected with HCV genotype 1 but not genotype 3 is associated with IFN-γ gene polymorphism at +2109A/G locus in Fars province, south of Iran

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Background: Hepatitis C virus (HCV) infection is a worldwide health problem, which associated with cirrhosis and hepato cellular carcinoma. With currently treatment regime, pegIFN plus ribavirine, sustain virological response (SVR) is achieved in only 50% of infected individuals. In HCV infection an inappropriate ratio of cytokine may effect on the benefit of antiviral therapy. Given the polymorphisms in regulatory regions of cytokines genes influence

cytokine production. The present study conducted to determine the frequency of genotype and allele of IFN-γ gene at +2109A/G locus in patients infected with HCV genotype 1 and 3 and investigates their association with response to therapy. **Methods:** 119 patients were treated with PEG-interferon and ribavirin is included in this study. The presence of HCV infection in patients was confirmed by RT-PCR. Genomic DNA of the participant was extracted using salting out method. IFN-γ gene polymorphisms were carried out by polymerase chain reaction using sequence specific primers and PCR-RLFP on genomic DNA. **Results:** Of 119 patients, 69 and 50 subjects infected with genotype 1 and 3, respectively. Of the patients, 104 were mail and 15 were female. The mean age of participants was 40.47. The frequency of AA genotype and A allele of IFN-γ gene at +2109 loci were significantly different between responder and non-responder subjects infected with genotype 1 (p=0.041; OD: 0.05; CI: 1.05-33.25). No association was found between this polymorphism and response to therapy in patients infected with genotype 3. **Conclusion:** Our finding indicated that heterogeneity at +2109 loci of IFN-γ gene could interfere to successful therapy in patients infected with HCV genotype 1 but not genotype 3.

Keywords: Hepatic C virus, Interferon-γ, Polymorphism

2243P

No association of IFN-γ (A+874T) gene polymorphisms withoutcomeof HCV infection in Fars province, Sothern of Iran: Spontaneous clearance versus chronic infection

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Background: Hepatitis C virus (HCV) infection is a worldwide health concern, which associated with end-stage liver diseases including cirrhosis and hepathocellular carcinoma. 10-40% of individuals infected with HCV may spontaneously clear the infection, generally within the initial 6 months. It has been reported that spontaneous clearance of HCV infection is associated with some polymorphisms at interleukin genes. Therefore we aim to determine the effects of IFN-γ (A+874T) gene polymorphisms on spontaneously clearance/chronic infection. **Methods:** 200 patients with chronic hepatic C and 44 individuals who spontaneously clear the infection were included in this study. The presence of chronic and/or spontaneously clearance of HCV infection in participants were confirmed using ELISA and quantitative and qualitative RT-PCR techniques. Genomic DNA of the participant was extracted using salting out method. IFN-γ gene polymorphism was carried out using PCR-RLFP method on genomic DNA. **Results:** The frequency of genotype and allele of IFN-γ gene at +874 loci were not significantly different between individuals with chronic infection and those who clear the disease. **Conclusion:** Our finding indicated that heterogeneity at +874 loci of IFN-γ gene could not interfere with chronocity/spontaneous clearance of HCV infection.

Keywords: Hepatic C virus, interferon-γ, Polymorphism, Chronic infection, Spontaneous clearance

3283P

Genetic polymorphisms of CCL22 and CCR4 in patients with lung cancer

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Background: The association of lung cancer and chemokines has been suggested in recent years. This study was aimed to investigate the association of 16C/A single nucleotide polymorphism (SNP) (rs. 4359426) in C-C motif chemokine 22 (CCL22), as well as C1014T SNP (rs. 2228428) in C-C chemokine receptor type 4 (CCR4), which serves as the receptor for CCL22, with lung cancer. **Methods:** Genotyping was performed in 148 lung cancer patients and 148 normal controls using Polymerase Chain Reaction-Restriction-Fragment Length Polymorphism (PCR-RFLP). The data were verified by direct automated sequencing. **Results:** Frequencies of CC, CA and AA genotypes of 16C/A SNP in CCL22 gene were 112 (75.7%), 33(22.3%), and 3(2.0%) in patients, and 119 (80.4%), 24 (16.2%) and 5(3.4%) in controls, respectively. No significant differences were observed in genotype frequencies at this position between cases and controls (P= 0.34). Moreover, there was no significant association between CCL22 polymorphism and the types of lung cancer in patients. The distribution of CC, CT and TT genotypes of C1014T SNP in CCR4 gene, was 76(51.4%), 60(40.5%), and 12(8.1%) in patients and 80 (54.1%), 49 (33.1%), and 19(12.8%) in controls, respectively. No statistically significant differences were observed in genotypes frequencies of CCR4 gene between patients and controls (P=0.24). The genotype inherited by patients observed not to be associated with the type of lung cancer (P>0.05).**Conclusion:** Our results reveal that CCL22 gene polymorphism at position 16C/A, and CCR4 gene polymorphism at position C1014T, appear not to be associated with susceptibility to lung cancer.

Keywords: Genetic Polymorphism, Lung cancer, Chemokine CCL22, CCR4 receptor

2244P

Lack of association between rs1799983 polymorphism and Parkinson's disease in Iran

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Background: There are many common risk factors such as rs1799983 polymorphism that play key roles in the development of Parkinson disease. It has extensively established that nitric oxide (NO) is involved in Parkinson pathogenesis. Numerous genetic risk factors have been related with Parkinson, but no study has unraveled a possible association between Parkinson and rs1799983 polymorphism. Evidence suggests that NOS3 might play a role in Parkinson, as a result we Studied eNOS common polymorphisms (rs1799983 polymorphism) in Iranian with Parkinson. **Methods:** We conducted study including a clinically well-defined group of 52 Parkinson patients to test the association between rs1799983 polymorphism and Parkinson in Iranian population. In the present case control study, the eNOS polymorphisms (rs1799983 polymorphism) have been investigated in 52 patients with Parkinson and 100 healthy subjects

by using ARMS-PCR methods. Then, the data were analyzed by pasw statistics 18 (SPSS) software. **Results and Conclusion:** The results of this study did not show considerable association between Parkinson's disease and rs1799983 polymorphism in Iranian population.
Keywords: Gene Polymorphism, Nitric oxide, rs1799983 polymorphism, Parkinson.

2388P
Association of the G894T polymorphism of the endothelial nitric oxide synthase gene with migraine: an Iranian case-control study

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Background: It has extensively established that nitric oxide (NO) is involved in migraine pathogenesis. Numerous genetic risk factors have been related with migraine, but no study has unraveled a possible association between migraine and G894T polymorphism. There are many common risk factors such as NOS3 gene G894T polymorphism that play key roles in the development of migraine disease. Polymorphisms in the endothelial nitric oxide synthase (NOS3) gene have been associated to migraine in a some populations. However, other groups failed to replicate this finding in other populations. Evidence suggests that NOS3 might play a role in migraine, as a result we Studied eNOS common polymorphisms (G894T polymorphism) in Iranian with migraine. We conducted study including a clinically well-defined group of 67 migraine patients to test the association between NOS3 G894T polymorphism and migraine in Iranian population. In the present case control study, the eNOS polymorphisms (G894T polymorphism) have been investigated in 67 patients with migraine and 100 healthy subjects by using ARMS-PCR methods. Then, the data were analyzed by pasw statistics 18 (SPSS) software. The results of this study did not show significant association between migraine and NOS3 gene G894T polymorphism in Iranian population.
Keywords: Gene Polymorphism, Nitric oxide, Nitric oxide synthase, G894T polymorphism, migraine

3025P
Association of HLA DRB1 gene variant in patients with multiple sclerosis in south west of Iran

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Multiple sclerosis (MS) is an inflammatory disease of the central nervous system. It is presumed to be an autoimmune disorder, causing severe neurological disability as a result of demyelination. It is likely that a combination of genetic and environmental factors is involved

in this condition. As in other complex diseases with autoimmune features, a genetic association with the human leukocyte antigen (HLA) complex is well documented. The most promising candidate gene associated with MS is HLA-DRB1. The HLA-DRB1(GeneID: 3123) gene is part of a family of genes called the human leukocyte antigen (HLA) complex. The HLA complex helps the immune system distinguish the body's own proteins from proteins made by foreign invaders such as viruses and bacteria. The *1501 allele of HLA is present in approximately 48% of MS patients, indicating that it is likely involved in the pathogenesis of the disease. The individual risk for developing MS is about twice as high in individuals who carry two copies of the *1501 allele.Variations in the HLA-DRB1 gene have been associated with an increased risk of developing multiple sclerosis. This condition affects the brain and spinal cord (central nervous system), causing muscle weakness, poor coordination, numbness, and a variety of other health problems. One variant of this gene, called HLA-DRB1*15:01, is the most strongly linked genetic factor for the risk of multiple sclerosis. **Methods**: The 50 MS patients and 50 controls will be include in this study will be diagnose by neurologists. Blood samples will be obtain from all patients with MS and will be store at −20ºC. DNA will be extract from blood leukocytes by the standard phenol-chloroform method. DNA will be dissolve in sterile double distilled water. HLA DRB1 alleles for MS patients will be genotype using a polymerase chain reaction (PCR) assay with amplification of the second exon of the genes. HLA DRB1 alleles will be genotype using the PCR assay with sequence specific primers (HLA DRB1*-PCR). The amplified products will be determine by means of agarose gel electrophoresis. **Results:** The aim of this study is analysis of the associations between HLA DRB1 alleles, especially HLA DRB1*15, and the onset, progression, or severity of the disease in north-west population of Iran, and comparison of results with similar results in other regions of Iran and world. Association between the HLA DRB1*15 allele and MS suggests that immunological changes affecting the prognosis of the disease might be regulated by genetic factors, i.e., supports an idea of genetic origin of MS.

2438P

Effect of silencing hdm2 expression by small interfering RNA on radiosensitivity of esophageal squamous cell carcinoma

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Background: Esophageal cancer is highly aggressive and is the sixth leading cause of cancer death worldwide. The hdm2 oncogene has now been shown to be amplified or overexpressed in many human cancers including esophageal cancer. It also has been suggested that hdm2 levels are associated with poor prognosis of cancers. The most exciting finding is the hdm2-p53 autoregulatory feedback loop that regulates the function of the p53 tumor suppressor gene. The hdm2 gene is a target for direct transcriptional activation by p53, and the hdm2 protein is a negative regulator of p53. The p53 tumor suppressor has an important role in cancer therapy, with p53-mediated cell growth arrest and/or apoptosis being major mechanisms of action for many clinically used cancer chemotherapeutic agents and radiation therapy. Therefore, the

hdm2-p53 interaction may be a target for cancer therapy. If the hdm2 feed-back inhibition of p53 is interrupted, a significant increase in functional p53 levels will increase p53-mediated therapeutic effectiveness. To explore the influence of silencing hdm2 expression by small interfering RNA (siRNA) on hdm2 protein expression, cell apoptosis rate and radiosensitivity of esophageal Squamous Cell Carcinoma this research managed. **Methods:** A siRNA segment for hdm2 gene was designed and synthesized, then introduced into the TE1,TE8,TE11 esophageal carcinoma cell lines by liposomal transfection. hdm2 gene and protein expression detected by real time PCR and Western Blotting, respectively. TUNEL and MTT assays and also colony forming assay were used to determine the effects of hdm2- siRNA on TE1,TE8,TE11 cells apoptosis rate and radiosensitivity following 1,2,3, and 4 Gy irradiation. **Results:** Here our results showed that hdm2-siRNA silenced its target mRNA specifically and effectively in human esophageal cancer cells, reduced tumor cell proliferation and induced apoptotic cell death ($p<0.05$). In the transfected TE1,TE8,TE11 cells, there was a significantly increased apoptosis rate than the control and negative siRNA transfection groups with and without radiation after 24 hours($p<0.05$). Cell survival and proliferation were measured by clonogenic survival assays. D_0, and SF values in hdm2-siRNA transfection group were all lower than those in the control group.MTT results showed that hdm2-siRNA transfected cells have a higher cell apoptosis rate than negative vector transfected cells or untreated cells after treatment with 1,2,3,4 and 6 Gy radiation.
Conclusions: Radiotherapy is a well established modality for treating many forms of cancer. However, despite many improvements in treatment planning and delivery, the total radiation dose is often too low for tumor cure, because of the risk of normal tissue damage. Gene therapy provides a new adjunctive strategy to enhance the effectiveness of RT, offering the potential for preferential killing of cancer cells and sparing of normal tissues. The hdm2-siRNA can effectively inhibit the expression of hdm2 gene, enhance the radiosensitivity and apoptosis of gastric cancer TE1, TE8, TE11 cells, having potential clinical benefits prospective.
Keywords: Esophagus cancer, TE1, TE8, TE11 cell, hdm2 gene, Radio sensitization, RNA interference

2474P

Genetic fusions of cfaB-ST toxoid, cssA/B and ltB of Enterotoxigenic Escherichia coli elicit neutralizing serum antibodies in mouse model that protect immunized animals against ETEC challenge

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Background: Enterotoxigenic Escherichia coli (ETEC) strains are the most common cause of bacterial diarrhea in children in developing countries and travelers to these areas. Enterotoxins and colonization factors (CFs) are two key virulence factors in ETEC pathogenesis and the heterogeneity of the CFs is the bottleneck in reaching an effective vaccine. In this study a candidate subunit vaccine which is composed of CfaB, CssA and CssB, structural subunits of CFA/I and CS6 colonization factors, LTB, the binding subunit of LT and ST toxoid, was

designed to provide broad spectrum of protection against ETEC. **Methods:** The chimeric gene, *c3l*, was synthesized and expressed in BL21 (DE3). The chimeric recombinant protein was purified with Ni-NTA columns and after dialysis was injected to mouse model. The ELISA and immunoblot analysis indicated the levels of anti-chimeric IgG and IgA. The challenge study was done with pathogenic bacteria for test of protection potential of elicited immunity. **Results:** In ELISA and immunoblot assays elicited antibodies detected chimeric protein and its subunits suckling mouse assay showed the ST neutralization potential of anti-chimeric serum. In the challenge study the immunized mouse were protected against pathogenic bacteria. **Conclusion:** The designed chimeric protein could be a candidate vaccine against ETEC infection.

Keywords: Enterotoxigenic *Escherichia coli*, candidate vaccine, virulence factors, enterotoxins, Immunity.

2547P

Single nucleotide polymorphisms (SNPs) of *CDH1* and *FGFR2* genes are correlated with increased risk of breast cancer

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Background: Strong heritable background has been related to breast cancer, which approximate cases who exhibit a family history of the disease are 15%. Mutations in genes such as **BRCA1**, **BRCA2** and **TP53** make people subject to an autosomal dominant inherited cancer possibility. A number of mutant alleles have been identified in genes such as **CHEK2**, **PALB2**, **ATM** and **BRIP1**. Cadherin-1 also known as E-cadherin (CD324) is a protein that in humans is encoded by the *CDH1* gene which is a tumor suppressor gene. Fibroblast growth factor receptor 2 (FGFR2) also known as CD332 is a protein that is encoded by the *FGFR2* gene, is a receptor for fibroblast growth factor.*CDH1* and *FGFR2* genes play a role in carcinogenesis. It has already been demonstrated that polymorphisms in *CDH1* and *FGFR2* genes is related to various malignant disorders. The aim of present study was to reveal the affection possibility towards breast cancer in relation to SNPs of *CDH1* and *FGFR2* genes. **Methods:** Tumor biopsy of 50 breast cancer affected women and whole blood of 30 healthy controls were collected. Afterward, genomic DNA content of the samples was extracted. Genotyping for detection of SNPs was performed through PCR-SSCP, PCR-RFLP, and sequencing. **Results:** Detected SNPs significantly increased risk for breast cancer was found for allele carriers. The single-nucleotide polymorphisms (SNPs) rs1219648, rs2981582 in *FGFR2* gene and rs1212348, rs2981382 were identified. **Conclusion:** Nucleotide changes comparison between breast cancer contracted patients and healthy controls depicted that there was SNPs frequency significantly in patients. This study supports earlier suggestions that SNP in the *CDH1*and *FGFR2* is a risk factor for breast cancer.

Keywords: SNP, CDH1, FGFR2, breast cancer

2661P

Insilico analyses of genome variation in regulatory genes involved in MS

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Background: Multiple Sclerosis (MS) is an autoimmune demyelinating disorder in which Interleukin 17 (IL-17)-producing helper T cells (TH17 cells) play a critical role. Micro RNAs are a new group of non-coding RNAs, take part in post-transcriptional regulation of gene expression by pairing with 3'UTR of their mRNA targets and inhibition of their translation. It has been demonstrated that micro RNA function in various cellular processes such as differentiation, proliferation, and apoptosis. It is possible that variations in micro RNA coding region may influence disease susceptibility. **Methods**: by using prediction data-bases and previous studies, mir-23a was chosen as an effective micro RNA in pathogenesis of MS disease. mir-23a gene has SNP in its regulatory region that can influence its expression. RFLP method was selected for this evaluation, and oligo7, beacon desiger7 and restriction mapper3 have used. **Results:** mir23a located in ZSWIM4 (zinc finger, SWIM-type containing 4) gene on the chromosome 19. based on NCBI database we elicit about450bp sequence that involves rs3745453, after that, we designed specific primers for amplification oligonucleotide contain variation by using oligo7. Restriction mapper3 was used for determining which restriction enzyme could display the variation. **Conclusion:** The result of this study could enable us to introduce the appropriate marker in mir23a gene region for molecular diagnoses of MS in the Iranian population.

Keywords: mir23a, MS, SNP, Iranian population

3030P

HLA Class I allele frequencies in Khuzestan population

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Background: Several genes have been identified to be involved in autoimmune disease susceptibility and unambiguously MHC genes are associated with these disorders, such as multiple sclerosis, arthritis rheumatoid, diabetes, and etc. The MHC geneson the short arm of chromosome 6 (including HLA) are integral to normal function of the immune response. Many of these genes play important parts in various immunological processes. Based on the structure of the antigens produced and their function, there are two classes of HLA antigens, termed accordingly, HLA Class I and Class II.HLA-A*03(HLA-A3) is one of HLA class I haplotypes that is unambiguously associated with autoimmune disease. Our aim was to study the frequency of this haplotype in Arab and non-Arab population from Khuzestan Province. **Methods:** In this study, the frequency of HLA-A3 allele was investigated in 200 people in Khuzestan. HLA-typing for HLA-A3 was performed by sequence specific primer polymerase chain reaction (SSP-PCR) amplification. **Results:** The frequency of HLA-A3 allele in this population is 26%. 25% of females and 30% of males have HLA-A3, and 26% of Arabs,

and 21.7% of non-Arabs have HLA-A3. **Conclusion:** This study indicates the frequency of HLA-A3 in Khuzestan. The highly polymorphic Human Leukocyte Antigen system encompasses different loci that have been studied in transplantation as well as autoimmune diseases and population associated research. Using the frequencies of different alleles one can calculate the genetic distances which can help in the classification of certain populations.
Keywords: HLA-typing, autoimmune disease, MHC , HLA-A3, Khuzestan population

2917P
Studying of M694V gene mutation in susceptibility of SLE disease

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Background: Immune system related diseases constitute a large spectrum of monogenic disease like Familial Mediterranean Fever (FMF) to complex genetic disease such as systemic lupus erythematouse**.** SLE is a systemic autoimmune disease characterized by inflammation, overproduction of antibodies. MEFV gene encodes Pyrin or Marenostrin that is an inflammatory regulator, which acts as pro-inflammatory and anti-inflammatory factor that is the main causative gene in FMF and yielding increased caspase-1 activation and IL-1β secretion. M694V, is a mutation in exon10 of MEFV gene which is occurred by substitution of G instead of A in nucleotide sequence that lead to conversing Methionin to Valin that is the most severe and common mutation, which is associated with inflammation and amyloidosis in patients. **Methods:** Present research was done on 31 SLE patients and 31 controls and aimed to identify the association between M694V mutations of MEFV gene with susceptibility of SLE disease. ARMS-PCR technique was used to determine M694V mutation. **Results:** significant differences between cases and controls were observed in the M694V mutation. To verify the results of ARMS-PCR technique, a number of samples were randomly sequenced. **Conclusion**: Due to these findings, the MEFV directly or indirectly affects the susceptibility of SLE disease. Although the large number of patients are required to confirm this association.
Keywords: SLE, FMF, MEFV,M694V, ARMS-PCR

2925P
To study association between the 1188 A/C rs3212227 polymorphism of Il-12β gene and hepatitis B disease in Iranian patients

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Background: Hepatitis B disease caused by hepatitis B virus is one of the major health problem globally. In case of hepatitis B, the T-cell mediated immune responses have pro vital role in controlling of the disease. But the weak T-Cell mediated responses cause chronic hepatitis B in patients. Interleukin-12 is required for the optimal generation of T-cell mediated cells and has critical role in the clearance of intracellular pathogens. So the aim of this study was to investigate the relation between polymorphism 1188 A/C rs3212227 of Il-12β gene and hepatitis B infection to better understanding of association between HBV and T-cell mediated immune responses. **Methods**: The study consisted of 300 subjects including 150 HBV infected patients and 150 sex and age matched healthy controls. Molecular techniques based on PCR-

RFLP were done to detect the 1188 A>C rs3212227 IL-12 gene polymorphism. Finally, statistical analyses logistic regression, Chi-square, and descriptive statistic were performed by SPSS software. **Results:** In control group, the frequency of AA, AC and CC genotypes of rs3212227 were 54.7%, 38.9% and 6.4%, in compare with 64.5%, 31.8% and 3.69% in patient group, respectively (*p*: 0.315, OR:1.335, CI 95% for OR: 0.6025- 2.959). **Conclusion:** Our results indicates no difference between frequency of 1188 A>C rs3212227 gene polymorphism in Iranian hepatitis B patients compare with healthy population. Although further studies of known IL-12 gene polymorphisms are necessary to confirm any association with hepatitis B.
Keywords: Interleukin 12, gene polymorphism, Hepatitis B

3072P
Association between the il-12a gene polymorphism rs 2243115 and hepatitis b in Iranian patients

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Background: Cytokines, involved in the T-helper 1 system, play a role in the regulation of hepatitis B virus (HBV) clearance and the immune response to HBV antigens during natural infection or planned vaccination. Our aim was to examine whether the polymorphic variants of IL-12 are equally associated with Resistance to Hepatitis B virus. Thus, the aim of this study is to investigate the relationship between the single nucleotide polymorphisms of 5' noncoding region of IL-12 p40 subunit gene on position-564, and susceptibility to hepatitis B. **Methods:** Our study was performed on 433 HBV-infected persons and 161controls. Genotyping procedures for identifying the rs2243115 (5'UTR T>G) polymorphism were affected by the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method. **Results:** The frequency of GG, GT and TT genotypes of this gene at position-564was respectively calculated 2.1, 21.7, 76.2 in group of patients and 1.3, 24.8, 73.9 in the control group. No significant difference was found between both case and control groups. P value: 0.599 OR: 1.265,CI 95% for OR: 0.548-2.917. **Conclusion:** Our results indicated that the IL-12A G>T polymorphism was not associated with the development of HBV infection in the Iranian population. Further studies are needed to identify the host genetic factors in immune defense including cytokine gene polymorphisms of both IL-12A and IL-12B.
Keywords: Hepatitis B, IL-12, Single nucleotide Polymorphism,

3210P
A novel nonsense mutation in ITGB2 gene causing severe leukocyte adhesion deficiency in an Iranian patient

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Background: Leukocyte adhesion deficiency type 1 (LAD-1) is a rare, autosomal recessive

inherited immunodeficiency disease caused by mutations in the ITGB2 (CD 18) gene and characterized by recurrent severe bacterial infections with lack of pus formation. **Methods:** In this report, we investigated ITGB2 gene (CD18) mutations in a four-month-old girl with recurrent hospitalizations because of delayed umbilical cord separation and necrotic tissue in the external ear she had no CD18 positive neutrophyl cells in Flow cytometry. These were all compatible with a diagnosis of LAD-1. All of coding regions of the ITGB2 gene were screened by direct sequencing of genomic DNA. **Results:** Gene analysis revealed a novel homozygous c.1821C>A nonsense mutation in exon 13 of ITGB2 gene, causing the substitution of Tyrosine to stop codon at the 607 amino acid. His parents were both heterozygous carriers. **Conclusion:** Our data indicated that this premature stop codon causing a complete absence of CD18 protein and lead to severe LAD-1 clinical phenotype. These findings will be useful for future studies of LAD-1 and genetic diagnosis in other patients.
Keywords: Leukocyte adhesion deficiency; nonsense mutation; immunodeficiency

3211P

IL-22 gene polymorphism in Iranian patients with Inflammatory bowel disease

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Background: Inflammatory bowel disease is a chronic inflammatory disorder of unknown cause that affects the digestive system. Recent studies showed that interleukine-22, a member of IL-10 cytokine family, is involved in inflammatory processes during the disease. The purpose of this study was to investigate the relationship between IL-22 gene polymorphism (rs2227503) and the susceptibility to inflammatory bowel disease. **Methods:** 89 patients and 201 healthy individuals referred to the Namazi Hospital of Shiraz, Iran were entered in this case-control study. Blood samples were collected and their genomic DNA was extracted. Restriction fragment length polymorphisms-Polymerase chain reaction technique (PCR-RFLP) was performed. Data were analyzed using a Chi-square test. **Results:** Allele frequencies and genotypes in patients and controls were calculated. Although the frequency of G allele was greater and A allele was lower in patients compared with controls, these data were not statistically significant ($p>0.05$). Also, there was no relationship between the rs2227503 related genotypes and IBD. **Conclusion:** This is the first study of evaluating rs2227503 in patients with IBD. More studies are required to clarify the exact role of IL-22 in pathogenesis of inflammatory bowel disease.
Keywords: Inflammatory Bowel Disease, Interleukin-22, Polymorphism.

3221P

Association of IL-23 Receptor Gene Polymorphisms with Juvenile Idiopathic Arthritis

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Background: The role of IL-23 and its receptor (IL-23R) in differentiation of CD^+ T cells into Th17 cells, induction of IL-17 production, and induction of inflammatory pathway has opened a new area of study to investigate the association between IL-23R polymorphisms and many autoimmune and inflammatory disorders. The aim of this study was to determine the association between IL-23R polymorphisms and Juvenile Idiopathic Arthritis (JIA). **Methods:** A case-control study on 55 patients with JIA and 78 healthy controls was performed. All samples were genotyped for three single nucleotide polymorphisms of *IL23R* (rs1495965, rs10889677, and rs1004819), using real-time PCR assay. **Results:** The frequency of the C allele of rs1495965 was significantly higher in the JIA group, compared to the control group (53.6% *vs.* 39.1%, p=0.026, OR=1.8, 95% CI=1.099-2.95). Both rs10889677 and rs1004819 alleles and genotypes showed no significant differences between the JIA patients and healthy controls. **Conclusion:** This study provides the evidence that the rare allele of rs1495965 has positive correlation with JIA disease.

Keywords: IL-23 Receptor, Juvenile Idiopathic Arthritis, Polymorphism

2647P

Genetic variation in the transforming growth factor-B1 gene is associated with multiple sclerosis disease

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Background: Multiple sclerosis (MS) is a neurodegenerative autoimmune disease characterized by recurrent episodes of demyelination and axonal injury mediated by immune cells. Cytokines released by them play an important role in the pathogenesis of the disease. Cytokines have pro-inflammatory and immunosuppressive characters. Among them, transforming growth factor-b1 (TGF-b1) has an important role in suppression of the immune system in autoimmunity. The pivotal function of TGF-b in the immune system is to maintain tolerance via the regulation of lymphocyte proliferation, differentiation, and survival. There are some reports about variation in -509 nucleotide in certain disorders. Defects in TGF-b1such as in its expression may have correlation with the onset of multiple sclerosis and we interested to determine whether the **TGF-b1** promoter region (rs1800469) polymorphism is related to the molecular pathogenesis of multiple sclerosis in Iranian population. **Methods:** The blood samples were collected from

50 MS patients and PBMCs gathered. Genomic DNA from PBMCs extracted, PCR primers designed for promoter region of TGF-b1 and the length of the amplicon is 680 bp, then PCR amplification executed. Digestion of PCR products were done by Tai1 restriction enzyme and agarose gel electrophoresis runned to separate digested fragments. Our data analyzed by SPSS. **Results:** rs1800469 SNP exists significantly in multiple sclerosis patients. **Conclusion:** rs1800469 is significant in Iranian patients with multiple sclerosis and cause of its position, which is in promoter region; we claimed that -509 SNP is able to have a significant track on the TGF-b1 expression.

Keywords: SNP, TGF-b1, Multiple Sclerosis

1972P

Evaluation of ficolin 3 gene mutation (+1637delC) in normal Iranian subjects

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Background: Ficolins are proteins that bind to carbohydrates, act as opsonins, and play an important role in innate immunity and antimicrobial defense. Polymorphism in ficolin3 gene can lead to complement deficiency and recurrent infections. The aim of this study was to determine the polymorphism of Ficolin3 in Iranian normal subjects. **Methods:** DNA extraction was performed using salting out method on 37 Iranian normal subjects. RFLP method was designed using Apa I restriction enzyme to determine deletion of Ficolin-3 gene (FCN3+1637delC). PCR products were evaluated using electrophoresis on 2% L-agarose gel. **Results:** Evaluation of the results indicated that in Iranian subjects, 91.9% (34) had wild type Ficolin gene and 8.1% (3) showed FCNdelC. Additionally, all three cases of FCNdelC were seen in male subjects. **Conclusion:** In this study, we showed that the frequency of FCN3+1637delC was low and only two variants of wild type and heterozygote form of FCN3+1637delC were seen in the studied subjects of Iran.

Keywords: Ficolin-3, Polymorphism, Iranian Subjects

1923P

Molecular mechanism of differentiation action for Etanercepte and Infliximab in inflammatory bowel disease in U937 monocytic cell line

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Background: Pro-inflammatory Cytokines such as TNF alpha, IL-6andanti-inflammatoryIL-10areevaluatedby ELISA. Measuremen of Caspase weareable to evaluate apoptosis and inflammation. In this study, the activity of NF-Kb p65protein expression analysis of NF-kappa The effect of these drugs is evaluate using immunoblotting after. It infliximab connected to T cells

is apoptosis or decreased expression of proinflammatory cytokines, is where as etanercept does not have the power. **Methods:** To specify a different mechanism of action of these two drugs, the effects of increasing and decreasing the CARD15gene expression in U937 monocyte cell inhibition of pro-inflammatory and anti-inflammatory cytokines produced by these drugs are reviewed separately. Increase and decrease the CARD15 gene expression using Western-blotting and Real time PCR is evaluated. Expression of Peroxisome proliferator-activated receptor gamma (PPARγ), Real-time PCR and immunoblotting techniques are studied. **Results and Conclusion:** Infliximab mono-clonal antibody that is directed against TNF-α chimeric proteinis made. These antibodies bind to TNF-α can stop its activity directly. Etanercept, a Fusion protein by genetic engineering of human origin, and the two chains are similarly designed. Studies have shownthatmacrophagesasM1orclassicallyactivatedmacrophagethatcancauseinflammation. Interestingly,thecells have specific molecule called Peroxisome proliferator-activated receptor gamma (PPARγ), When there ceptoris activated M2 macrophages may result in M1 macrophages. PPAR and M2 is reduced inflammatory response. Increased expression (PPARγ) is associated within flammation. Etanercept is not effective in the treatmentof inflammatory bowel disease, while Infliximab effective.

Keywords: Molecular mechanism, Etanercept, Infliximab

Immunohematology

Oral Presentations:

3004O

Application of cell free foetal DNA (cffDNA) in non-invasive prenatal diagnosis (NIPD) of foetal *RH* group status

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Background: Based on the sources and method of sampling for extraction of foetal DNA, blood group genotyping assays can be categorized in two groups: (1) invasive e.g. amniocentesis, chorionic villus sampling (CVS) and percutaneous umbilical cord blood sampling (PUBS) and (2) non-invasive e.g. using cffDNA from maternal plasma. However application of the latter has its own advantages and limitations, which will be discussed. The ultimate aims of this study was to evaluate the specificity and sensitivity of Real-Time PCR and dual-labelled TaqMan probes for NIPD of foetal *RHD7*, *RHC*, *RHc*, *RHE* and SRY status using cffDNA. **Methods:** To evaluate the specificity and sensitivity of Real-Time PCR assays for NIPD of foetal *RHD7*, *RHC*, *RHc*, *RHE* and *SRY* status, previously published and/ or in-house designed sets of gene-specific primers and dual-labelled TaqMan probes were employed. NIPD foetal blood group genotyping by Real-Time PCR was evaluated using maternal plasma samples at different gestation ages (12 to ≥40 weeks) from 51 alloimmunised pregnant women. The QIAamp DSP Virus Kit (QIAGEN) was used for cffDNA extraction from the plasma samples. **Results:** The assays showed 100% concordance for *RHD7*, *RHC* and *RHE* assays; 95.7% concordance for *RHc* and 99% concordance for *SRY*. **Conclusion:** Overall the *RH* genotyping assays and the *SRY* assay were found to be reliable and sensitive for use diagnostically and as an internal positive control, respectively but obviously for male foetuses only. Negative Real-time PCR results for NIPD blood group genotyping cannot be used diagnostically if the *SRY* gene is not detected.

Keywords: NIPD, cffDNA, *RH* Genotyping, Real-time PCR, *SRY*

2851O

Microvesiculation during storage of Irradiated red cell concentrates

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Background: Metabolic and protein changes occur via irradiation to Red blood cells (RBCs) and progressively develop during storage. The vesiculation of the RBCs is known part of the storage lesion. The aim of this study was to determine a link between Microvesiculation of RBCs in RCCs (red cell concentrates), during storage and protein changes, particularly at the RBC membrane. **Methods:** Twenty leukoreduced RCCs were produced from whole blood in citrate-phosphate-dextrose anticoagulant using the buffy coat method. one RCC was irradiated (25 Gy) 24 hours after blood donation, while the other remained as an untreated control. Vesicles were quantified using glycophorin A on a FACScan flow cytometer. Ghost cell fractions were prepared by the Dodge method. RBC microvesicles (MVs) were prepared by low speed centrifugation of RBCs, followed by filtration and ultracentrifugation. Western blot analyses on RBC membrane and microvesicles were conducted with antibodies against heat shock protein (HSP) 70, band 3 and IgG. **Results:** Significant differences was shown between irradiated and untreated RCCs. Western blot analysis of irradiated samples revealed an elevated recruitment of HSP 70 to the membrane compared to untreated samples. Furthermore, band 3 was found to decrease in ghost preparations at days 28 and 42, while IgG, which is known to bind to neo-antigens on band 3 under conditions of cellular stress, was found to increase in ghost preparations. Both of these protein changes were more pronounced in irradiated RBCs. Lastly, our observations suggest that these proteins are increased in MVs released during storage; again to a greater extent in irradiated *vs.* untreated RBCs. **Conclusions:** The in vitro data demonstrate accelerated storage lesion in irradiated RBCs, and this is reflected in increased vesiculation and alterations in the protein composition at the RBC membrane. The length of storage will influence RBC microvesicle generation and significant changes to the RBC membrane. Vesiculation during storage of RBCs may enable the RBC to shed altered or harmful material.

Keywords: Irradiation, RBC storage lesion, Microvesiculation

2691O

Identification of the first Rh null phenotype in an Iranian family in Ghazvin

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The first type occurs either when the Rh AG (glycoprotein associated with Rh) gene is absent or there is a mutation. People with the Rh null phenotype are very rare, about 1 in 6 million and more often in family marriages. It is very difficult to find compatible blood for them due to the possibility of people with similar characteristics in other family members in their household. 41-year-old woman with weakness and lethargy referred to Razi Hospital of Qazvin diagnosed with blood type A negative and hemolytic anemia. Due to incompatibility in cross-matched samples tested, serum samples of patient were checked at room temperature and 37˚C with a cell panel of 11cell and showed reaction with Anti-Human Globulin as 3 + or 4 + while the auto-control tube and direct Combus were negative, indicating the likelihood of high prevalence of antibodies against the patient›s serum. Due to the lack of specific antisera in a database samples for further testing blood was sent to the headquarters lab in Tehran. The patient›s serum was tested with antisera anti-D, anti-C, anti-c, anti-e, anti-E blood groups

and finally considered to be Rh null. This is the first case in Rh null patient determined by screening of serum antibody tests. After reviewing of other family members, his brother was found to be the second Rh null.

Keywords: Rh null, Rh antigen, Blood transfusion

2502O

Recombinant factor VII injection to rabbit, approval and purification of polyclonal anti factor VII antibodies generated in rabbit to prepare the immunoaffinity chromatography column

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Background: So far various methods for purification of rFVII produced in various cells has been applied that one of these methods is immuno affinity chromatography. In this study for purification of rFactor VII produced in Lizard Leishmania, we prepared CNBr-activated sepharose 4B resin immune affinity chromatography column by rabbit stimulating to produce antibody against rFactor VII and purification of specific antibody in rabbit serum. **Methods:** First, 80 µl of inactivated recombinant factor VII in three steps injected to the four months Rabbit: with Freund's complete adjuvantFreund's incomplete adjuvant and purified protein, in one month interval. Fifteen days after the first injection, for confirmation of produced polyclonal anti rFVII in rabbit dot blot assay was performed on rabbit serum. Rabbit blood was collected and serum total IgG isolated by Protein Aresin. SDS-PAGEandWestern blot assays performed for confirmation of isolated total IgG. finally for purification of specific anti rFVII, immunoaffinitychromatography was applied and checked by dot blot assay. **Results and conclusion:** Fifteen days after first injection Polyclonal anti rFVII production in rabbit confirmed by dot blot assay with negative serum control. After third injection serum total IgG was isolated by Protein Aresinand purified IgG nature was confirmed by SDS-PAGE and Western blot. Then specific antibodies against FVII, purified with CNBr-activated sepharose 4B resinimmuno affinity chromatography and dot blot results confirmed the nature of purified specific antibodies. Finally anti rFVIISepharoseresin4Bcolumn was prepared and stored in 20% alcohol in 4˚C.

Keywords: Recombinant factor VII, Immuno affinity chromatography, Polyclonal antibodies

1914O

Immunomodulatory effects of blood transfusion on tumor size, metastasis, and survival of fibrosarcoma in Balb/C mice

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Background: One of the adverse effects of blood transfusion is the transfusion-related

immunomodulation.The increased incidence of cancers is described as a main outcome of such immunomodulation. The aim of this study was to investigate the effects of blood transfusion on the growth, survival and metastasis of tumor in Balb/c mice with fibrosarcoma. **Methods:** The number of 35 male Balb/c mice was divided into five groups, and then were induced with fibrosarcoma by subcutaneous administration of 1.2×10^6 WEHI-164 cells. After expansion of the tumor (day 7), hemorrhage-induced anemia was developed by retro-orbital blood sampling (200-300 µl). Twelve hours later, blood deficit was replaced with fresh allogenic, fresh syngenic, storage allogenic, and storage syngenic blood. After 13 days, the tumor size and survival rate were investigated. The mice were sacrificed 20 days after injection and their lymphatic tissues were observed for blood transfusion-induced metastasis. **Results:** The tumor size increased significantly in the groups received allogenic and storage blood as compared to those received fresh syngenic ones and also the control group. Regarding to the survival rate, the control group was not significantly different from the experiment groups. Two samples obtained from the inguinal lymphatic nodes of mice that underwent storage allogenic blood transfusion were observed to be metastatic. **Conclusion:** The results obtained in the present study may be considered as a new approach in blood transfusion medicine.

Keywords: Allogenic blood transfusion; Fibrosarcoma; Transfusion-related immunomodulation.

1514O

Evaluation of natural autoantibody against red blood cells in healthy individuals

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Background: Natural autoantibodies (N-Auto-Abs) are a class of antibodies that are formed without any previous provocation against their own. Currently an important role has been proposed for these antibodies in immune homeostasis; as studies have shown that N-Auto-Abs are increased before the onset of symptoms. We can study them as biological markers to predict onset of disease. For the first time, we studied the prevalence of N-Auto-Abs against RBC antigens as well as the prevalence of cold antibodies in blood donors. **Methods:** 300 healthy individuals who donated at the Tehran Blood Transfusion services were selected randomly; they blood samples were collected included 289 individuals (96.3%) males and 11 individuals (3.7%) female. Their age range 17-64 years. EDTA and plasma was evaluated for the study of N-Auto-Abs that reacted at 4° C. **Results:** In this study, 28 individuals (33.73%) showed positive reaction with their own cell suspension. Of these, only 13 individuals (46.43%) were positive for natural autoantibodies. In addition, 15 individuals (53.57%) were positive for N-Auto-Abs and cold antibodies. 55 individuals (66.26%) showed positive reaction with the cell type one, two and three. They showed a negative reaction with their own cell suspension. 217 individuals (72.4%) showed a negative reaction. **Conclusion:** Results showed that there are N-Auto-Abs against RBC in healthy donors with a relatively high frequency. The identification and assessment of risk in patients with hematological diseases, especially those related to RBC diseases can be used as a marker for predicting the diseases.

Keywords: Natural autoantibody, Red blood cells, Blood donors

1541O

Basic Principals for Pre-Transfusion Testing Methods for Relevance of Antibody Screening

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Background: pre-transfusion testing is performed in order to select blood components that will not cause harm to recipient and will have acceptable survival when transfused. Strategies for platelets, plasma and white cell components will be discussed separately. Regular pre-transfusion tests performed properly with confirmation of ABO, Rh type, antibody screening, antibody identification and compatibility testing. Despite of vital role of blood and its components as only curable treatment its transfusion is accompanied with many complications. Therefore it is essential to determine those patients need to blood transfusion. **Methods:** We would discuss the interpretations of the CTR in Alzahra University Hospital for minimizing of the blood preparation for elective operative surgery. This study was done in summer 1389 in Sant. Alzahra University Hospital in Isfahan. 683 cases were evaluated in their elective procedures in two departments. The data collected in check list and then the data was analyzed by SPSS v16. In this study pre-transfusion testing such screen test with local check cells and routine cross match has been done for all patients. **Results:** The data has shown that in cross match transfusion ratio (CTR) in Alzahra University Hospital was 1.9. This study shown that in our elective operative surgery we need2360 unit blood and we used 1115 unit blood for cross match. **Conclusion:** Use a new procedure in our hospital blood bank witch play a key role in the management of the surgical patients. We omit the reservation of blood and blood products for the surgeries, there fore we can decrease the ratio of CTR to minimum rate.

Keyword: CTR, Transfusion, Cross-match

Poster Presentations:

1856P

The prevalence of HCV infection in thalessemia patients

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Background: In spite of implementation of blood screening for viral infections, there is a risk of viral infections in thalessemia patients due to their need to long term blood transfusion. Hepatitis C(HCV) is the major cause of post transfusion hepatitis. This study was planned to investigate the prevalence of HCV infection among thalessemia patients in Iran.**Methods:** In this cross sectional study, 1821 thalessemia patients referred to IBTO research center from Jan. 2010 to Aug. 2012 were included. Serological assays for anti HCV were performed by ELISA method. Reveres transcription polymerase chain reaction assays were done to determine HCV RNA in plasma sample of patients. **Results:** Out of 1821 thalessemia patients, 879(48.3%) were male and 942(51.7%)were female. The mean age +/- SD was 26.12+/- 7.93 years. The

overall prevalence of anti-HCV was 58.5%. Two hundred and fifty eight out of 1066 (24.2%) anti HCV patients were HCV RNA positive, without consideration of any treatment. HCV RNA positive patients were older than HCV RNA negative patients (P<0.000).**Conclusion:** Although with implementation of blood screening for viral infections, the risk of HCV infection has decreased during last decade in Iran, more improved techniques are necessary to detect viral infection in blood and blood components and more attention on hygienic measures in medical centers should be performed.

Keywords: Thalassemia- Anti HCV- HCV RNA- Viral infection- Blood screening

2080P

The Study of Blood Utilization in Vali-e Asr Teaching Hospital in Birjand – Iran

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Background: Blood and blood components are routinely ordered in medical care services. The effective managements of blood utilization are the key element to reserve the blood units for the patients who are in need of transfusion. The aim of this study was to asses the efficiency of blood utilization in a teaching hospital. **Methods:** This is a descriptive, cross sectional study. Blood requests in 16 wards of a teaching hospital over a 3-months period were included. The cross-match transfusion ratio (C/T), the transfusion index (TI) and transfusion probability (T%) were calculated. Data were analyzed in SPSS and statistical significance was set at P=0.05. **Results:** In total 1617 patients received blood with the mean age of 41.55 + 23/94 years. Out of 4043 blood units were requested only 1394 units were transfused. The mean number of blood units requested for each patient was 2.9, whereas the mean number transfused was 0.86. The overall C/T ration, TI index and T% were 1.5, 1.3, and 60.1% respectively.

Keywords: blood utilization. C/T ratio, transfusion

2079P

Steps to successful implementation of haemovigilance in Birjand teaching hospitals

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Background: Haemovigilance is a national system to detect, gather and analyse unwanted events related to blood transfusion. The first haemovigilance system was estabilished in 1993 in Japan and the first one in Europe was initiated in France in 1994. Although haemovigilance is currently functioning in many countries but implementation of a national haemovigilance system is not a straight forwards task and usually takes long time of planning. Iranian blood transfusion organisation has been put the haemovigilance programme into practice from 2012 and it is now operational. It was implemented nationally and targeted the hospital blood banks. The aim of this study was to assess a newly implemented haemovigilance system in Birjand university hospitals. **Methods:** A set of standard preformatted form for the requesting blood, and notification of an adverse reaction in a transfused patient has been developed by the Iranian blood transfusion organisation. We assessed the Simplicity and workability of these

forms relating to capturing transfusion reaction events in real time. Proportion of returned information for each on the forms was determined. Further evaluation was done by means of validated and reliable questionnaire for understanding the viewpoint and expectation of the nurses and blood bank staff involved in blood transfusion regarding the haemovigilance programme. **Results:** The data showed some imperfections in hemovigilance establishment in Vali-asre hospital. Basically most of these deficiencies are related to the national level of designing haemovigilance programme rather than regional and local limit. **Conclusion:** Haemovigilance system is operating from its implementation in Birjand university hospitals, but regular supportive supervision is needed to improve the effectiveness of the system. The data need to be collected and analysed and collaboration at regional level between blood centres and hospitals also required more strengthen.

Keywords: Haemovigilance, transfusion incident, blood

1577P

Lack of correlation between the CCR5-Δ 32 mutation and acute myeloid leukemia in Iranian patients

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Background: Chemokines and their receptors are crucially in pathogenesis of Acute Myeloblastic Leukemia (AML). CC chemokine receptor 5 (CCR5) is a specific chemokine receptor for CC ligand 3 (CCL3), CCL4 and CCL5 chemokines which all play key roles in identifying cancer properties and localization of infiltrated in leukemia cells. It has been demonstrated that the popular mutation in CCR5 gene (CCR5-Δ32) leads to mal-expression of the receptor and affect its function. The aim of this study was to determine the rate of CCR5-Δ32 mutation among Iranian AML patients. **Methods:** In this study, blood samples were obtained from 60 AML patients and 300 healthy controls. The CCR5-Δ32 mutation was evaluated using Gap-PCR technique. **Results:** Our results showed that only one of the patients and one out of the controls, had hetrozygotic form of CCR5-Δ32 mutation, while, the rest of studied samples had the wild form of the gene.

Keywords: CCR5, myeloid leukemia, mutation

1671P

Phenotype and genotype discrepancy of Duffy blood group in patients with thalassemia

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Background: Serologic blood typing is difficult after blood transfusion, due to donor red cells presence in recipient's circulation. The aim of this is study was to compare molecular and serology methods to determine Duffy blood group in patients with thalassemia major. **Methods:**

Lymphocytic DNA was purified from peripheral blood of 20 apparently healthy subjects with no family history of thalassemia or blood transfusion and **35** patients with thalassemia major (consisting 19 patients with hemolytic blood transfusion reaction and 16 patients without complication). The samples were analyzed for FYA/FYB using PCR-SSP by handmade primers in comparison to serologic method in parallel. **Results:** Phenotype and genotype were not similar in two healthy subjects. There were two mismatches between phenotype fy(a^+ b^-) and FYB genotype. In patients group, 15 discrepancies was found between molecular and serologic method. **Conclusion:** Mutation of FYB gene promoter makes the protein absence in erythroid lineage without any effects on non-erythroid cells (like lymphocytes). Therefore the FYB gene mutations, disappears the antigens on red blood cell which is compatible with the (fya+/fyb-) phenotype. But in thalassemia patients with discordant phenotypes probably is due to the presence of this antigen in patient' blood.

Keywords: Thalassemia patients, Duffy phenotype, FYB gene

2045P

PCR Based Rh Blood Grouping of Blood Donors in East Azarbaijan

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Background: In last decade, methods based on molecular approach have been considered as a good tool for detection of gene polymorphisms responsible for most of blood group antigens. These methods are used to investigate the polymorphism prevalence among populations and clarifying serological blood grouping discrepancies. The application of molecular blood group genotyping could help to clarify profile of minor antigens in blood donors which help the process of compatibility tests for alloimmunized patients. **Methods:** Molecular based tests have been designed to clarify the different alleles of Rh blood group system genes based on PCR and RFLP methods. The results showed that that different alleles of the Rh (D, c, C, e, E) genes could be amplified along with controls in designed PCR reactions. The phenotype of samples for Rh blood group system was proven using standard serological method. **Results:** The study performed with 430 informed blood donorwith 430 n using stanAzarbaijan blood transfusion headquarters demonstrated that all samples could be genotyped using applied methods. The concordance of genotypes and phenotypes for the 4 antigens in 430 samples was 98.8 percent. **Conclusion:** The study results revealed that molecular extended genotyping could be used for genotyping of Rh blood donor samples in a facilitated PCR reaction and has almost same efficiency for Rh blood grouping as standard serological methods. The applied tests could help to determine the different allelelerd serolog of Rh blood group genes in population.

Keywords: PCR, Rh blood group, Genotyping

2680P

Elevated serum levels of cell death circulating, M30 and M65, in patients with β-thalassemia major

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Background: Deposition of iron in visceral organs, mainly in the liver, causes tissue damage in β-thalassemiamajor (β-TM) patients. Keratin 18 (K18) represents one of the major caspase substrates duringapoptosis of hepatocytes. **Methods:** To better characterize the hepatic apoptosis and/or necrosis in β-thal patients,the circulating levels of M65 (soluble intact K18) and M30 (the caspases-generated K18 fragment)were measured in 40 β-TM patients and compared with 40 healthy controls. The ratio of M30/M65(caspase-cleaved to total K18) was also determined in thalassemic and normal subjects. **Results:** Results of the ELISA assays revealed that the serum levels of hepatocyte death markers, M65 and M30, were significantly increased in β-thal patients compared to healthy controls ($p < 0.0001$). M30 serum levelswere also positively correlated with the serum levels of liver transaminases including aspartateaminotransferase (AST) ($r = 0.337$, $p = 0.047$) and alanine aminotransferase (ALT) ($r = 0.391$, $p = 0.02$).

Keywords: Apoptosis, Keratin 18 (K18), Necrosis, Aminotransferase

2529P

Effects of glycosylation on the function and phagocytosis of platelets during storage of platelet concentrate in cold

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Background: Cold storage caused the clustering of gpIbα molecules on platelets. Consequently, the lectin domains of αMβ2 receptors on the liver macrophages recognize these molecules and ingest them. In this study, the effects of glycosylation of platelets was studied on the ingestion of them after storage in 4°C as a model for the clearance of 4°C-stored platelets in body. **Methods:** Twenty-one units of PCs were prepared from blood bank of Iran. Platelets were divided into two portions and UDP-galactose was injected to one of the portions for glycosylation. The bags were kept at 4°C. The parameters of platelet counts, aggregation and the binding capacity of macrophages (THP1 cell line) to platelets and their ingestion were studied. The data were analyzed using the nonparametric test; Wilcoxon. **Results:** The results showed that platelet counts and aggregation values remained almost stable in 4°C-stored platelets, in both test (glycosylated) and control samples. It was revealed that glycosylation and storage in cold did not significantly prevent ingestion and phagocytosis of 4°C-stored platelets. **Conclusion:** Counts and the functional capacity of platelets remained relatively stable during their storage in cold with or without glycosylation. Although glycosylation and cold storage of platelets didn't affect their phagocytosis by THP1cells, it seemed that storage of PCs in cold was an attractive area in future research.

Keywords: Platelet concentrate, Glycosylation, phagocytosis

1661P

Study on detecting antibodies to Toxoplasma Gondii and Epstein-Barr virus in serum of blood donors by ELISA

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Background: Toxoplasma gondii (T. gondii) and Epstein-Barr virus (EBV) infection in blood donors could represent a risk for transmission in blood recipients. T. gondii can stay alive in citrated blood for 50 days at 4 ^{0}C. On the other hand transmission of EBV through transfusion of blood products and Platelet-rich plasm has been reported. There is scarce information about the epidemiology of T. gondii and EBV infection in blood donors in Iran. Therefore, we sought to determine the prevalence of T. gondii and EBV infection in a population of healthy blood donors referred to Gonabad Blood Transfusion Organization (East Iran). **Methods:** Three hundred blood donors in blood banks of Gonabad City were examined for T. gondii and EBV infection between Aprils to July 2011. Blood donors were tested for T. Gondii and EBV antibodies by using ELISA. **Results:** Forthy eight (16%) of 300 blood donors had IgG anti-T. gondii antibodies. Five (1.6%) of them had both IgM and IgG and two (0.6%) had only IgM anti-T. gondii antibody. Thus the prevalence of IgG and IgM anti T. gondii antibodies was 17.7% and 2.3% respectively. Forty six percent were positive for VCA- IgG EBV antibody. The seropositivity rates for females and males were found as 48.4 % and 46.3%, respectively. Positive persons were 36 first-time blood donors, 34 sporadic and 69 regular donors. **Conclusion:** Our study showed a relatively high incidence of T.gondii antibodies in blood donors of Gonabad. About EBV the results of this study confirmed lower distribution pattern to compare those reported in other studies.

Keywords: Toxoplasma Gondeii, Epstein-Barr virus, Serology Blood Donors

3288P

Frequency of irregular red cell all antibodies in thalassemia major patients with post transfusion Immunologic reaction referred to blood transfusion organization, Kerman center

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Background: Alloimmunization is a common problem in chronically transfused patients. This problem has been reported to be less common in transfused hospital-based patients. Alloimmunization can lead to some difficulties varying from delay in the provision of similar blood types to delayed hemolytic transfusion reactions. The aim of this study was to analyze alloimmunization against RBCs among chronically transfused patients. **Methods:** Retrospective analysis of 60 chronically transfused patients was conducted with antibody screening test and identification of patients' all antibodies with the panel cell test. For patients with alloantibody, the demographic data for sex, age and history of transfusion were collected. Finally, the data were analyzed by SPSS software version 18. **Results:** sixteen out of 60 patients (mean age 15±10 years) had alloantibody (prevalence rate of 26.6%). The most common clinically significant all

antibodies found were anti-E (75%), anti-K (68.7 %), anti-c (31.25%) anti-P1 (12.5%) anti-jk^b (6.25%). The most common clinically significant all antibodies identified in males and females were anti-E, anti-K and anti-c respectively. Eight patients were concomitantly positive for anti-E and anti-K all antibodies. Three patients were concomitantly positive for anti-E, anti-K and anti-c all antibodies. Two patients were concomitantly positive for anti-E, anti-K, anti-jk^b and fy^a all antibodies. **Conclusions**: According to our result, Alloantibody prevalence rate did not show any correlation with age and sex and was more common in patients with transfusion record. Important factors contributing to the higher prevalence of the above all antibodies are the higher prevalence of the related antigens in the population, higher antigenicity power. We conclude that red blood cell matching, at least for Kell and Rh systems, is necessary to prevent alloimmunization in thalassemiacs.

Keywords: RBC antigens and antibodies, Serological testing, Blood Transfusion

1528P

Effect of silymarin on soluble apoptosis markers in serum samples of iron overloaded thalassemia patients: A comparison with desferrioxamine

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Background: Iron overload is the most common clinical complication in thalassemia patients. Iron deposition in visceral organs causes tissue damage despite appropriate chelation therapy e.g. desferrioxamine.The generation of reactive oxygen species (ROS) through Fenton reaction may lead to cell death and apoptosis. To better characterize molecules involved in apoptosis, we measured circulating levels of apoptosis markers in thalassemia patients. **Methods:** Serum levels of soluble CD95 (sCD95), sCD95L, sTNF receptor type 1 (sTNFR1), sTRAIL and cytochorome C, were measured in two groups of 40 thalassemia patients using ELISA kits. The first group received desferrioxamine and the second group received combined desferrioxamine and silymarin. **Results:** Serum levels of apoptosis markers, were lower in thalassemia patients, but the difference was not significant in desferrioxamine versus combined desferrioxamine and silymarin groups. **Conclusion:** To find out the exact mechanism of apoptosis and the effect of treatment with silymarin on apoptosis in thalassemia patients, expression of membrane death receptors must be determined in tissues that have been damaged due to iron overload.

Keywords: thalassemia, iron overload, desferrioxamine, silymarin soluble apoptosis markers

2788P

Premarital Screening of beta thalassemia minor in north-east of Iran

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Background: Beta thalassemia is a preventable disease. Iran has about 20,000 patients who are homozygote for β-thalassaemia and 3,750,000 carriers. The aim of this study was to determine the prevalence of beta thalassemia minor among men who underwent premarital screening in Quchana city in Khorasan Razavi region of Iran. **Methods:** This research is a descriptive cross-sectional study. From 2010 to 2011, all participants (1000) under marriage coming to health center of Quchan underwent routine mandatory tests. Participants were considered to

have beta-thalassemia minor on the condition that hey had a mean corpuscular volume (MCV) <80fl and a mean corpuscular hemoglobin (MCH) <27 pg and a hemoglobin A2 level >3.5%. Venous blood was taken into an EDTA tube and the complete blood count and red blood cell indices were measured with a Coulter automated cell counter. Electrophoresis was performed on cellulose acetate. **Results:** Mean and SD of hemoglobin, MCV and MCH were 16±2.9, 91±4 and 28.4±2, respectively. Hemoglobin A2 Higher than 3.5 percent was reported as 3.5%.The prevalence of beta-thassemia minor with high hemoglobin A2 and microcytic hypochromic anemia was 3.5. **Conclusion:** In countries with high prevalence of hemoglobinopathies, a premarital screening program is helpful for identification and prevention of high-risk marriages. Detecting carrier couples with premarital screening program is an effective way of controlling thalassemia major.

Keywords: Prevalence, beta-Thalassemia, Premarital Examinations

2852P

Can irradiation change ATP level in red cell concentrates during storage?

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Background: Red blood cells (RBCs) develop a progressive storage lesion characterized by metabolic changes There are reports about irradiation effect on ATP level changes in RCCs (red cell concentrates) during storage. The aim of this study was to compare the in vitro quality of gamma-irradiated and non-irradiated RCCs during storage and determine a link between biochemical hallmarks of RBC storage lesion and RBC ATP level. **Methods:** Twenty leukoreduced RCCs in saline-adenine-glucose-mannitol (SAGM) preservative were produced from whole blood in citrate-phosphate-dextrose anticoagulant using the buffy coat method. In a pool and split design, one RCC was irradiated (25 Gy) 24 hours after blood donation, while the other remained as an untreated control. RCCs were sampled on storage days 2, 7, 14, 21, 28 and 42. Biochemical parameters were determined using an ADVIA 120 hematology analyzer, blood gases were measured on a Gem Premier 3000 and hemolysis was assessed using the Harboe method. ATP levels were measured using high performance liquid chromatography. **Results:** Significant differences in known in vitro measures of RBC storage lesion were seen between irradiated and untreated RCCs; lactate levels increased during storage to a higher level in irradiated RBCs vs. untreated ($p < 0.05$), and a greater increase in extracellular potassium ($p < 0.001$) and higher hemolysis levels ($p < 0.001$) were seen in irradiated vs. untreated RCCs. ATP levels decreased more rapidly in irradiated RBCs vs. untreated ($p < 0.01$). **Conclusions:** Our in vitro data demonstrated that irradiated RBCs shows accelerated lesions during storage lesion. Gamma irradiation of SAGM-preserved RCCs leads to serious deterioration of the ATP of erythrocytes during storage, which may partly explain the decreased in vivo survival of irradiated RBC.

Keywords: gamma-Irradiated RCC, storage lesion, RBC ATP

3007P

Resolving discrepancies during validation of genotyping versus phenotyping in human blood grouping

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Background: Haemagglutination, the classical and gold standard technique for blood group antigen typing is a simple, quick and inexpensive method. However, it has technical issues such as low-throughput, limited availability of reagents, and unreliability in determining the phenotype of recently-transfused patients. Substantial technological progress and greatly increased knowledge about the molecular background of the 30 blood group systems has facilitated development of molecular technologies including PCR-SSP, microarray Bead Chips, Real-Time PCR and DNA sequencing techniques for genotyping in combination or as alternative tools for the determination of blood group antigen status. The ultimate aim of this study was to evaluate not only feasibility but also resolving of discrepancies between phenotyping versus genotyping empirically. **Methods:** Genomic DNA from buffy coat (n=222) of apparently healthy donors for different purposes; e.g. blood group genotyping using in-house developed PCR-SSP protocols and validation of microarray BeadChiptechnology were extracted using in-house and commercial kits (QIAGEN). All the blood samples,including control donor samples were phenotyped serologically for ABO, RhD, RhCDE, RhC/c, RhE/e, K1, K2, Fy^a, Fyb, Jk^a, Jk^b, Co^a, Co^b, Di^a, Lu^a, Lu^b, M, N, S, and s using antibodies/antiserum from Alba BioScience except for Di^a, Co^a, and Co^b (ImuMed).Confirmatory antisera were from ImuMed and DIAGAST. All the genotyping results for each sample were compared with their serologic results. Every single discrepancy was investigated empirically. **Results:** Overall there were 9genuine discordant samples(*FYB* = 7, *JKA* = 1, *RHC* = 1). Six of the sevenFY discordants were due to the presence of FY^x (Fy^b/Fy^x: 265C>T) mutation and one was the consequence of a 67T>C mutation in the *GATA-1* motif in the promoter region of the *FY* gene (-67T>C). The two *JKA* and *RHC* discordant samples however, need further investigations. **Conclusion:** Resolving discrepancies between genotyping and phenotyping empirically is an essential in these kinds of investigations, and will be discussed in more details.

Keywords: Blood group, Phenotyping, Genotyping, PCR-SSP

2084P

Evaluation of thyroid dysfunction in thalassemia patients admitted in Zanjan hospitals during 1391

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Background: Thalassemia are forms of inherited autosomal recessive blood disorders that originated in the Mediterranean region. Thyroid dysfunction usually appears in the second decade of life and is thought to be associated with iron overload in patients with thalassemia

major In thalassemia, the disorder is caused by the weakening and destruction of red blood cells. Thalassemia is caused by variant or missing genes that affect how the body makes hemoglobin. The aim of this study was to determine the prevalence of thyroid dysfunction in patients with thalassemia and to determine its correlation with ferritin levels. **Methods:** This descriptive study is a retrospective review of 61 patients with a mean age of 10.23±5.78 years, 45 patients were evaluated and 16 were selected as controls. 45 patient have thalassemia and 16 were without any disorder. Diagnosis of beta-thalassemia major was based on family history, complete transfusion dependence and hemoglobin electrophoresis. Thyroid function were evaluated by measurements of serum total thyroxin (T3), serum total triiodothyronine (T4), thyroid-stimulating hormone (TSH) and serum Ferritin levels. Total serum T3, T4, TSH and ferritin levels were studied in blood sample employing ELISA method. The study was designed according to the patient refer to Zanjan hospitals. Statistical analysis was performed by SPSS version 17. **Results**: Of 45 patients, 25 (55.5%) in Group A , and 20 patients (44.5%) in Group B (In Group A, patients with serum ferritin lower than 1500 ng/ml. In Group B ,Patients with serum ferritin more than 1500 ng/ml) . There are 16 patients in the control group's. In Group A, of 25 patients, 18 patients (72 %) had normal thyroid and 7 (28 %) had thyroid dysfunction, and 20 patients in Group B, 4 patients (20 %) with normal thyroid and 16 patients (80 %) had thyroid dysfunction. Average T3 and T4 and TSH group, a control group was not significantly different. While the second group T4 and TSH lower than the control group. **Conclusion:** The findings in this study suggest that the incidence of hypothyroidism in thalassemia patients with serum ferritin levels greater than 1500 is more common. As a result, these patients are more at risk of hypothyroidism. In other word, better control of serum ferritin in patients with thalassemia major reduced incidence of hypothyroidism. Early recognition and hence prevention of these complications might help improve the quality of life of these patients. However, the limited number of patients in this study had a larger sample size is recommended for this study.

Keywords: Thalassemia, Ferritin ،TSH ،T4 ،T3

Immunoinformatics

Oral Presentations:

2843O

Designing truncated recombinant adjuvant base on Toll like receptor 5 (TLR5) agonist fused to polytopic E2 glycoprotein construct of Hepatitis C Virus (HCV)

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Background: Bacterial flagellin effectively induces humoral/cellular immune responses against foreign particles and has adjuvanticity by toll like receptor (TLR)-ligand activity. Here, we attempted to design truncated recombinant adjuvant base on TLR5 agonist which will fused to B cell designed construct for more efficacy of vaccine. **Methods:** The docking interactions TLR-5 and the truncated forms of FliC were evaluated using Hex docking sever. The interaction region of FliC truncated and then, geometry optimization and validation of structures were carried out. In following, truncates fused and modeled. Best of fusion protein models linked to B cell construct that were achieved by prediction of conformational immunodominant epitopes by hybrid approaches, selecting consensus epitopes, fusion them by linker and three-dimensional (3D) analysis. Finally, recombinant adjuvant fused to B cell construct and intact recombinant protein were visualized in 3D structure and analyzed. **Results:** four truncated structures were selected and the Ramachandran plots showed all of the residues of all four truncated structures located within the allowed regions. Also, truncates 3, showing the best interaction status to TLR-5. The binding site was located in the central binding region of TLR-5. Finally, visualization and last checking revealed suitable results of recombinant adjuvant fused to B cell construct for in vitro utilization. **Conclusion:** The rational design of new adjuvans has focused on Toll like receptor (TLR) agonists, triggers maturation of dendritic cells effectively and acts as bridge between the innate and adaptive immune responses. Ideally, combination of recombinant adjuvant which fused to polytopic construct will help to better and successful immune responses of vaccine.

Keywords: Recombinant adjuvant, Toll like receptor 5 (TLR5), FliC, E2 glycoprotein, immune responses

2752O

In-silico conformational epitope mapping of Iranian hepatitis C Virus (HCV) E2 glycoprotein

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Background: Hepatitis C Virus (HCV) encodes structural proteins (core, and envelope proteins E1 and E2) and nonstructural or NS proteins. E2 glycoprotein is highly variable protein and has major role in stimulation humeral immune responses. In Iran, this virus is a significant public health concern and therefore, the development of an effective vaccine against HCV base on Iranian isolates is important issues. Here, by using hybrid approaches, we aimed to predict most probable and reliable conformational epitopes of E2 protein of Iranian isolates. **Methods**:After evaluating most frequent HCV genotypes in Iran, followed steps were; retrieving sequences of E2 protein, overall analysis/editing of sequences, finding the highly variable regions (HVRs) and, then selecting target sequence for next analysis by similarity matrix and phylogeny tree. Tertiary structure was predicted and energy of modeled structure optimized. In following, wide array of immunoinformatic tools applied for prediction/mapping of conformational B cell epitopes. For conformational epitope by respect to obtaining reliable and effective consensus immunogenic epitope, we used ElliPro, DiscoTope 2.0, SEPPA, CBTOPE, BCEP servers. **Results:** By aid of immunoinformatics tools, 5 unique and reliable B cell immunogenic regions for E2 protein of Iranian HCV isolates were identified. These epitopes located at different regions of E2 protein and their average lengths were 25 to 50 Amino acids. **Conclusion**: Essential step in achieving successful protective and therapeutic polytopic, mosaic or chimeric vaccines is reliable and accurate prediction of epitopes in targeted protein.T herefore, we applied hybrid approache to find consensus epitopes in different tool results.

Keywords: Hepatitis C virus (HCV), E2 glycoprotein, Epitope, Conformational, Prediction

2239O

Victory model and theory; simulation of structures and functions of antibody and MHC by innovative hands, forehand and arms modeling techniques

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Background: Hand, forehand and arms (forelimb) molecular modelingforshowingofantibody and MHC structures and functions named “Victory model”in this article, that encompasses immuno-anatomical models used to mimic the behavior of biological molecules.”Victory model” name come from “Y”-shaped of antibody when we showing it by forelimb. **Methods:** The study consists of the three major phases: (a) conceptualization of the modelbased on the literature reviews in field of immunology, biochemistry, anatomy and computer science, (b) visualization themodels by forelimb, and (c) refinement of the modelsand checking their quality of displaying concepts. **Results**: About 72 different modelsobtained for showing different antibody structures andisotypes(IgM, IgGand etc.), Igvariable and constant regions, disulfide

bonds, Immunoglobulin diversity, Somatic hyper mutation and affinity maturation, Activation of complement and interaction with other molecules and cells. Also, 25 forelimbmodelsobtained for MHC class I and IIstructure and functions. In this study some hidden aspects of antibody interactions and functions, cause touchability of models with eyes, were defined that never have been understood. **Conclusion**: The findings of the study presents a models for designing innovative training projects that enable teachers to better transfer concepts to student in immunology classes and studentscan simply exerciseand have deep, accurate and touchable insight to structure and functions of antibody and MHC. Besides, it may raising discussions that forelimb may be takes some evolutions patterns and shaping forms from specific antigen recognizing proteins as both have responsibility in control of objects.

Keywords: Antibody, MHC, Simulation, Immuno-anatomy, modeling

1433O

The new CD20 chimeric protein

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Background: When designing a therapeutic approach for Non-Hodgkin's lymphomas (NHL), we face the issue of selecting the best target antigen. The depletion of B cells by anti CD20 monoclonal antibody Rituximab used in immune therapy leads to systemic side effects and should be significantly improved. The CD20 marker is a 33-kDa surface protein that expressed by mature B cells and most malignant B cells. This protein is first expressed by human pre-B cells in the bone marrow, predominantly after Ig heavy chain rearrangement, but not by pre-B cells or differentiated plasma cells. **Methods:** In this study we designed a new chimeric DNA containing twice repeated nucleotide sequence of CD20 external loop which attached to each other by a linker. The synthetic gene was cloned into E. coli expression vectors adjacent to T7 promoter. After that the expression vector was transformed into E.coli BL21 (DE3). The induced chimeric protein by IPTG was confirmed in immunobloting technique using anti Histaq antibody and specific antibody against CD20. **Results**: The bioinformatics results of structural prediction showed that induced protein could expose to immune system as separated section. The recombinant plasmid was also identified by double digestion successfully. The results of Western blotting and ELISA of immune mice showed that purified chimeric protein is a good powerful antigen.**Conclusion:** Taken together our results showed that plasmid pET28-CD20 has a good expression in prokaryotic cells and give a satisfied construction of CD20 which could be a good candidate for antibody production.

Keywords: CD20, Chimeric protein, Bioinformatics

1782O

Computation based-drug design and discovery against CXCR4 for stem cell transplantation

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Background: CXCR4 is a G protein-coupled receptor (GPCR) that has multiple critical functions in normal and pathologic physiology that include regulation of the metastatic behavior of mammary carcinoma, utilization as a co-receptor for infection by T-tropic strains of human immunodeficiency virus-1 and mobilization of stem cells from bone marrow to blood stream . In the current investigation, in silico and molecular modeling studies were used to design the novel and potent CXCR4 antagonist.**Methods**:CXCR4 crystal was retrieved from protein data bank and subsequently minimized by molecular dynamic package, Gromacs. Then, 150000 compounds were docked against the CXCR4 binding site. Based on the free energy of binding and Lipinski rules, five compounds were selected and the most potent one was investigated in the molecular dynamic study within 30 nanoseconds. To simulate the biological interaction truly, CXCR4 was embedded in the POPC. Finally, the dynamic output files and nature of interaction were analyzed and interpreted. **Results**: The binding energy of selected compound was -13.5 kcal/mol. By the binding of antagonist to CXCR4, gyration radius was decreased. Also, the backbone RMSD was about 2.7 Å after 9000 ps and was not increased significantly during the simulation. Analysis of ligand-receptor interaction showed that the most important amino acids involved in CXCR4-antagonist interactions are including Lys^{38}, Glu^{32} and Trp^{94}. **Conclusion**: Designed and discovered compound would be synthesized experimentally and used to hematopoietic stem cell transplantation as a stem cell mobilizing agent for patients with multiple myeloma and non Hodgkin's lymphoma.

Keywords: CXCR4, drug design

2101O

Computational Design of Small Molecule Inhibitors of Programmed Death 1 (PD-1), an Important Immune Checkpoint Protein in Tumor-Induced Immune Suppression

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Background: Programmed death 1 (PD-1) is a key immunecheckpointreceptor expressed by activated T cells, which can cause immunosuppression in the tumor microenvironment. Inhibition of the interaction between PD-1and PD-L1 can enhance T-cell responses and mediate antitumor activity. Anti PD-1 monoclonal antibodies are being developed and are shown to be effective in PD-1 inhibition. However, their toxicity is under estimation. Also, monoclonal antibodies used as therapeutic approaches, need high costs and effort. Small molecule inhibitors are easy to handle, use and design and thus can be a promising approach in the mentioned field. **Methods:** The 3D structure of PD-1/PD-L1 complex was used to find

the interacting residues. A four-residue sequence of PD-L1 was chosen as the most important part in its interaction with PD-1. Small molecule peptidomimetics were designed based on the four-residue peptide. The most appropriate peptidomimetics were evaluated using a precise docking technique. Similarity search was also performed to find other potential small molecule inhibitors. **Results:**Four small molecule peptidomimetics were designed and docked separately with PD-1. Comparing to the docking score of the four-residue sequence with PD-1, two of the four peptidomimeticsshowed acceptable docking scores. Three small molecules were also introduced as a result of the similarity search for each of the two peptidomimetics. **Conclusion:**By analyzing the results, a high potency was observed for the final two small molecule inhibitors described here in blocking PD-1. The knowledge-based computational design of inhibitors can accelerate the rational inhibitor design in developingtherapeutic strategies in cancer therapies.

Keywords: Immune checkpoint, PD-1, Small molecule inhibitor, Peptidomimetics, computational design

1619O

Immunological evaluation of predicted linear B-cell epitopes of human CD20 antigen

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Background: The importance of CD20 as a target for immunotherapeutic depletion of B cells is irrefutable. Several anti-human CD20 (hCD20) monoclonal antibodies are expressed at different stages of development. However, resistance to anti-CD20 therapy has made the search for new alternatives imperative. Identification of B-cell epitopes within hCD20 using in silico tools can provide new opportunities to develop monoclonal antibodies with different binding sites. Furthermore, identification of the relationship between amino acid sequences of predicted B-cell epitopes and immune responses facilitates the determination of immunogenic regions of proteins by using their primary structure. Experimental evaluation of predicted linear B-cell epitopes as candidate peptides and bioinformatics allow us to explore this relationship. **Methods:** In this study, we predicted and selected three linear B-cell epitopes within extra membrane loop of human CD20 antigen by utilizing ABCpred, Bcepred, BCPREDS, ANTIGENIC, BepiPred, MHCPred and SVMHC web servers. Mouse humoral responses to keyhole limpet hemocyanin (KLH)-conjugated candidate peptides (B-cell epitopes) were evaluated in ELISA and flow cytometery experiments.**Results:**P1 raised a strong immune response and responses of P2 and P3were moderate. The staining of Raji cells (CD20+) and no staining of Jurkat cells (CD20-), demonstrating the specificity of the antibodies to the B-cell epitope peptides.**Conclusion:**In this study, the results ofin vivoexperiments confirm the accuracy of thein silico prediction. We conclude that the P1, P2, and P3 B-cell epitopes are effective for immunization of mice in conjugation with KLH.

Keywords: Antipeptide antibody, B-cell epitope, Human CD20, Immunoinformatics.

2300O

Computational Molecular Features of Envelope Glycoprotein E1 of GB Virus C/Hepatitis G Virus

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Background: E1 envelope protein of GB virus C (GBV-C) plays important role in viral biological functions and its molecular structure. The purposes of our study were multi-aspect molecular evaluation of GB virus C E1 protein from its characteristics, mutations and structures that it will help to new directions for future researches. **Methods:** Briefly, steps that followed here were; retrieving sequences of E1 protein,finding the mutational /conservative regions, analysis potential glycosylation, phosphorylation sites, prediction primary, secondary and tertiary structure, and then followed by amino acid distributions and transmembrane topology evaluation. **Results:** Based on entropy plot, 5 hyper variable regions (HVR) observed which located in residues 55-60, 67-70, 245-250, 262-267 and 341-343, respectively. Analysis primary structure revealed neutral nature, stability and its low hydrophilicity. Transmembrane topology prediction showed residues 48-125 and 217-219 presented outside while residues 25-47, 126-148, 194-216 and 220-242 were transmembrane. Also, two N-glycosylation and 12 potential phosphorylated sites were found. Secondary structure prediction showed 5 α-helix,25 β-strand and 29 Coil regions in E1structure. Finally, tertiary structure revealed more details about its molecular conformation. **Conclusion:** The comprehensive analysis of a protein with important roles has never been easy, and in case of HGV E1 envelope glycoprotein, there is no much data on it's the molecular features and function in GBV-C virus. So, results present study may explain some structural and functional properties of this protein in GBV-C, as well as help to better understanding of E1 protein characteristic other members of Falvivirus family, especially HCV.

Keyword: GB Virus C/Hepatitis G Virus; Envelope glycoprotein E1; Molecular features.

2605O

In silico characterization of the functional and structural of the hemagglutinin (segment 4) protein from the Asian influenza virus A (H3N2)-2013

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Back ground: Influenza A (H3N2) viruses caused regional outbreaks in Asia with widespread activity as announced by the World Health Organization. Hemagglutinin (HA) is the most important protein in molecular epidemiology and pathogenesis of influenza viruses.
To determined virulence and pathogenicity of the virus, we characterized the functional modules of the surface glycoprotein hemagglutinin (HA).**Methods:** For analysis of the mutations in 2013$H_3N_{2\ \text{influenza virus}}$, all 90 HA sequences from the 2013 H_3N_2 outbreak were downloaded from GenBank.We analyzed receptor binding sites, basic patch, neutralization antibody epitopes and T cell epitopes by several bioinformatics softwars.**Results:**Amino acids sequences used to define the HA functional and structural modules and prediction human specific receptors.

The mutation in HA, which is found in some isolates, may confer dual binding specificity. HA variant suggesting difference in membrane fusion function. HA in some isolate has extra glycosylation site. T cell epitopes patterns in various HA molecules were determined.
Conclusion: These results are useful and critical for understanding the pathogenicity of the virus and host immune response against the H_3N_2 influenza virus.
Keywords: H_3N_2, in silico, hemagglutinin

3012O

B Cells Identification of Linear and Conformational Epitopes of Toxin B Produced by *Clostridium difficile*: A Bioinformatics Study

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Background: *Clostridium difficile*is an anaerobic, sporogenous, rod-shaped gram positive bacterium which produces two important toxins, called Toxin A and Toxin B, which are common factors of causing Antibiotic-Associated Diarrhea (AAD). It is also the most common factor for causing diarrhea in patients admitted to hospitals for a long time. This bacterium can also lead to Pseudomembranous colitis. Use of antibiotics by patients affects their intestinal flora, and by long term consumption of these medicines, ideal environment would be created for *Clostridium difficile* growth. Growth and colonization of this bacterium leads to toxin production which would be released in intestinal. These two released toxins would attach to intestinal cell membranes and by preventing their natural function would cause inflammation and scar in intestinal. These toxins are among large toxins with high molecular weight. Predicting and characterizing the immunogenic areas of each toxin may help us to develop anti-microbial and/or immune based protocols against *Clostridium difficile*. In this study, the immunogenic regions of toxin B are predicted by using bioinformatics tools. The immunogenic regions, moreover, were scored based on the best immunogenic potential for downstream studies. **Methods:** For identification of B cells linear and conformational epitopes,Ellipro and Discotop servers were applied. To identify linear epitopes Ellipro server was used and to identify conformational epitope Discotop server was used. These two servers make the prediction through the protein three dimensional structures. **Results:** The bioinformatics studies showed that there are number of regions in toxins B which can be used for experimental investigations. **Conclusion:** Prediction and characterization of immunogenic regions have many advantages such as providing a basis for constructing recombinant probiotic bacteria with high potential for immune system stimulation against pathogens. Moreover, through creating recombinant adjuvant proteins accompanying these recombinant components, we may be able to increase the effect of toxin B to develop anti-microbial and/or immune based protocols against *Clostridium difficile*.
Keywords: *Clostridium difficile,* Bioinformatics, Immunogenic regions, toxins B

2582O

Bioinformatics identification, cloning and partial purification of antigenic regions of Clostridium novyi alpha toxin for immunization

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Background: Clostridium novyi alpha toxin, which increases vascular permeability and causes cell rounding in cell culture, has around 200 kDa molecular weight . Since a small fragment of an antigen can induce an immune response against whole antigen , cloning and production of the regions with high antigenicity has opened new possibilities for immunity against lethal toxins . A critical requirement of this strategy is the identification and selection of antigenic regions of the toxin. In this study, we describe a combination of bioinformatics , molecular biology and purification methods for the production of protein fragments expected for high antigenicity. **Methods:** the fragments were selected based on bioinformatics study and protein secondary structure. Specific primers were designed and used for amplification of the regions. The amplified fragments were inserted into cloning and expression vector. The desired plasmid construction was confirmed by the PCR. a positive clone was expressed and the recombinant protein was partially purified by ammonium sulphate precipitation, followed by chromatography through gel filtration column and their antigenic characteristics were evaluated by ELISA and Western blot and was used for animal immunization. serum was used for cross -reaction with toxin and recombinant antigen. **Results:** the cloning step was verified by PCR. Results of immunological tests confirmed that the purified recombinant protein with the selected antigenic fragments showed more antigenicity compared to natural whole protein. **Conclusion:** it shows that bioinformatics-based approach can enhance the optimal selection of potential targets of immune response. This strategy provides a method for production of subunit and synthetic peptide vaccines and antibodies or antisera for competition assays, as reagents for affinity – chromatography ligands for purification of antibodies or proteins.

Keyword: Bioinformatics antigenic region, Alpha toxin, Clostridium novyi , Gel filtration

2604O

An in-silico comparison of Large S protein antigenic and immunogenic properties in all of HBV genotypes

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Background: Hepatitis B virus (HBV) is the most common infectious agent among hepatitis viruses and remains as a global health problem. HBV is coated with large (L) middle (M) small (S) surface proteins of HBV. LHSP consists of pre- s1, pre-s2 and s protein. The sequence of this region has variation are in different genotype and subtype. **Methods:** Protein data bank (PDB) and National Center of Biotechnology Information (NCBI) databases and Chimera, Predict Protein, Multalign and CASTp softwares used for DNA sequence and protein properties analysis. Physico-chemical properties were computed by Expasy's ProtParam server and PROTSCALE. SOPMA, CYS_REC, Motif Search and SOSUI server were applied to predicate Secondary structure prediction and functional characterization. Tertiary structure and structure Validation

analyzed with the help of SWISSMODEL, ERRAT, PROVE, PROCHECK, WHATCHECK and Verify 3D.**Results:** Data analysis was shown fundamental differences between LHSPs among different genotypes of HBV. Difference included various mutation (deletions and point mutation) which may influence the physical and chemical parameters, structure and functions. **Conclusion:** LHSPs of each genotype have a different chemical and/or structural function, strongly suggesting that each has a different three-dimensional structure. These data might be useful for future research on design new vaccine for HBV and also various pathogenesis of different HBV genotypes.

Keywords: HBV, LHBs, Genotypes

Poster Presentations:

1507P

Codon Optimization of B30 EG95 gene from Echinococcus granulosus in order to increase its expression level in recombinant E.coli

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Background: Echinococcal disease is a zoonosis by the tenniid tapeworm Echinococcus granulosus. Oncospheres are the infective stage Echinococcus granulosus in intermediate host that an immune response against oncospheres –derived antigens is excepedearly stages of the infection, therefore we can use oncospheres as antigen in serological test for diagnosis of echinococcusis in intermediate host. since most of parasit's antigens have low specifity, in this study we considered recombinant antigen of oncospheres. **Methods:** Optimization of codon usage of transgenes to match the host's preferred codon usage is a strategy for enhancing foreign protein expression in heterologous species. In this study the sequence of B30 EG95 genewas obtained from NCBI. After ORF analysis and translation into amino acids was codon optimized using Vector NTI software. **Results:** Nucleotide replacement was performed without change in amino acids. For example E.granulosusonly use AAT codon for asparagine in B30 EG95 genesequences, while the E.coli prefers AAC for coding asparagine or in the case of val, thr, ala and argE.granulosus, this parasite preferred different codons than Bacteria. **Conclusion**: However there are some similarity in preferred codon between this two organisms, for instantsno E.granulosus and E.coli prefered CGA, CGG and AGG codons for argenin. It is expected that codon adaptation of B30 EG95 geneincrease the stability and expression level of this gene in recombinant E.coli and affect as a recombinant antigen for E.granulosus.

Keywords: Echinococcusgranulosus, Recombinant antigen, Codon optimization, B30 EG95 gene

2744P

Tuberculosis Disease Diagnosis Using Artificial Immune Recognition System

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Background: There is a high risk of tuberculosis (TB) disease diagnosis among conventional methods. This study aimed to determine the TB disease using hybrid machine learning approaches. **Methods:** The patient's epicrisis reports taken from Pasteur Laboratory from north of Iran was used. All 175 samples have twenty features. The feature s are classified based incorporating fuzzy logic controller and artificial immune recognition system. The features normalized through fuzzy rule based on labeling system. The labeled features categorized into normal and tuberculosis classes through Artificial Immune Recognition Algorithm.
Results: In overall,the highest classification accuracy was reached for the 0.8 values of learning rate (α). The AIRS classification approaches using fuzzy logic also yielded better diagnosis results in terms of accuracy of detection and when compared to other empirical methods. The accuracy of classification is 99.14%, sensitivity of 87.00%, specificity of 86.12%.

Keywords: Artificial Immune Recognition System, Fuzzysystem, Tuberculosis, Safety

2058P

Three dimentional structure prediction of *Acinetobacter baumannii* FepA

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Background: *Acinetobacter baumannii* is a gram-negative bacterium that causes serious infections in compromised patients. Iron repressed proteins such as FepA (ferric enterobactin protein) represent outer membrane siderophore receptors. 3 dimentional protein structures could be employed in drug and vaccine designs and conformational epitope predictions. Since experimental determination of 3D protein structures is expensive and time consuming, other approaches are ought to be considered. Nowadays, bioinformatic tools are of interesting advantages for biologists. Prediction of 3D protein structure is one of the wide applications of these tools. In this in silico study 3D structure of *Acinetobacter baumannii* FepA protein, an appropriate vaccine candidate is predicted. **Method:** Protein sequence was extracted from NCBI database. A template for structure prediction of protein was selected with protein sequence blast against PDB database. Protein template was selected focusing on its coverage length and identity percentage with the query. 3D structures of this protein was determined with various web servers. **Results:** 3D structures were evaluated by Qmean, a composite scoring function which is able to derive both global and local error estimates on the basis of one single model. Top ranked three structures were refined with KoBaMIN web server that reduces the structure errors. Refined structures were evaluated with Qmean score and the quality of structures were observed to have improved. **Conclusion:** The best refined structure with highest score was selected as final structure. This structure was validated with other relevant software. 3D structure of *A.baumannii* FepA is predicted via *in silico* tools.

Keywords: *Acinetobacter baumannii*, FepA, 3D structure, vaccine design, conformational epitope prediction

1841P

Computation based-drug design and discovery against CXCR4: implications for designing new drugs for peripheral blood stem cell transplantation

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Background: CXCR4 is a G protein-coupled receptor (GPCR) that has multiple critical functions in normal and pathologic physiology that include regulation of the metastatic behavior of mammary carcinoma, utilization as a co-receptor for infection by T-tropic strains of human immunodeficiency virus-1 and mobilization of hematopoietic stem cells (HSC) from bone marrow to blood stream. Mozobil is the well-known inhibitor of CXCR4 which has been recently approved by FDA for mobilization of HSC in patients with multiple myeloma and non-Hodgkin lymphoma. As Mozobil is very expensive, designing of new potent and cheaper inhibitors are certainly necessary. In the current research, we used in silico and molecular modeling studies to design novel and potent CXCR4 antagonists.**Methods**:CXCR4 crystal was retrieved from protein data bank and subsequently minimized by molecular dynamic package, Gromacs. Then, 150000 compounds were docked against the CXCR4 binding site. Based on the free energy of binding and Lipinski rules, five compounds were selected and the most potent one was investigated in the molecular dynamic study within 30 nanoseconds. To simulate the biological interaction truly, CXCR4 was embedded in the POPC. Finally, the dynamic output files and nature of interaction were analyzed and interpreted. **Results**:The binding energy of selected compound was -13.5 kcal/mol. By the binding of antagonist to CXCR4, gyration radius was decreased. Also, the backbone RMSD was about 2.7 Å after 9000 ps and was not increased significantly during the simulation. Analysis of ligand-receptor interaction showed that the most important amino acids involved in CXCR4-antagonist interactions are including Lys^{38}, Glu^{32} and Trp^{94}.**Conclusion**:Designed and discovered compound be could be synthesized experimentally and used for mobilization of HSC for peripheral blood hematopoietic stem cell transplantation in patients with multiple myeloma and non Hodgkin's lymphoma. Further experiments are ongoing in the lab.

Keywords: CXCR4, drug design.

1563P

Vaccinoinformatics: Combination of Vaccinomics and Bioinformatics as an Accelerant for Peptide-Based Vaccine Designing

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Background: Recently, the world have witnessed many emerging and re-emerging pathogens especially viruses as life-threateninginfectious diseases. Undoubtedly, vaccination is the most effective medical tool to prevent and control viral infections. However, the time consuming and expensive assays, together with a heavy workload of vaccine design and development, will make the process too uphill to be handled experimentally. Thus, this can be a situation

wherein silico techniques come to effectively accelerate the vaccine designing procedure. **Methods:** Critical requirements of this strategy are including the identification of the most appropriate antigen component within the pathogen, predicting its tertiary structure using in silico modeling methods,predicting the T-cell and B-cell epitopes of the antigen, assuring that the predicted peptidic epitopes can interact efficiently with the important immune system molecules and many other processes. All these requirements together with their importance and details are reviewed in this paper.**Results:**Predicted peptidic epitopes of the pathogen's most important antigen which are verified to have certifiable interactions with the immune system components are highly potent to be real vaccine candidates. These potent candidates can now be examined experimentally. Therefore, thanks to bioinformatics the need of examining all possible peptidic epitopes will be nullified. **Conclusion:**Since computational vaccinology or vaccinoinformatics and immunology are closely related, by having sufficient knowledge in immunology we can make use of the ever-growing bioinformatics tools more efficiently in vaccine design.

Keywords: Bioinformatics, Peptide-based vaccine design, Epitope prediction, Immunology

2262P

Investigating the probable role of miR-9 in Multiple Sclerosis by bioinformatics methods

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Background: Multiple sclerosis (MS) is an autoimmune neurodegenerative disease that manifested by chronic inflammatory demyelination of the central nervous system (CNS). Interleukin 17 (IL-17)-producing T helper cells (TH-17 cells) are increasingly recognized as key participants in various autoimmune diseases, including multiple sclerosis.Although sets of transcription factors and cytokines are known to regulate TH-17 differentiation, the role of noncoding RNA is poorly understood. MicroRNAs are endogenous noncoding RNAs that comprise post-transcriptional regulation of gene expression. The aim of this work is to identify miRNAs in the pathway of Th17 differentiation by means of bioinformatics databases. **Methods:** Bioinformatics studies revealed several miRNAs played important roles in the naïve T cell differentiation to mature Th17 cells. These miRNAs could induce or inhibit the pathway of Th17 differentiation. For this purpose, we gathered information about different miRNAs in variant autoimmune diseases that Th17 was involved with. In other studies we have identified 64 genes which affect different pathway of Th17 differentiation. Eventually, the interaction between miRNAs and genes by means of 10 variant database was analyzed.**Results:**Based on our results, we have several miRNAs that One of them was miR-9. miR-9 probably inhibit negative regulators of Th17 differentiation and induce this pathway. One of the genes that miR-9 may interact with is: PIAS3.**Conclusion:**miR-9 could be a key miRNA in progression of symptoms of MS by inducing the differentiation to Th17. However in-vitro and in-vivo experiments are needed to confirm our computational analysis which is an ongoing research of our team.

Keywords: miR-9, Th17 subset, differentiation, autoimmune disease, Multiple Sclerosis

2145P

Bioinformatic analysis of recombinant Cryptosporidium parvum p23as a target for vaccine

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Background: Cryptosporidium parvum is a parasitic protozoan, which develops inside the parasitophorous vacuole between the microvillous membrane of enterocytes in a wide variety of vertebrates, including human. Cryptosporidiosis is an important parasite that causes of severe diseases in the immunodeficient people especially AIDS patients. Cryptosporidiosis has been also reported as a common serious primary cause of outbreaks of diarrhea in newborn calves. The aim of this study was to establishcharacterization of a recombinant P23 as an immunogenic antigen in domestic isolates of C. parvum. **Methods:** Stool specimens of naturally infected calves were used for molecular detection of C. parvum by nested PCR. To obtain the recombinant P23 protein, totalmRNA was isolated from oocyst of C. parvum, and cDNA was synthesized. The cDNA was then amplified using specific primers for P23 gene. The RT-PCR product was cloned in pGEX-5X-2 vector and sequenced.The blast algorithm was used for homologous gene search and then P23 structure was predicted based on homology modeling and threading algorithm. The Immune Epitope Database (IEDB) was utilized for epitope identification and finally structural and functional annotations of recombinant P23 were compared with other homologous proteins. **Results:** The sequence analysis showed 98%-100% nucleotide sequence identityand 97%-100%amino acid homologyto the known P23 sequences in GenBank. Epitope prediction of P23 reveled several peptide sites that can be a good candidate site for vaccine design. **Conclusion:** Since P23 is an immunodominant surface glycoprotein expressed in the early phase of infection and theimmunogenic epitopes are found in its residual chain of amino acid sequence, the recombinant P23 could be recommended as a favorable candidate for chemotherapy or prevention against C. parvuminfection.

Keywords: Cryptosporidium parvum, Recombinant P23, Bioinformatic analysis.

2622P

Mir-19a and its role in Multiple Sclerosis according to bioinformatical prediction

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Bachground: Multiple Sclerosis is an autoimmune and destruction of the myelin disease , including the central nervous system . In this disease the immune system get impaired with TH17 cells malfunction. The recent findings say of the presence of multiple miRNA in diseases.

miRANs are small RNA with short sequences, can have pivotal roles in the regulation of cellular diverse functions and control their impact on the level of mRNA by post-transcription methods. **Methods:** This scheme is based on the possible role of vital miRNAs in the process of differentiation TH17 which finally to be effective in the treatment of patients.Bioinformatic studies suggest that miRNAs can be involved in the differentiation of naïve T cell toT helper17. Here ,we aim to a few examples of miRNAs which involved in differentiation process of TH 17 cells that contribute in several autoimmune diseases including MS and is thought be effective on 64 different involving genes during the differentiation**. Results:** Among the datas , Mir-19a is one of these miRNAs which its role in other autoimmune diseases like lupus-like autoimmunity and IBD has been detected . This Mir is probably a inhibitor of negative regulator on these genes like Socs3. **Conclusion:** Based on our findings, Mir-19a can be one of the most important miRNA which interferes in the distinction TH17 cells in MS patients and can be the determining factor in MS. Finally, more research will be required in-vitro and in-vivo to confirm our computational analysis which is an ongoing research of our team .

Keywords: Mir-19a , Th17 subset, differentiation, autoimmune disease

2654P

miR-27a function in multiple sclerosis based on Bioinformatics methods

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Background: Multiple sclerosis (MS) is a neurodegeneration and defective immune regulation diseases with the myelin damage in the Central Nervous System(CNS). Studies revealedthat T_H17 hasa key role in the pathogenesis,inflammation and autoimmunation of several autoimmune diseases.IL-17 is a hallmark production in T helper 17 (T_H17) cells that recognized as pro-inflammatory cytokine. MicroRNAs are small endogenous non –coding RNAs that can affect cellular function by regulating post transcriptional gene expression. Recently, researchers interest to select them as therapeutic targets. **Methods:** We usecomputational analysis to find microRNAs that they canaffectdifferentiation of T_H17 cells from naïve T cell.So we candidate 64 genes that deregulated of them cause different autoimmune diseases. So our Biobioinformatic analysis indicated that 8 microRNAs such as miR-27a havestrong interaction with some genes that have roles in T_H17 cells development and differentiation.These data investigated from 10 databases. **Results:** Our Bioinformatics data demonstrated miR-27a showupregulation in MS patients. Several genes such as PPARγ, NR2F6act asnegative regulators that inhibit naïve T cells differentiation into T_H17 cells. So miR-27a has interaction with them and reduces their expression, therefore induces T_H17 differentiation and increases the risk of causing of multiple sclerosis. **Conclusion:**According of our bioinformatics studies miR-27a can be used as therapeutic targets to decrease progression symptoms of MS. However we need to confirm our finding by invitro and in vivo experiment.

Keywords: autoimmune disease, miR-27a, T_H17, differentiation, deregulation.

2514P

Identification of antigens of CCHF virus for vaccine production using bioinformatics methods on isolates in hard ticks obtained from Khorasan-Razavi province, 2012 and comparing it with other proteins of CCHF

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Background: The Crimean-Congo haemorrhagic fever (CCHF) virus belongs to the genus Nairovirus of the family Bunyaviridae and is the agent of a severe haemorrhagic fever in humans. The disease is endemic in many countries in Africa, Europe and Asia. A safe and effective vaccine that can be used widely in humans has not been produced so far. **Methods:** The current study was achieved for detection of the virus genome from ticks of Khorasan-Razavi province and samples were sent to the Institute Pasteur of Iran to perform RT-PCR other proteins of cchf obtained from UNIPORTKB and NCBIP. Then by using of immunoinformatics servers, B cell and T cell epitopes were predicted. annotation and prediction of antigenic regions were performed by Geneious (version 5.1.6).**Results:**11 epitopes were predicted for B cell. 7 antigenic regions and 10 transcription factors Obtained. The sequence in this paper revealed only one T cell peptide with 195 in length. But the other proteins had smaller peptide. For instance: Envelope glycoprotein had three peptides (60, 60, 25), Nucleoprotein (14, 60), Nucleocapsid protein (35, 60), Nucleocapsid S (17, 60), L protein 66 peptides and Structural capsid protein showed three peptides (29, 60, and 60).**Conclusion:**In Eastern Europe, Russia and Bulgaria work has been done on cchf vaccine, but the vaccine is safe and effective as far as it can be used widely in humans has not been produced, Bioinformatics analysis results showed that these sequences have several antigen.

Keywords: CCHF virus, Immunoinformatic, Khorasan-Razavi, Antigen, Vaccine

2266P

Allergenicity Analysis of some Cat and Dog proteinsby using Bioinformatics tools

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Background: Sensitization to animal allergens is one of the most important risk factors for developing allergic diseases such as asthma, rhinitis, and atopic dermatitis.As furry pet ownership in indoor environments increases, sensitization to animal allergens from domestic exposure is a concern. **Methods:** The amino acid sequence of some canis and felis proteins retrieved from UniprotKB and NCBIpdatabases, and then saved into a FASTA file format. Then physic-chemical properties of sequence were evaluated. At third step, by using of immunoinformatics servers allergenic peptide (epitopes) were predicted and analyzed. Also, we predicted B-cell epitopes and antigenicity testing by Kolaskar & Tongaonkar method. **Results:**In this study some proteins such as Fel d 1-A, Fel d 1-B, Fel d 4, Fel d 7,Serum albumin,Cystatin-A in cat and Can f 2, Can f 1, Can f 4 and Lipocalin-Can f 6 in dog were used.Epitopes were predicted for B cell and compare with allergenic epitopes and Kolaskar & Tongaonkar.Results revealed no allergenic epitope (IgE epitope) in amino acid lengths. But all these proteins showed Bcell epitopes.**Conclusion:**Allergies are among the most common chronic conditions worldwide. Bioinformatics analysis results showed that these proteins have several peptides that can be responsible for its allergenicity and we can use these epitopes for other purposes.

Keywords: Cat, Dog, Allergenicity, Immunoinformatic

2224P

Identification of allergensof Honeybees (Apismelliferaand Apiscerana) and some Ticks,Mites, and Fleas using bioinformatics tools

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Background: Allergy is defined as an immune-mediated inflammatory response to common environmental allergens that are otherwise harmless. The immune processes of allergy usually rely on the production of IgE antibodies specific to common allergens. Allergic diseases are caused by the activation of mast cells and basophils through cell-surface-bound IgE. **Methods:** The amino acid sequence of some proteins of honeybee, mites, ticks and fleas retrieved from UniprotKB and NCBIp databases, and then saved into FASTA files format. Then physic-chemical properties of sequence were evaluated. At third step, by using of immunoinformatics servers allergenic peptide (epitopes) were predicted and analyzed. **Results:** We studied 9 various proteins for both Apismellifera and cerana. results revealed that there were not many allergenic epitopes for Apismellifera and Apiscerana, just one IgE epitope for H9KA47_APIME and Major allergen Api g 1, isoallergen 1 protein in Apismellifera in position 168 and position 146, respectively. Blast result revealed a hit found with ARPs database for most of proteins. we did not find any allergenic protein for fleas and ticks. But among the 14 proteins for mites 4 protein called Peptidase 1 in Dermatophagoidespteronyssinus, Peptidase 1 and Paramyosin in Dermatophagoides farina and Peptidase 1 in Euroglyphusmaynei were allergen. **Conclusion:** The prevalence of allergic diseases worldwide is rising dramatically in both developed and developing countries. Bioinformatics analysis results showed that these proteins have several peptides that are responsible for allergy and we can use these epitopes for other purposes.

Keywords: Bioinformatics, Allergens, Immunoinformatics, Epitopes

2745P

Immunogenic specificity of a recombinant peptide from Helicobacter pylori - an in silico study

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Back ground: Helicobacter pylori is a gram-negative, motile, spiral and microaerophilic bacteria that colonizes on the human gastric mucosa and causes gastric infection. H.pylori infection may lead to gastritis, gastric or duodenal ulcer and gastric carcinoma. Studies in animal models have shown that Vaccination with recombinant H.pylori vacuolating cytotoxin A (VacA), cytotoxin associated antigen, and neutrophil activating protein (NAP) confers protection against H. pylori infection. **Methods:** By Using several bioinformatic tools, 12 segments of the three mentioned peptides were attached together based on antigenic properties and propose as a new recombinant protein for further immunoinformatic analyses. B cell epitope mapping, T cell epitope mapping, allergic properties, 3D structure, antigenicity and solubility were determined. **Results:** Analysis of antigenic properties, solubility, and stability of recombinant peptide showed that the regions of interest are proper for practical studies. It can be expected that antibodies against these regions may effective in protection against

Helicobacter pylori infections. **Conclusion:** Our study demonstrated that recombinant peptide is useful for immunogenic research and can be a good candidate vaccine against H.pylori infection.

Keywords: Helicobacter pylori, recombinant peptide, in silico

2658P

In silico Optimization of Signal Peptides for the Development of Secretory Renilla luciferase as a Useful Gene Reporter in Cancer Immunotherapy Studies

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Background: There are few techniques available for quantifying immunologic studies such as systemic metastasis in preclinical models and cancer immunotherapy studies. Real time methods for detection of tumor progression or assessing the treatment response are also greatly needed. Luciferase enzyme is commonly used as a gene reporter for in vivo imaging process to establish cancer studies. Renilla luciferase is a promising candidate to be used in these studies as it has low molecular weights and also its emission spectrum is appropriate. If we become able to express luciferase a naturally secreted protein then there will be advantages such as increasing the sensitivity of experimental tests, real time and non-invasive monitoring of treated responses and many others. **Methods:** 100 signal peptide sequences were extracted from human and mouse secretory proteins and were then added to renila Luciferase sequence. Final sequences were tested by SecretomePserver to find the most potential secretory sequence. Before checking with SEcretomePserver, moreover, all sequences were checked to assure that they are not potentially transmembrane using TMHMM web server. **Results:** 14 signal peptides were proved to have appropriate scores. They were introduced as being highly efficient to make the target protein secretory. Using the above server, scores more than 0.5 and less than 0.9 were mentioned to be secretory. **Conclusion:** The potential signal peptides can be used to make the renilla luciferase secretory. Therefore their gene can be cloned within the recombinant luciferase cassette. By having the secretory form of renilla luciferase, it can be efficiently used in cancer studies and immunologic screenings.

Keywords: Signal peptide, Renilla Luciferase, Secretion, Cancer.

2655P

De-Immunization of secretory form Renilla lusiferase : a computational approach

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Background: Luciferases are a reporting tool for monitoring biological processes. Secreted

luciferase is used for quantitative assessment of cells in vivo by measuring its levels in blood. Designing of a secreted form of Renilla luciferase (RLuc) can improve application of this reporter. Since immunogenicity of this protein may be disturbing, we can reduce the immunogenicity by the replacement of critical residues. This RLuc blood assay complements in vivo bioluminescence imagingin small animals which are used in immunology research. **Methods:** B cell epitopes and T cell epitopes from Renilla luciferase was predicted by DiscoTope server and nHLAPred, respectively. Effects of 30 single amino acid substitutions in B cell epitopes were evaluated on protein function and stability by SNAP and Mutpred web servers. Four neutral mutations were selected that reduce B-Cell epitope residues after checking the 3-D structure of RLuc mutants out by DiscoTope server. Homology modeling of RLuc mutants was performed using M4Tserver. **Results:** nHLAPred scores showed negligible T cell epitopes, thus the B cell epitpes were undergone our approach. Therefore we changed some residues of B cell epitope out of the catalytic site. Consequently, mutations which had the least effect on the protein function and stability were chosen. These replacements can indirectly reduce B-Cell epitopes. However, these substitutions do not eliminate antibody binding, but have significant effects on the extent of the binding.**Conclusion:** The secreted RLuc with low immunogenicity can be used as a strong and sensitive tool in immunology and cancer studies.

Keywords: Renilla luciferase, mutation, immunogenicity, B cell epitope

3149P

In-silico analysis and evaluation of confirmed eukaryotic signal peptides effect on EGFP-scfv secretion

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Background: Signal peptides are small sequences which direct proteins to their proper cellular and extracellular locations or culture media in both prokaryotic and eukaryotic cells. Computational prediction of signal peptides (SPs) and their cleavage sites is of great importance in evaluation of potential signal peptide and determining whether a protein is secretory or not. In this study, With the aim of improving EGFP-scfv secretion, the effect of some eukaryotic confirmed signal peptides were predicted and evaluated. **Methods:** The sequence of EGFP-scfv was analyzed using online program SignalP 4.1 (http://wwwcbs.dtu.dk/services/Signalp/) for cleavage site prediction within the amino acid sequence based on Hidden Markov Models (HMM) algorithm. In the second step, the sequence of some confirmed homosapiens signal sequences including signal sequence of growth hormone variant, IL-10, cathepsin D, E and F, trypsin-1, apolipoprotein B-100, granzyme B, complement factor 1, metalloproteinase inhibitor 1,2 and 3 and some mus musculus signal peptide including growth hormone receptor and leukemia inhibitory factor were downloaded from Spdb (http://proline.bic.nus.edu.sg/spdb/) and added to the N-terminal end of sequence and then analysed using SecretomeP 2.0(www.cbs.dtu.dk/services/SecretomeP/).**Results**: No signal sequence was found in EGFP sequence. After adding the different eukaryotic signal peptides it was shown that appolipoprotein B-100 has the highest NN score and consequently the best potential

ability for secretion.**Conclusion:** Insilico prediction and optimization of signal peptide is a vital option to increase the overall yield of recombinant protein production before producing experimentally.

Keywords: In silic, Signal peptides, EGFP-scfv, Computational

2355P

Bioinformatic prediction of anti-human CD4 EGFP- scfv antigenic properties

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Background: Antigenic determinant is structural component of an antigen molecule that frequently found on regions of the molecule that have an unusually high degree of exposure to solvent. After entering an antigen into the body, it's antigenic determinants are presented by MHC molecule to T-cell which takes active part in host immunereactions. Anti-human CD4 EGFP- scfv is a bioluminescent-based reporter to identify $CD4^+$ cells. In this study, Antigenic properties of anti-human CD4 EGFP- scfv were predicted and the antigenic potential of each motif was determined. **Methods:** The antigenic properties of the sequence from anti-human CD4 EGFP- scfv were analyzed and antigenic epitopes were determined using Predicting Antigenic Peptides (http://imed.med.ucm.es/Tools/antigenic.pl). This web program predicts those segments from within a protein sequence that are likely to be antigenic by eliciting an antibody response. **Results:** The sequence of anti-human CD4 EGFP- scfv has 502 amino acids and shows 18 antigenic determinants. Average antigenic propensity of anti-human CD4 EGFP- scfv was 1.0113 based on antigenic plot and only a small sequence in the N-terminal region of EGFP- scfv shows a significant average antigenic propensity. **Conclusion:** Predicting and analyzing antigenic properties of monoclonal antibodies in fusion with bioluminescent reporters has great importance due to the vast application in clinical and medical research. Regardless of whether the anti-human CD4 EGFP-scfv has the suitable potential to prevent or detect some diseases such as AIDS or not, it seems that its immunogenicity is small enough for further in vitro studies.

Keywords: Bioinformatics, Anti-human CD4 EGFP- scfv, MHC, T-cell

2360P

Design and prediction-based evaluation of an antigenic domain to increase antigenicity of anti-human CD4 EGFP- scfv

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Background: The antigenic regions on the protein surface that are recognized by immunoglobin are called B-cell epitopes. Prediction and identification of epitopic regions on protein surface is of vital importance for developing synthetic monoclonal and polyclonal antibodies. Most

of algorithms which predict the position of epitopes, work based on certain protein properties like hydrophilicity, mobility/flexibility, surface accessibility, structure and etc. In this study, tandem hydrophilic sequences were added to N-terminal end of EGFP molecule with the aim to increase its antigenic properties.**Methods**: The amino acid sequence of EGFP was submitted to Predicting Antigenic Peptides (http://imed.med.ucm.es/Tools/antigenic.pl) and its epitopic regions were determined. In order to increase antigenic properties, a hydrophilic domain composing of sequential antigenic sequences was added to N-terminal end of EGFP and submitted again and then the results were compared together. **Results:** The natural sequence of EGFP has 239 amino acids and includes 8 antigenic determinants with antigenic propensity (1.0198). However the new designed EGFP- based domain with high immunogenic potential has 349 amino acids with 12 antigenic determinants. Moreover it's antigenic propensity is about 1.0353, and perhaps the new domain is an ideal option for production new antibodies for EGFP with diagnostic purposes. **Conclusion:** Prediction and design epitopic domain is an early step in producing specific antibodies. Here designing a hydrophilic domain for EGFP With the aim of increasing the antigenicity and it's antigenic propensity was done. The potential antibodies may have multiple applications in molecular biology, including western blots, immunopreciptation, ELISA, flow cytometry and immunohistochemistry.

Keywords: Immunoglobin, Epitopes, Enhanced Green fluorescent protein (EGFP), Anti-human CD4 EGFP-scfv

2554P

The Prediction of Antigenic Properties of Human Growth Hormone and GHR Antagonist

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Background: Growth hormone (GH) is a protein that contains 191 amino acids with two disulfide bonds and four α-helixes. Its molecular mass is approximately 22 kDa. Significant physiological effect of GH is the promotion of postnatal longitudinal growth. GH also has been associated with alterations in lipid, carbohydrate, nitrogen, and mineral metabolism.A single amino acid substitution in the GH gene at the position 120 was found to transform the consequent protein from an agonist to an antagonist at the growth hormone receptor (GHR). Inthisstudy,we consideredthe effects of mutagenesis on the antigenic properties ofgrowth hormone and its antagonist derivative using bioinformatics. **Methods:**The protein sequence of growth hormone was obtained from NCBI (www.ncbi.nlm.nih.gov) and was manipulated at position 120 to achieve the antagonist of GHR. Then, the antigenic properties of growth hormone and the new antagonist were studied using a web-based database (http:// imed.med.ucm.es/tools /antigenic). **Results:**There are seven antigenic spots in the primary structure of growth hormone.The antigenicpattern is similar in the sequence of the antagonist of GHR and the average antigenic propensity for both proteins including nativeGH and the new antagonististhe same, and being 1.0317. **Conclusion:**Manipulated GH has antagonistic properties as well as therapeutic potential for the treatment of some diseases such as cancer and acromegaly.We have shown that altering the glycine 120 may notalter theantigenicity of the growth hormone, since itis not in antigenic regions. Therefore,the GH antagonist may be used in human trial as a safe drug.

Keywords: Human Growth Hormone, antagonist, antigenic spots, bioinformatics

3016P

The Effect of Mutations on the protein folding rates of Renilla Luciferase8; a Bioinformatics Study

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Background: Renilla Luciferase (RLUC) catalyzes the oxidation of coelenterazine to produce light and is an expansive employment asreporter genes in biological research. RLUC8 is the mutant form of Renilla Luciferase, and a total of eight favorable mutations are combined to produce this mutant luciferase, that comparedwith the parental enzyme, is 200-fold more resistant to inactivation in serum and exhibits a 4-fold improvementin light output. these mutations including A55T, C124A, S130A, K136R, A143M, M185V, M253L, andS287L.
Since a major issue in molecular biology today is to understand how a protein folds into its characteristic three-dimensional structure and Proteins can fold into their native structures at very different rates of folding, varying from several microseconds to even an hour, Here to consider whether mutations can effect on the folding kinetic and folding rate of the enzyme, a bioinformatics study is carried out and the folding rate of RLUC has compared with RLUC8.
Methods: The amino acid sequence of RLUC was obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and the sequence of RLUC8 obtained from (http://www.stanford.edu/~loening/plasmids/sequence/RLuc8-6xHis.fasta).then the three-dimensional structure of Both sequences were determinedusing (http://swissmodel.expasy.org), and finally the folding rates of both enzymes were analyzed using prediction of protein folding rates server (http://psfs.cbrc.jp/fold-rate).**Results:**As a result of this study, it was found that the protein folding kinetics of both enzymes are Multi-States and the folding rate of RLUC8 ($\ln(k_f)$ = -9.7275212/sec), is about 0.18/sec more than the folding rate of RLUC ($\ln(k_f)$ = -9.9051571/sec).
Conclusion: therefore this characteristic affected by mutations as well as the other properties of the enzyme such as thermal stability and the ability of light emission have improved.

Keywords: RenillaLuciferase(RLUC), Renilla Luciferase8, mutation, Protein Folding Rate, Protein Folding Kinetic.

2451P

The Effect of Cumulative Mutations on the Solubility of Renilla Luciferase; a Bioinformatics Study

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Background: Renilla luciferase (RLUC) catalyzes the oxidation of coelenterazine to produce light and is a popular reporter enzyme for gene expression and biosensor applications. Super RLUC is the mutant form of renilla luciferase, and contains three manipulated sites. Cumulative mutations including V267I and K189V cause a more than two-fold increase in the

apparent kcat of light emission compared to wild-type RLUC and the third mutation, M185V, increased both the stability of enzyme activity in serum and the ability of light emission. Here, to consider whether cumulative mutations can improve the poor solubility of the enzyme, a bioinformatics study is carried out and the solubility of RLUC has compared with supper-RULC.Methods:The amino acid sequence of RLUC was obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and the sequence of supper-RLUC obtained by changing V267I and K189V, and M185V. Both sequences were analyzed using Recombinant Protein Solubility Prediction server (http://www.biotech.ou.edu/). **Results:** As a result of this study, it was found that both enzymes have a poor chance of solubility when overexpressed in E. coli. **Conclusion:**The results moreover, show new challenge for biotechnological innovations of RLUC. While many properties of the enzyme such as thermal stability and the ability of light emission have improved in RLUC, the insoluble nature of enzyme is an unsolved problem for further applications.

Keywords: Renilla Luciferase (RLUC), Super Renila Luciferase, Mutation, Enzyme Solubility

2490P

A Search for Antigenic Patches on Gaussia luciferase; a Bioinformatic Analysis

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Background: Gaussia Luciferase (GLuc) is a naturally secreted protein by the copepod, Gaussia princeps. With 19kD, it is the smallest and “brightest” luciferase and an ideal reporter protein, either as a standalone expression monitor or fusion partner with other proteins such as antibodies during in vivo imaging studies. Although, multiple disulfide bonds contribute to the Gluc stability at relatively elevated temperatures, the antigenic properties of Gluc is absolutely unknown. In the current study, the antigenic property of the Gluc has been studied using bioinformatic studies that may help us for further protein manipulations to obtain more effective reporters for in vivo imaging studies. **Methods:** The amino acid sequence of GLuc protein was obtained from Genbank (www.ncbi.nlm.nih.gov). The sequence was analyzed for determining potential antigenic epitopes using a web-based program (http://imed.med.ucm.es/Tools/antigenic.p). The results were analyzed based on biochemical properties of each region to recognize the most antigenic spots on GLuc. **Results:** GLuc contains 175 amino acids and there are 7 antigenic regions in the primary structure of GLuc. The predicted binders were shown by a peak in graphical interface or as colored residues in HTML interfaces. Average antigenic propensity for this protein is 1.0305.**Conclusion**: Prediction of antigenic regions GLuc as a reporter gene is important for two reasons. A) Design and or production anti-luciferase antibodies for use in immunocytochemistry and Western blot applications. B) Protein manipulations to reduce the antigenic property of the Gluc. Therefore, in the first step, the antigenic regions of GLuc have been analyzed in this study.

Keywords: Gaussia luciferase (Glu), Antigenic regions, Protein manipulation, Bioinformatics

2491P

An in-silico search for the improvement of the luciferase-based secretory reporters; a comparative study

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Background: Signal peptide is a short peptide in the N_terminus end of most secretory proteins, directing them to their proper cellular and extracellular location in both prokaryotic and eukaryotic cells. Predicting the effects of signal peptide on secretory protein is important in determining the efficiency of the secretory proteins. The aim of this study is comparision between different eukaryotic signal peptides on the secretory potential of Gaussia luciferase(GLuc). **Methods:** The sequence of native GLuc and a collection of manipulated GLuc with some human signal peptides such as IL_2, chymotrypsinogen, albumin and trypsinogen_2 were analyzed using a web-based program, secretomp2.0 server namely(http://www.secretomp). The results were ranked based on the efficiency of secretion and compared with some available experimental results in literatures. **Results**: There were some minor differences betwee in-silico and experimental results from literatures. In experimental studies, the native secreted GLuc has highest potential for secretion but the results obtained from the in-silico studies showed that the highest NN score is for human chymotrypsinogen. The NN scores for others theoretical manipulated GLucs, moreover, are not similar to that we respected and has been reported in experimental studies.**Conclusion:** The ability of bioinformatic servers to use in experimental studies is always in doubt. Such as other web-based tools, the secretomp server is not universal tool for prediction of the secretion efficiency of all proteins. However, the question that which one of signal peptides is definitely useful for designing a luciferase-based secretory reporter remains unanswered. This possibly answered by further experimental studies.

Keywords: Gaussia luciferase (GLuc) , In silico , Secretomp, Protein secretion

2558P

Comparing the Solubility of Renilla Luciferase and Renilla Luciferase 8 Enzymes using Bioinformatics Studies

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Background: Bioluminescence is a fascinating process in which living organisms
Convert chemical energy into light. Reports have shown that bioluminescence occurs in many different organisms includingluciferases. Theluciferases that use coelenterazine as their substrate are more appropriate for application as bioluminescent tags, as these enzymes are not ATP dependent and in general require only molecular oxygen in addition to coelenterazine for luminescence. From this group of proteins,Renillaluciferase(RLUC), a monomeric 36kDa protein, catalyzes coelenterazine oxidation to produce light. Renilla luciferase 8 (RLUC8) contains eight favorable mutations that, compared with the parental enzyme, is more resistant

to inactivation and has more light output. Although RLUC is a fascinating reporter enzyme in many studies, the insoluble nature of RLUCis a limiting factorfor its applications. In this study, the solubility of both RLUC and RLUC8 has been considered using bioinformatic studies.The results may be useful for designing more soluble RLUC.**Methods:**Theamino acid sequence of the protein was analyzed using Recombinant Protein Solubility Prediction server (http://www.biotech.ou.edu/) to predict the probable Solubility of RLUC and RLUC8enzymes.**Results:** Results from biochemical analysis have shown both RLUC and RLUC8are highly insoluble and mutagenesis has negligible effect on the solubility of RLUC 8.**Conclusion:** The present study attempted to compare RLUC with RULC8 to seewhich one, when overexpressed in E. Coli., would be more prone to solubility in particular.In addition, it was expected that RULC8 with greater stability and light output could be moresoluble.But, both of them had no chance of solubility in this respect.

Keywords: Enzyme Solubility, Mutant, Renilla Luciferase, Bioluminescence

2859P

Design new inhibitors for HTLV-1 protease with docking and QSAR methods

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Background: Human T lymphotropic virus type 1 (HTLV-1) plays role in a wide variety of diseases such as adult T-Cell Leukemia (ATL), HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM / TSP), and causes neurologic disorders and other disorders opportunistic pulmonary infections with immune deficiency such carinii penomonosisty, CMV, Gammapathy monoclonal, chronic renal failure, etc. Protease is a one of the important enzymes in the maturation of the virus replication cycle of HTLV-1. Unfortunately, the made drugs can't suppress the virus, completely and it is essential to design new drugs to find cure and better treatment. **Methods:**Hence, our new compounds were created based on peptidomemitics and QSAR properties for this virus protease as inhibitors. The ligands were created by Hyperchem and QSAR properties of them studied and evaluated by molinspiration website (www.molinspiration.com). Docking was performed by using the Autodock software. One of the designed ligands to act more effective was chosen.**Results:** Molecule binding energy equals to -8.35 Kcal/mol and inhibition constant equal to752.7 nM. **Conclusion:**QSAR and docking results showed us that peptidomemitics based compounds are very suitable compounds for rational drug design. They can be used to produce new drugs for HTLV and HIV virus.

Keywords: HTLV-1 protease,QSAR method

2818P

A Simple Stereological Method for Estimating the Number and the Volume of the Pancreatic Beta Cells

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Background: Number of the beta cells as well as their volume is a fundamental variable in the pancreatic research.This study describes a simple method for stereological estimation of the volume of the pancreas, the total volume of the islets, and the total number, as well as the mean volume, of the beta cells in rat. **Methods:** The primary volume of the pancreas was measured using the immersion method. Also, tissue shrinkage was estimated and the final pancreas volume was corrected without the need for serial sectioning. A limited number (i.e., 10-13) of the isotropic uniform random slabs of each pancreas was embedded in the same block. One 5 µm and one 20 µm sections were obtained and stained with a modified aldehyde fuchsin. The point counting, optical dissector and point-sampled intercept methods were used to estimate the volume density of the islet, the numerical density of the beta cells, and the mean cell volume, respectively. **Results:** After calculating the tissue shrinkage, the mean primary volume of the pancreas (628 mm3; CV: 25%) was corrected to obtain the final volume (442 mm3; CV: 39%). The mean islet volume was reported as 3.8 mm3 (CV: 22%). Besides, the total number of the beta cells was estimated as 2.9x106 (CV: 20%). Moreover, the mean volume of the beta cells was obtained (1,158 µm3; CV: 9%).**Conclusion:** It takes almost one hour to estimate the volume of the islets and two hours to count the cells and estimate the intercepts per animal.

Keywords: Stereology, Beta Cells, Volume, Pancreas

3178P

An insilico chimeric multisubunit vaccine targeting virulence factors of Enterohemorrhagic Escherichia coli (EHEC)

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Background: Pathogenic strains of E. colifamilycause millions of dead especially between children each year. The pathogenesis of EHEC is very high because of colonization potency in human intestine and its toxinthat lead tohemorrhagic diarrhea, HC and HUS diseases. So, a good candidate for vaccination against this pathogen is correct combination of different virulence factors which involved in its pathogenicity. **Methods**: Gene sequences of EspA, Stx2B and Intiminproteins were adapted from valid gene bank. A good oligopeptide linker with rigid structure was choice between subunits. Then the different combinations of chimer subunits werestudied by Protparam online software. The secondary and tertiary structures of chimeric protein with and without linker were studied with I-TASSER online software. The antigenicity, B and T cell epitopes of this chimer were analyzed with Vaxijin, B&T cell pred online softwares. mRNA properties also analyzed by appropriate software. **Results**: Physicochemical parameters from Protparam showed that EspA must be the first subunit in the the chimer construct. 3D structure prediction showed that linker separate three subunits from each other and conformational epitopes are exposed very well. mRNA structure has good properties after codon optimization. **Conclusion**: From the immunoinformatic studies we found that EspA-Stx2B-Intimin combination sequence as a chimeric protein has the best score between others combinations of genes.

Keywords:EHEC, Virulence factors, Immunoinformatic, Insilicovaccine design

3341P

In silico analysis of virus coat protein epitopes for monoclonal antibody production against of different strains

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Background: Different strains of virus detection using ELISA require specific monoclonal antibodies against epitope of each strain. Any change in the amino acid sequences of protein, can change epitopes or generate new epitopes and therefore change the kind of antibody recognizer of that epitopes. Challenge of finding specific monoclonal antibody, in spite of high costs, this technique requires a great deal of time and energy and on the other hand uncertainty duplicate hardness of it.Then it is necessary to analysis of type, amount and site of variation in all strains and only in the event that the existing diversity, leading to the creation of epitope is different than other strains may be used with the corresponding antibody identification with extremely high speed and accuracy to the diagnosis of viral contamination. In this experiment, the grapevine fanleaf virus in Iran that is almost in the great prevalence of vineyards, selectedand According to the last research regarding the distinction between the virus Iranianstrains in terms of protein ofin phylogenetic tree, information relating to this protein from the NCBI gaderedand all epitopesevaluated using BCpred Server. With the use of this server and perform three FBCPred,BPPred and AAP, analysis methods, two differentand specific epitopes of Iranian strains were identified which can be used in the production of antibodies specific strains.

Keywords: Epitope analysis, Virus strain, Antibody

3310P

Three dimentional structure prediction of of fliC in Salmonella enteric

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Background: Salmonella enterica is a zoonotic pathogen causing Typhoid fever in humans and animals. It is an important cause of food borne infections in humans throughout the world. A recent study estimated approximately 22 million cases of typhoid each year with at least 200,000 deaths. FliC encoding Flagellin plays a role in pathogenesis and is an important antigens in vaccination. In this study 3D structure of FliC was predicted using bioinformatic tools. **Methods:** The protein Sequence of fliC was obtained from NCBI database. The in silico gene analysis was performed using Expasy's ProtParam. In silico Linear and conformational epitopes for fliC were determined. Tertiary structure prediction was done by homology modeling via I-TASSER software and PSIPRED. **Results:**All best epitopes were selected and conformational epitopes for B cells were predicted by CBTOPE and Discotope servers. Comparative and ab initio modeling of the sequence was exploited to produce three-dimensional structures models of the protein. The 3D modeled structure for protein was generated by Swiss model and I-TASSER software. **Conclusion:**The best refined structure with the highest score was selected as the final structure. This structure was validated with other relevant software.

Keywords: In silico, 3D structure, fliC, epitopes, Salmonella

3212P

In-silico analysis and evaluation of confirmed eukaryotic signal peptides effect on UNC-46 secretion

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Background: γ-Amino butyric acid is the major inhibitory neurotransmitter of the CNS, and GABA transporter is critical in maintaining a GABA reservoir and associated function. Mutation in unc-46gene in Caenorhabditis elegans causes defects in all behaviors that are mediated by GABA.UNC-46 is a sorting factor that localizes the vesicular GABA transporter to synaptic vesicles. The UNC-46 proteins are a type 1 transmembrane protein with 259 amino acids in length, which comprise a cleavable signal peptide. **Methods:** The sequence of signal peptide of UNC-46 was downloaded from Spdb (http://proline.bic.nus.edu.sg/spdb/). This sequence substitute with the sequence of some confirmed homosapiens signal sequence including signal sequence of apolipoproteinB-100, cathepsin D, cathepsin F, adhesion, apolipoprotein A-1, growth hormon –releasing hormon receptor and add to N-terminal end of sequence and then analyzd using Secretome P 2.0 (www.secretomep). **Results:** The results were analyzed to rank the obtained secretory reports based on the efficiency of secretion. It was shown that adhesion has the highest NN score, so the signal peptide of this protein has the best potential ability for secretion. **Conclusion:** Increased secretion UNC-46, GABA transmission efficiency is increased.Insilico prediction, use for production of high efficiency recombinant protein before producing experimentally.

Keywords: In silico, γ-Amino butyric acid, GABA transporter,UNC-46

3340P

Prostate cancer metabolome profiling by using proton nuclear magnetic resonance spectroscopy.

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Background: Prostate cancer is the second leading causes of death among men. It is the most common cancer after lung cancer. Metabolomics is new approaches for early detection of diseases with discovery of new biomarkers. Using 1H NMR spectroscopy and feed forward neural network modeling, it is possible to detect and predict the disease. In this investigation, we examined the chance of neural networking as tools in diagnosing this disease.**Methods:**In this study, serum samples from15 men with prostate cancer, and 15 healthy male with same range of age were collected. 1H NMR spectroscopy with CPMG protocol were recorded by Bruker 400 MHZ and data were analyzed. Feed forward neural networking was run with seventy percent of samples. This model was tested with other thirty percent of samples. ROC test were also used to find out the possibilities of new marker. **Results:** Analyzing the data showed that there was variation in metabolites concentration and metabolism pathways in both healthy and cancer patients. Using ROC test showed 85% of sensitivity in differentiation of two groups with 100 metabolites and 0.2875-error rate by using Artificial neural network **.Conclusion:** Thirty-one metabolic pathways showed changes but the major one were seen

in the aminoacyl tRNA biosynthesis, nitrogen metabolism, arginine and proline metabolism, alanine, aspartate glutamate metabolism

Keywords: prostate cancer, metabolite, NMR spectroscopy, Artificial neural network

Immunology & Clinical Laboratory

Oral Presentations:

3059O

Determination of the HAMA (Human anti-mouse antibody) reference range in the Kermani men population

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Background: The human immune system in the face of other animals antigens and antibodies including mice reacted and produce HAMA antibody. Since the murine antibodies are used for diagnostic and treatment purposes so HAMA antibodies in patients due to possible interference with test results, and subsequently the wrong diagnosis and treatment are important. Because the interpretation of laboratory measurements need to be aware of the normal ranges and expected ranges for these results, Therefore, the aim of this study is that we can to determine HAMA reference range in the Kermani Men with measurement of the HAMA concentration in this population and to set it to use in studies related to reviews of HAMA antibody. **Methods:** In a cohort study, 40 persons who had no occupational relation to the mouse with the age range 24-58 years were studied. The concentration of Human anti-mouse antibody (HAMA) in the studied samples of serum using ELISA techniques was measured. Demographic information using the form collecting information was obtained. Analysis of data was done using SPSS version 18. **Results:** According to the results of the present study, the lowest and the highest amount of HAMA in the studied population were (with an average age of 40.6), respectively, 0.07 and 3.93ng/ L. **Conclusion:** Since the mean concentration of each analyte in different population groups in terms of various demographic factors such as age, sex, occupation, race, geographic location and position of habitat and etc is different, Therefore, Determination of reference range for each analyte based on the effective demographic factors nationally and locally is essential and necessary. Parallel to this research, tests on the sample of people with direct encounter in mice and their secretions (animal house workers) by Dr. Mohammadi and his colleagues have done that its results are being reviewed.

Keyword: HAMA, Reference range, Kermani men

2673O

Modification and evaluation of avidity IgG Testing for differentiating of Toxoplasma Gondii infection in early stage of pregnancy

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Background: Toxoplasma gondii infection, an intracellular parasite, is often asymptomatic or is caused by different clinical diseases without being detected. Evaluation of IgG, IgA, and IgM in order to diagnose the pending Toxoplasmosis may confront some problems. Several researches has showed that Toxo IgG avidity can be useful in the recent active Toxoplasmosis. In current study, modification and importance of improved Toxoplasma Avidity IgG testing has been evaluated for differentiating Toxoplasma gondii infection in early stage of pregnancy. **Methods:** This experimental study included 300 pregnant women with risk of Toxoplasmosis in their initial months of pregnancy. We randomly divided 300 serum samples into A group (n=60) with high avidity and B group (n=40) with borderline avidity. The samples with Toxo IgG levels were classified to four subgroups. IgG avidity was evaluated by enzyme-linked immunosorbent assay (ELISA) method. **Results:** The mean absorbance of 100 samples in two groups was calculated, and then, two dilution curves with plotted absorbance against dilution were drawn for each serum sample. The results of this study showed that in groups with different concentrations of toxo IgG, appropriate dilution of serum is suitable for testing of Avidity. Our findings revealed the subgroups of 1, 2, 3, and 4 with serum dilutios of 1/3 , 1/6, 1/9, and 1/18 respectively, had real and good avidity. **Conclusion:** One of the issues affectig the results of avidity is high concenteration of Toxo IgG in serum sample. As shown in this study, the best points of dilution for well avidity in both high and borderline avidities are marked with arrows in figures 1-8. This study confirmed that improved methods of measuring Toxo Avidity IgG are very important.

Keywords*:* Toxoplasmosis, Pregnancy, ELISA, IgG

1439O

Immunodiagnosis of Vibrio Cholera by OMPw monoclonal antibody

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Background: Cholera is an acute intestinal disease with watery diarrhea, vomiting, high dehydration, acidosis, circulation disorders caused by Vibrio cholera and routine microbiological and biochemical analysis need three working days. On the other hand, In spite of many publications related to immunological and molecular methods for cholera diagnosis most assays require enrichment by previous culture of bacteria, which increases the time needed or involves the use of costly equipment and reagents. **Methods:** In this research we used the recombinant bacterial outer membrane proteins (OMPw) for immunization of balb\c mouse and rabbit to have monoclonal and polyclonal antibody respectively. Hybridoma cells were expanded in usual manner, its supernatants were collected and stored at -20^{0}C for further experiments. The specific anti OMPw antibody (IgG2a) production was determined by enzyme-linked immunosorbent assay (ELISA) using heat-inactivated bacteria and recombinant OMPw as coating antigen. Its cross reaction with Salmonella, E.coli, and shigella was also examined

by ELISA and immune-blotting. **Results:** Rabbit Polyclonal and mouse monoclonal antibody raised against OMPw antigens showed very high absorbance values in ELISA at high dilutions between 1:250 and 1:8000 with recombinant OMPw antigen. Our immuno-blot results with bacterial antigen of Salmonella, E.coli, and shigella showed that anti OMPw antibody have some cross reaction with these agents. **Conclution:** The purpose of this study was to obtain a sensitive and specific immune diagnostic assay for detection of vibrio Cholera. Our results demonstrated that OMPw is not a good and specific candidate for this purpose.
Keywords: Vibrio cholereae, Ompw, Monoclonal antibody.

1438O
CTX monoclonal antibody might be a good candidate for immunodiagnostic of Vibrio Cholera

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Background: Vibrio cholera (V.cholera), a food borne pathogen, is still a serious problem in some countries in Asia and African regions. The epidemic and pandemic cholera in various regions was mainly caused by V.cholera serogroups O1 and O139. Diagnosis of V.cholera is the key importance to initiate effective therapy and to institute proper epidemiological measures. Detection of V.cholera toxin is one of the important diagnostic key.**Methods:** In this research we used the recombinant V.cholera toxin (CTX) for immunization of balb\c mouse and rabbit to have monoclonal and polyclonal antibody respectively. Hybridoma cells were expanded in usual manner, its supernatants were collected and stored at -20^{0}C for further experiments. The specific anti CTX antibody (IgG2a) production was determined by enzyme-linked immunosorbent assay (ELISA) using recombinant CTX as coating antigen. Its reaction and cross reaction with V. cholera, Salmonella, E.coli, and shigella was also examined by ELISA and immune-blotting respectively.**Results:** Monoclonal antibodies specific to V.cholera CTX toxin were successfully generated. The monoclonal antibody reacted with recombinant CTX also bound to isolates of V.cholera and react with little cross reaction to Salmonella, E.coli, and shigella. The sensitivity of the antibody ranged from 1X10^6 to 101x10^7 c.f.u. ml-1 for V.cholera obtained from pure cultures.**Conclusion:** The purpose of this study was to obtain a sensitive and specific immune diagnostic assay for detection of V.cholera. Our results demonstrated that CTX toxin in conjunction with OMPw could be good candidate for this purpose.
Keywords: Vibrio cholereae, CTX, monoclonal antibody.

3133O
Interferon-γ release assays and tuberculin skin testing for diagnosis of latent tuberculosis infection

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Background: IFN-γ release assays (IGRAs) have been recently introduced as a complement or alternative to tuberculin skin testing (TST) for diagnosis of latent tuberculosis. TST might lead to false positive results due to previous vaccination or cross reaction with other mycobacterium while IGRAs only test for specific tuberculosis antigens. The aim of this study was to evaluate patients suspicious to have tuberculosis with TST and IGRAs considering their clinical and paraclinical data. **Methods:** Ninety patients (mean age 44 years) were included and IGRA (Quantiferon TB Gold) and TST were performed according to manufacturers' instructions. **Results:** TST was negative, suspicious and positive in 63%, 1% and 35% of patients. IGRA was negative, indeterminate and positive in 73%, 2% and 25% of patients. In 53% of patients, IGRA and TST were negative and in 16% of patients both tests were positive. The size of TST was significantly ($p=0.007$) higher in patients with positive IGRA (18±12mm) compared to patients with negative IGRA (7±8). 8% of patients had positive IGRA results and negative TST. IGRA was significantly more positive in patients with radiological images confirming tuberculosis ($p<0.0001$). 20% of patients had positive TST and negative IGRA. **Conclusion:** While IGRA and TST results are similar in majority of patients, there are cases in which these tests do not confirm each other. As the gold standard tests for tuberculosis are not easily available, sensitivity and specificity of IGRA cannot be easily measured. In populations with BCG vaccination, IGRA might be more specific and also it can patients with latent tuberculosis but negative TST results. The cost effectiveness and sensitivity and specificity should be assessed in each population.

2008O

Assessment of Lactobacillus acidophilus and Bifidobacterium animal effects on the expression of cytokines IFNγ and TGFβ in U-937monocytic cell line

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Background: Probiotics are group of bacteria that has regulatory and stimulatory effect in immune system. These effects, affect the whole immune system response pattern and their consumption regulates the immune circumstance and decrease sign and symptoms of several immunologic disorders. Lactobacillaceae and bifidobacteriaceae families are most common applicable bacteria among probiotics. The purpose of this study was to investigate the influence of L.acidophilus and B.animal on the expression of inflammatory cytokine, IFN-gamma and anti inflammatory cytokine, TGF-beta in monocyte 937U. **Methods:** After cultivation of L.acidophilus and B.animal, bacterial suspension was prepared in PBS then inactivation was carried out by heat process. Cell line of 937U was cultured in RPMI media up to logarithmical growth phase. Than four groups of cell were prepared (empty cells, junta L.acidophilus, junta B.animal and junta B. animalL. acidophilus). Cells were cultured in RPMI media for 72 hours with or without bacterial constituents. Then separation of cells from media follows by RNA extraction and cDNA synthesis. Gene expression rate was measured by real -time PCR.**Results**: Results indicated that the expression of IFN-gammais decreased and beta-TGF cytokine is increased significantly. These expression pattern can prevent differentiation to TH1 and TH17 cells while let the Tregand TH2 cells to be appeared.**Conclusion**: In this study it was shown

that the consumption of certain species of probiotics can inhibit the Th1 and Th17 response but they stimulate Th2 and Treg responses. Outcome of this process was to suppress inflammatory reactions in the body that can be a way to prevent and treat autoimmune diseases in the body.
Keywords: L.acidophilus, B.animal, Monocyte, gamma-INF, Beta TGF.

3248O

Normal ranges of lymphocyte subpopulations in a sample of adult Iranians

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Background: Nowadays flowcytometry is frequently used for immunephenotyping in clinical and research laboratory. Normal lymphocyte ranges are used for interpreting the data of the patients. Despite frequent use of flowcytometry in Iran, no study, to date, investigated the normal ranges of lymphocyte subtypes in Iranian population. The aim of this study was to evaluate the range of lymphocyte subpopulations in healthy Iranian individuals between the ages of 15 and 55 years old. **Methods:** Blood samples were obtained from 86 healthy subjects including 43 female and 43 male with the mean age 32.95 $\pm$ 10.62 and 30.30 $\pm$ 9.8, respectively. The numbers of patients in different age groups was calculated based on the data on the website of national organization for civil registration. Flow cytometry analysis was used to determine the range of lymphocytes subpopulations in peripheral blood samples using a lysed whole-blood technique. **Results:** The mean percentage of lymphocytes subpopulations in peripheral blood of individuals was 68.8±9.3 for total T ($CD3^+$) cells, 11.9±4.1 for B ($CD19^+$) cells, 39.9±7.7 for helper T ($CD3^+CD4^+$) cells, 33.1±6.6 for cytotoxic T ($CD3^+CD8^+$) cells, 6.7±3.6 for activated T ($CD3^+HLA\text{-}DR^+$) cells, 12.6±7.3 for NK ($CD3^-CD16/56^+$) cells and 5.1±2.8 for NKT ($CD3^+CD16/56^+$) cells. **Conclusion:** The mean percentage of lymphocytes subpopulations in healthy southern Iranians seems no to be significantly different from those reported in the West. These data may be used for the evaluation of health and disease status and also response to antivirus treatment in patients with HIV.
Keywords: Normal Lymphocytes Subpopulation, Flowcytometry

1846O

The C1p Receptor, C1qRp, Could be a New Marker for Diagnosis of Multiple Myeloma

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Background: CD93 is a highly glycosylated transmembrane protein and has been demonstrated as one of the C1q receptors known as C1qRp. CD93 is expressed on monocytes, neutrophils, endothelial cells and has been shown to play an important role in clearance of apoptotic cells by monocytes/macrophages. We have recently demonstrated that CD93 is strongly expressed on human hematopoietic stem cells from bone marrow, umbilical cord blood as well as mobilized peripheral blood stem cells (Jalili A, Transfusion 2010:50;2002). In addition, recent studies

have shown that CD93 is expressed on B lymphocytes during their early development and is downregulated along B cell maturation. However, it was demonstrated that CD93 is expressed on mice plasma cells (PC) particularly long-lived PC. Moreover, animal studies have shown that CD93- deficient mice are impaired in antibody secretion and the number of plasma cells in the bone marrow, indicating that CD93 is crucial for maintenance of plasma cell in the bone marrow. However, the expression of CD93 on myeloma cells as long-lived malignant plasma cells have not been yet studied. **Methods**: First, by employing flow cytometry and RT-PCR we examined the level of CD93 on a myeloma cell line, U266, and found that it highly expressed on this cell line. Next, we have sorted primary myeloma cells as they are positive for CD138 by magnetic beads and examined the expression of CD93 in these sorted. Our data demonstrate that in contrast to $CD138^-$ cells, $CD138^+$ primary cells strongly express CD93. Finally, to determine the expression of CD93 protein on primary myeloma cells from patients with multiple myeloma, a three-colored flow cytometry analysis was performed and bone marrow samples were stained with anti-CD93-PE, Anti-CD38-PerCP and CD45-FITC. **Results**: Interestingly, we observed that CD93 is highly expressed on myeloma cells, $CD38^{hi}$/$CD45^{low}$ cells). **Conclusion**: we are demonstrating for the first time that CD93 is expressed on myeloma cells and that CD93 could be new marker for diagnosis of multiple myeloma.

Poster Presentations:

2201P

Phytohaemagglutinin and anti-CD2/CD3/CD28 macsibeads stimulate $CD4^+$ lymphocyte proliferation extensively

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Background: Lymphocytes proliferate considerably following appropriate stimulation in vitro. The aim of this study is to define proliferative capacity of two different stimulation methods on $CD4^+$Lymphocytes. **Methods:** Lymphocyteswere isolated from healthy donor blood sample after removing adherent cells (monocytes). We compared the efficacy of macsibeads coated with anti-CD2, anti-CD3, anti-CD28 (anti-CD2/CD3/CD28), and Phytohaemagglutinin (PHA) on $CD4^+$ lymphocyte proliferation by using CFSE dye and Flowcytometry in cell culture media. **Results:** Each of this stimulus methods resulted in extensive $CD4^+$lymphocyte proliferation. The proliferative capacity of PHA was more compared with stimulation by anti-CD2/CD3/CD28 macsibeads. Stimulation by PHA led to 90.9% proliferation in $CD4^+$ lymphocyte population, but macsibeads induced 70.3% proliferation. **Conclusion:** Our results show that macsibeads along with PHA can be used to obtain a large number of expanded $CD4^+$ lymphocytes.

Keywords: Lymphocyte, Proliferation, PHA, Macsibead

1631P

Prevalence rate of Herpes Simplex virus type 1 and 2 IgG antibodies in Gilan province

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Background: Herpes simplex virus IgG antibodies are common worldwide. The most common manifestations of HSV-1 infections are oral lesions, neonatal disease, genital lesions, encephalitis , ocular infection , asymptomatic infection , non-oral ,non-genital skin lesions . Data on prevalence of HSV-1 and HSV-2 IgG antibodies are limited in Asia, especially in Iran.**Methods:** We did random blood sampling on 800 cases referred to Gilan's clinical laboratories. Demographic data gathered by a well-designed questionnaire and for serological studies, blood samples centrifuged.HSV-1,2 and HSV-2 specific ELISA kits used to determine IgG type specific antibodies in sera samples. **Results:** HSV-1 and HSV-2 IgG antibodies were positive in 467 (58.4%) and 28 (3.5%) subjects, respectively. According to our study, there was significant correlation between age, marital status , job , symptoms , history of disease and HSV IgG antibodies seroprevalence. **Conclusion:** This study is the first, to our knowledge, to present the comparative seroepidemiology of HSV-1 and HSV-2 IgG antibody in Gilan province. Our findings were in agreement with prior studies in which HSV-1 IgG was more prevalent than HSV-2 IgG antibody and seropositivity increased with age. Prevalence rate of HSV IgG antibodies in Gilan is lower than most European countries and higher than African countries. The high prevalence of HSV infection underlines the need for focusing on preventive efforts and education among the population.

Keywords: HSV-1, HSV-2, ELISA, IgG antibody, Gilan

1394P

Prevalence of HTLV-I Infection in Patients with Thalassemia Major in Mazandaran, North of Iran

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Background: Human T-lymphotropic virus typeI (HTLV-1) is one of the health threatening problems in endemic areas and can be transmitted by different routes such as blood transfusion. In order to correct chronic anemia in thalassemia subjects, they may need to get blood unit sperm onth.Thus,they areatrisk of infection with blood-borne viruses suchasHTLV-1. Inthe present study,we investigated the prevalence ofHTLV-1 infection among high risk patients with thalassemia in north of Iran, Mazandaran. **Methods:** A total number of 288 thalassemia patients including 151 females (52.4%) and 137 males (47.6%) with meanage of 21.45±6.6 years were tested for HTLV-1Ig Gand IgMantibodiesagainst gagproteins by enzyme linked immuniosorbent assay (ELISA). Polymerase chain reaction(PCR) test was applied to confirm HTLV-1infectionin DNA samples of antibody positive subjects. **Results:** In the primary screening by ELISA, 20 out of 288(6.9%) patients were positive for HTLV-1 antibody. The PCR results confirmed that four out of 20 samples (1.4%) were HTLV-1positive. **Conclusions:** The seroprevalence of HTLV-1infection in patients with thalassemia in Mazandaran province compared to other parts of Iran was not too high; however, HTLV-1 screening should beperformed prior to blood transfusions to decline the risk of virus transmission in these patients.

1393P

Chronic Urticaria: The Necessity of Laboratory Examination

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Background: Urticaria is a common dermatologic disease. About 20 per cent of the population experiences it in a life-time period. The aim of this study was to compare the various laboratory examinations of chronic urticaria patients and healthy individuals and to determine the necessity of laboratory tests in such patients. **Methods:** In this study 78 patients suffering from chronic urticaria and 67 healthy individuals (2-50 year-old) with analogous demographic features underwent ALT, AST, S/E, ESR, CBC, TSH, T4, C4, C3, CH50, ANA, anti-thyroglobulin, anti-peroxidase, and anti H. pylori antibodies testing. **Results:** Forty-one per cent of patients had increased IgE in comparison to 14.92% in normal subjects. Anti-thyroid antibodies were positive in 17.94% of cases while only 9% of normal individuals were positive ($p<0.05$). Anti H. pylori antibodies were positive in 69.23 % of patients (all above 18-year-old) and 61.19 per cent of normal population ($p>0.05$). No significant difference found in other variables. **Conclusion:** Urticaria is often diagnosed based on clinical grounds and no routine laboratory examination is required.

Keywords: Urticaria ,Laboratory tests, Thyroid Function

1861P

Higher serum levels of IL-27 in patients with acute myocardial infarction

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background: Cytokines play an important role in the pathogenesis of cardiovascular diseases. The aim of this study was to evaluate the serum levels of IL-27 in patients with acute myocardial infarction (AMI) to clarify any association. **Methods:** A total of 60 patients with AMI and 60 sex- and age- matched healthy subjects as a control group were enrolled to this cross-sectional study. Serum samples were collected from all participants (for AMI patients at 3-5 days after events) and tested for the levels of IL-27 by use of ELISA method. **Results:** The mean serum levels of IL-27 in AMI group (38.00 ± 14.38 Pg/ml) was significantly higher than that observed in control group (24.91 ± 14.96 Pg/ml; $P<0.0001$). The mean serum levels of IL-27 in AMI patients with or without a certain traditional risk factor including hypertension, dyslipidemia, diabetes smoking was significantly higher as compared to control group. **Conclusions:** These results showed that the higher serum levels of IL-27 were associated with AMI. The presence or absence of a certain traditional risk factors of IHD did not influence the serum levels of cytokine.

Keywords: Acute myocardial infarction, Interleukin-27

1874P

Potential of C-reactive protein (CRP) in early detection of neonatal septicemia

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Background: Early diagnosis and treatment of the neonates with suspected sepsis are essential to prevent severe and life threatening complications. It is a difficult procedure because symptoms and signs are usually non-specific. **Methods:** This prospective study was conducted on neonates admitted to neonatal intensive care unit (NICU) at Hazrate- Masumeh Hospital, Kermanshah from November' 2012 and December' 2013. 45 neonates were included with the age group of first mount of life in study, all of which were suspected to have sepsis in clinical settings. Automated complete blood count (CBC) performed for all patients. The peripheral blood smear (PBS) of neonate were reviewed by microscopic examination.CRP performed by semi quantitative latex agglutination method. Positive cultures were the "gold standard", against which the performance of CRP, abnormal white blood cell counts (WBC) were compared. **Results:** Among 45 septic screens, 26 (57.8%) neonates with sepsis had raised CRP levels of more than 6 mg/lit. The sensitivity and specificity of CRP was 57.8%% and 73.4% respectively. **Conclusion:** In this study CRP test is a valuable adjunct in screening for neonatal sepsis, complementing clinical decision-making. These findings support the usefulness of the CRP to establish an early diagnosis of neonatal sepsis.

Keywords: Neonatal Sepsis, CRP, Infection

1700P

Evaluation of ASO titer in the serum of pregnant women and compared with newborns cord

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Background: SLO is an extracellular toxin that is produced by Streptococcus Pyogenes. ASO, in some cases, can create Cross reaction with self-antigens that similar to SLO, causing reactions with heart tissue antigens in rheumatic fever or deposition of immune complexes and activation of the complement system in the kidney may cause glomerulonephritis. Therefore transfer the high titration of ASO from mother placenta to fetus can be interpreted in a number of transient myocardial dysfunction in neonates. **Methods:** To evaluate the ASO titer in baby and comparison with maternal blood, 3 cm^3 of the cord blood was collected from 50 newborns that their mother's blood was collected too .There are several ways to measure ASO, one of these methods is nutralization, which is dilutions were prepared (1/10,1/100,1/500).Other methods are Turbidometric immunoassay that the wavelength was evaluated in 340nm. Additionally such methods as Colorimetric Liposom Lysis, Nephlometry , Elisa , Latex Agglutination were used. **Results:** The mean titration of ASO in the control group was 177.54 and the mean titration among pregnant women was 164.962.Standard deviation of these 2 means was 148.784 and 142.566 respectively ,the difference between 2 means were not significant, and the results indicate that normal titers less than 200 units of frequency is higher among pregnant women. **Conclusion**: Pregnancy does not increase or decrease the ASO titer in body. Possible transfer rate of ASO from mother to fetus is related to the ASO titer in the mother's blood.

Keywords: SLO:Streptolysin O, ASO:Anti- SLO

2130P

Evaluation and Phylogenetic study of Human T-Cell Leukemia Virus Type 1 in referred to health centers Torbat hydarie in the northeast of Iran

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Background: Human T-cell lymphotropic virus type I (HTLV-I) is an oncogenic human retrovirus that causes adult T cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in only 2-3% of infected people. It was previously shown that HTLV-I infection is endemic in Khorasan Razavi province, particularly in Mashhad and Neyshabour. The region is presently the largest endemic area for this virus in Iran due to several factors such as environment, immigration patterns and individual risk behaviours. The purpose of this study was to determine the prevalence and phylogenetic analysis of HTLV-I in Torbat-e-Heydarieh located, Northeastern of Iran.**Methods**: Between April and June 2011, serum samples obtained from 400 randomly selected individuals screened for the presence of anti-HTLV-I antibodies by ELISA method (Dia.Pro/Italy). Genomic DNA was then extracted from peripheral blood mononuclear cells (PBMC) using PrimePrem™ genomic DNA isolation kit,(GeNet Bio, South Korea). PCR for HTLV-I Tax and LTR region was performed using specific primers. Three out of 5positive HTLV-I samples were selected for sequencing and phylogenetic analysis of LTR. The phylogenetic tree was built using PHYLM v2.4.5 integrated inside Geneious software. **Results** : In the primary screening of the samples by ELISA, eight (2%) samples were positive for HTLV antibodies, from which only five (1.25%) cases (three males and two females) were confirmed to be HTLV-I by PCR. A significant correlation existed between prevalence HTLV-I infection and increase age among positive cases. The results showed that HTLV-I in Torbat-e-hydarieh belonged to the cosmopolitan subtype. The present study showed Torbat-e-hydarieh may be a new endemic area for HTLV-I infection. **Conclusion:** Our results demonstrated that there HTLV-I infection in Torbat-e-Heydarieh. Thus, routine screening among blood donors along with other strategies are needed for prevention of the virus transmission in region. Also emphasizes that systemic HTLV-I screening of blood donors in other cities in Khorasan province is important and should be taken into account.

Keywords: HTLV-I, Seroprevalence, phylogenetic, PCR, Iran

1469P

Prevalence of serologic markers hepatitis B viruses in special patients in mazandaran

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Background: Hepatitis is a common disease placed in liver of human and proliferates. Doing sensitive blood tests define viruse proliferation in a body. This viruse is a serious danger to transfer in special patients (Thalasemia, Dialysis, hemophilia)And totally in patients who receive blood repeatedly. The aim of this study is definition of different (HBV)hepatitis B viruses serologic markers in special patients of Mazandaran. **Methods:** From the total of 94

serum samples in special patients of Mazandaran in the first 6 month of 1392, samples were collected and identified by the way of ELISA and were evaluated by using SPSS Statistical software.**Results:** Evaluating HBV serologic markers in these special patients about the number of Hbs Ab title was (57.4%), but Hbe Ab (12.6%), Hbc IgM (13.8%), Hbc total were (10.5%) respectively.According to accomplished test the errant of Hbs Ab in comparison of another test had a meaningful difference ($p<0.05$).**Conclution:** The finding of this study showed that considering all these patients receive vaccine, in negative people it was caused by no repeatation the vaccine reminder and because these people are in dangerous group, should be concerned.

Keywords: HBV, Thalasemia, Hemophilia, Dialysis, ELISA.

1470P

In vitro antifungal activity of Clotrimazole, Miconazole and Ketoconazol by binary mixture pattern against hospital isolates of Candida

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Background: The lack of variation in antifungal drugs ,and the misuse or inappropriate use usually causes resistant strains of the yeast in human‘s normal flora .Unfortunately ,a large number of Candida infection cases in immunosuppressed patients with insufficient treatment eventually can cause patient‹s death .**Methods:** The aim of this study was to evaluate the in vitro conventional antifungal azole compounds with binary mixture with appropriate ratios by susceptibility test using the laboratory as a mixture of two in vitro conditions. 10 isolates of Candida were admitted from patients with signs of cutaneous and mucosal infections. The binary mixure of common antifungal drugs Clotrimazole, Ketoconazole and Miconazole on equally proportion were used. The drugs were solved in various concentrations on the SDA medium and then Cndida isolates were cultured in the SDA plates. The minimum inhibitory concentration (MIC 90) and minimum fungicidal concentration (MFC) was determined. **Results:** The combination with Clotrimazole and ketoconazole in equal proportion had more effective than other drug mixtures against all isolates with the exception of isolate 3. In contrary ,the combination of Miconazole and Clotrimazole had the least effect ,and the MIC was calculated in the range of 3.12 to 50 µg/ml .Evaluation of MFC showed almost the same results .Lowest values of the MFC belonged to the combination of clotrimazole and ketoconazole which was obtained 6.25 µg/ml. **Conclusion:** It is concluded that the use of the combination of Clotrimazole and Ketoconazole in equal ratios has better antifungal effects against of the Candida infection.

Keywords:MFC, antifungal drugs, Candida infection

1836P

Serologic evaluation of toxoplasmosis in mazandaran province Tooba Lab during 2013

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Background:Toxoplasmosis is a parasitic disease that caused by Toxoplasma gondii. Cats are the primary source of infection to human hosts. Fecal contamination of hands is a significant risk factor. In various places throughout the world, it has been shown that up to 95% of populations have been infected with Toxoplasma. Infection is often higher than areas of the world that have humid climates and lower altitudes. **Methods:** Serum IgG and IgM antibodies against Toxoplasma gondii among 441 patients were measured by enzyme linked immunosorbent assay (ELISA) specific kit in a cross-sectional study**. Results:** The overall prevalence was 57.82%. IgG and IgM antibodies had higher levels than standard range with 57.59% and 0.45 % quantity respectively. **Conclusion**: In this study, we have shown that prevalence of toxoplasma in mazandran province noticeable. It is concluded that molecular studies necessary for toxoplasmosis.

Keywords: Toxoplasmosis, antibody, ELISA

1797P

Expression of the HBZ gene of HTLV-1 in HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) before and after Triple Therapy

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Background: Human T-cell leukemia virus type 1 (HTLV-1) is complex retroviruses, that causative agent of adult T cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopathy/ tropical spastic paraparesis (HAM/TSP). HTLV-1–infected T cells and activated cytotoxic T lymphocytes (CTL) that play a central role in virus activity and immunosyste alterations toward malignancy or autoimmunity in adaptive immune responses.. HBZ is expressed in HAM/TSP cells, suppresses Tax-mediated and plays a central role in disease pathogenesis. **Methods:** we measured the HTLV-1 (HBZ) mRNA load and mRNA/DNA ratio before,during, and after an alpha interferon(pegasys/Roche/Germany) based tripletherapy in HAM/TSP patients. pripheral blood mononuclear cells (PBMCs)were isolated using Ficol Hypaque(cederlan/ Canada) and the RNA was extracted from PBMCs using TriPureTM Isolation Reagent(Roche Diagnostics, Lewes, UK). complementary DNA was synthesized using TaqMan Gold RTPCR Kit. A real- time PCR Taqman method was desingned and optimized for evaluation of Tax gene expression. **Results:** HBZ mRNA expression per HTLV-1-infected cell was decreased after successful immunomodulatory treatment for HAM/TSP. These studies also demonstrated a relative stability of the HTLV-1 proviral load throughout the disease. Our findings provide new insights into the complex immune conditions underlying HTLV-I-associated diseases.

Keywords: HTLV-I, HAM/TSP, HBZ, Real-time PCR

2090P

Detection of Neisseria Gonorrhoeae and Chlamydia Trachomatis in patients with symptomatic urethritis using multiplex PCR, gram stain and urine culture

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Background: Chlamydia trachomatis and Neisseria gonorrhoeae are the most common causes of sexually transmitted infections. The aim of this study was detection of N. gonorrhoeae and C. trachomatis in patients with symptomatic urethritis using Multiplex PCR, gram stain and urine culture.**Methods:** This cross sectional study was conducted in 137 patients with symptomatic urethritis, referring to Yasuj Sahid Mofateh Clinic. After completing a demographic questionnaire, 10-15 ml of first void urine was obtained. After centrifugation of the urine, sediments were used for polymerase chain reaction based on plasmid primers and then cultured on chocolate agar medium. The data was analyzed using descriptive statistics and Chi-square test.**Results:** The patients included 28 (20%) male and 109 (80%) female. The frequency of infectionwith C. trachomatis and N. gonorrhoeae was 3.65% and 5.11%, respectively. N. gonorrhoeae was detected in two (7.14%) male and five (4.65%) female and C. trachomatis was observed in two (7.14%) male and three (2.6%) female. No coinfection with C. trachomatis and N. gonorrhoeae was detected. Using culture, gram stain and Multiplex PCR for detection of N. gonorrhoeae we found 5.11, 4.38 and 5.84% positive cases, respectively. Through Multiplex PCR assay for detection of C. trachomatis 3.6% of the cases were found positive.**Conclusion:** The results of this study showed a relatively low frequency of C. trachomatis and N. gonorrhoeae inYasuj.

Keywords: C. trachomatis, N. gonorrhoeae, urethritis, polymerase chain reaction

2015P

Cytokine Status in Ukrainian Children with Irritable Bowel Syndrome Residing in a Radioactive Contaminated Area

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Background: The effect of low dose radiation on immune system is shown. Ionizing radiation can affect cytokine production and polarization of T helper cells.**Method**s: Our study included 75 rural children population aged 4-18 yrs, who lived in a contaminated area exposed to natural environmental radiation with clinical irritable bowel syndrome (categorized in three groups) and 20 rural children participants aged 5-15 yrs who were living in areas with similar levels of radioactive contamination without clinical irritable bowel syndrome as control group. Internal radiation activity was measured by gamma-ray spectrometry. Serum levels of IL-4 and IFN-γ were measured by enzyme linked immunosorbent assay.**Results**: A trend towards increased levels of IL-4 was observed in children with clinical irritable bowel syndrome. In these children, IFN-γ levels were lower than that of the control group.**Conclusion**: The IBS symptoms in Ukrainian children residing in a contaminated area may have stemmed from Th1 to Th2 immune deviation and differential expression of IL-4 and IFN-γ.

Keywords: Chernobyl Children, Ionizing Radiation, Irritable Bowel Syndrome,Th2 Response

2955P

Study on IL-17A and IL-21 serum changes in schizophrenic patients versus healthy control subjects

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Background: Immunological alterations in schizophrenic patients have been received much consideration during last decade. Several studies have been revealed that serum levels of interleukin-6, TNF-α, Iinterleukin-18 increased in patients with schizophrenia. There are no remarkable reports on the changes of IL-17A and IL-21in schizophrenic patients. Therefore, the purpose of this study was to evaluate changes of serum IL-17A and IL-21 in schizophrenic patients in comparison with healthy controls. **Methods:** In the present study serum levels of IL-17A and IL-21 in 30 patients with schizophrenia before treatment and 3 months after treatment were measured by enzyme-linked immunosorbent assay (ELISA) and compare to 30 match healthy control group. **Results:** Serum levels of IL- 21 in schizophrenic patients was significantly higher than control group (P=0.001). IL-21serum levels in schizophrenic patient three months after treatment was not significant changed in Comparison with this group before treatment (p=0.06). Serum levels of IL-17A in the schizophrenic patients had no significant changes than the control group (P=0.4). Serum levels of IL-17A in patients with schizophrenia three months after treatment compare to patients group before treatment had no significant changes (P=0.7). **Conclusion:** Results of this study showed that IL-21 might be involved in the pathologic mechanism of schizophrenia.

Keywords: Schizophrenia, Interleukin-17, Interleukin-21, Immunity

K,kik-P

Survey on IL- 17 and IL-21 serum changes in polymyositis and dermatomyositis patients versus healthy subjects

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Background: Polymyositis (PM) and dermatomyositis (DM) are systemic chronic autoimmune diseases that lead to muscle atrophy and weakness. The immune system mediator molecules like cytokines are altered in these patients. There are no reports on serum levels of IL-17 and IL-21 in patients with PM and DM. Therefore, the purpose of this study was to evaluate IL-17 and IL-21 serum levels in patients with PM and DM before treatment and three months after treatment in comparison with healthy control group. **Methods**: Serum levels of IL-21 and IL-17 in 15 patients with PM and DM (5 DM and 10 PM) before treatment and three months after treatment was measured by enzyme-linked immunosorbent assay (ELISA) and compare to 15 healthy control group. **Results**: Serum levels of IL- 21 in PM and DM patients was significantly higher than control group (P=0.001).There was no significant changes on Serum levels of IL-21in PM and DM patients before treatment and three month after treatment (p=0.3). Serum levels of IL-17 in the PM and DM patients had no significant changes compare to control group (P=0.2). Serum levels of IL-17 in patients with PM and DM three months after treatment compare to this group before treatment had no significant changes (P=0.2). **Conclusion**: Results of this study support the hypothesis that IL-21 but not IL-17 is highly

active in PM and DM and may play a considerable role in the pathologic mechanism of the disease.

Keywords: polymyositis, dermatomyositis, interlukine-21, interlukine-17, autoimmunity

2739P

Comparison the degree of sensitivity and specificity of diagnostic methods, ELISA and immunofluressence for diagnosis of toxoplasmosis in pregnant women in Mashhad

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Background: The most important diagnostic methods Toxoplasmosis, using serological IFA (Indirect fluorescent assay) and ELISA (Enzyme Linked Immunoadsorbent Assay). The aim of this study is to compare the sensitivity and specificity of two serologic techniques IFA and ELISA in diagnosing Toxoplasmosis in pregnant women in Mashhad Azad University Hospital. **Methods:** In this observational study 167 pregnant women who were referred to Mashhad Azdad University hospital were randomly include and blood samples were obtained and examined for Toxoplasma antibodies with ELISA and IFA kits and the results were analyzed with paired t test and SPSS16 to compare the two tests (the first degree error is 0/05). **Results:** The mean age of patients was 26.7_+5.63 years and mean gestational age was38.26_+5.81 weeks. ELISA test for 100 patients (59.9%) was negative and for 67patient (40.1%) was positive. The IFA test was negative for 110 patients (65.9%) and positive for 57 patient (34.1%), the sensitivity ELISA was 96.4 respectively. Specificity of test was 89% for ELISA .positive predictive value was also determined as 82% for ELISA. **Conclusion:** Although the results of two techniques in this study were similar, but ELISA is preferred due to low expenses, lower time and because it is easy to do. So we suggest using ELISA for diagnosing toxoplasmosis in pregnant women.

Keywords: Toxoplasmosis, Sensitivity, Specificity, ELISA, IFA, Pregnancy

3027P

Determining correlation between prostate-specific antigen concentrations andserum C-reactive protein in men with and without prostate cancer

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Background:-The correlation between C-reactive protein (CRP), and cancer is not fully understood, especially since so many conditions can raise CRP without increasing cancer risk. The prostate-specific antigen (PSA) test measures the blood level of PSA, a protein that is produced by the prostate gland. The PSA test has been widely used to screen men for prostate cancer. The higher a man's PSA level, the more likely it is that he has prostate cancer. It appears that individuals with cancer do have an elevated CRP prior to developing and during cancerous illness, such as prostate cancer in men. We studied the correlation between prostate-specific antigen concentrations and serum CRP in men with and without prostate cancer.

Methods: In this study, the levels of prostate-specific antigen were examined in the PSA-based screening population including 267 men in Zanjan city. Among these men, 23 had serum PSA concentrations greater than 4.0 ng/ml some of whom were patients having symptoms of the illness. The PSA measurement was carried out by using chemiluminescence method in medical diagnostic laboratory of Zanjan. These men were selected and their serum CRP levels were measured. The results were analyzed with using correlation coefficient test, the non-parametric statistical, and Spearman test in SPSS 18 software. **Results:** The evaluation was performed and our data indicated that one of nonspecific markers of systemic inflammation, CRP, was not associated with change in PSA levels in the high concentration, in PSA men group, ($p > 0.005$). **Conclusion:** Inflammatory response and cancer are strongly correlated, and CRP may be more of an incidental player. However, we did not obtain a direct correlation between PSA marker concentration for prostate cancer and serum CRP levels.
Keywords: Correlation, Chemiluminescence, Prostate-specific antigen, Serum CRP levels

3121P

Neutralizing effects of immunotherapy on hepatic toxicity induced by scorpion envenomation

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Background: Antivenom immunotherapy is the specific treatment for scorpion envenoming because of the severity of scorpion envenoming and the rapid diffusion of its toxins. Unfortunately, the administration of antivenom remains empirical and their efficacy is controversial. In the present investigation, protective effects of polyvalent antivenom against histopathological perturbations in liver and marker (ALT and AST) changes induced by *Mesobuthus eupeus* scorpion venom were studied in experimental rabbits. **Methods:** Twenty four rabbits were randomized into four groups: six rabbits in control group were received 1 ml ultra-pure water subcutaneously (group 1). In group 2, sub lethal dose of *Me* venom (4 mg/kg) injected subcutaneously to animals. Simultaneously venom (subcutaneously 1ml of an ultra-pure water solution containing 4 mg/kg of *Me* venom) and antivenom (intravenously 5 ml) were administered in six rabbits considered as group 3 animals. In group 4 rabbits five ml of antivenom, 60 min after *Me* venom injection was administered intravenously. **Results:** Results showed in group two animals, venom injection caused histopathological abnormalities in liver such as central vein congestion, congested vessels in portal areas, dilated sinusoids and steatohepatitis. ALT level was significantly increased at 3 h following *Me* venom injection but there were no differences between control and envenomed animals regarding ALT serum level at 0 h and 1 h after venom injections. In addition, there were no alterations in AST levels in envenomed animals at 0 h, 1 h and 3 h following *Me* venom injections. Simultaneous administration of antivenom and venom intensively prevented histopathological damage and marker changes. In group four, Immunotherapy decreased histopathological damages and reversed ALT elevation back to normal. **Conclusion:** Antivenom immunotherapy can prevent and neutralize hepatotoxic impacts of *Mesobuthus eupeus* scorpion envenomation if used at

optimum time and route.

Keywords: polyvalent antivenom, *Mesobuthus eupeus*, histopathological complications, AST, ALT

2275P

Study of Arbutin and Pyrus Boissieriana Buhse leaf extract effects on urea and creatinine levels induced by Cyclosporine A in rat

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Background: Cyclosporine (CsA) is a potent immunosuppressant medicine that is generally used to prevent graft rejection after transplantation. Overproduction of reactive oxygen species is one of the side effects of it's that damage to the liver and kidney, CsA administration cause to **increase** in serum urea and creatinine too. Investigations have shown that Pyrus Boissieriana Buhse (grows in Mazandaran) leaves extract (Telka EXT) and Arbutin (ART) have antioxidant properties. In this study, we aimed to evaluate the protective property of ART and EXT in rats that received CsA. **Methods:** After categorizing sixty four male Wistar rats (250-300 g) in eight groups (n=8), administration and gavages of CsA (25 and 50 mg/kg), Arbutin (50 mg/kg), EXT (500 mg/kg) and DW (control) were done and serum urea and creatinine levels analyzed. **Results:** Serum urea in coadministration of ART and CsA at a dose of 50 mg / kg was lower than in coadministration of the EXT and CsA50 (p = 0.01), urea in administration of ART50 was lower than the coadministration ART50 and CsA50 (p = 0.003) and **there was no significant difference** between group that received only Arbutin and control group (p = 0.282). Serum Creatinine was higher in the administration of Arbutin 50 than the others (p < 0.05), but creatinine in the groups that received only EXT was significantly lower than the others (p < 0.05). **Conclusion**: Because of reduction in serum urea and creatinine by EXT, It may be an adjunctive agent for patients that receive CsA.

Keyword: Cyclosporine A, Pyrus Boissieriana Buhse, Arbutin, urea, creatinine

2261P

The CCL5/CCR5 axis itself but not CCR5 –Δ32 gene variant is involved in nephropathic complications of T2D

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Background: Association of nephropathy complication with T2D is a complex status depend upon several genetic and environmental factors. Present study was aimed to examine and

compare the serum levels of the CCL5 and relation it with CCR5 –Δ32 mutation in nephropathic and non-nephropathic type 2 diabetic patients. **Methods:** In this study, blood samples were obtained from 100 T2D patients, 100 NT2D diabetic patients and 100 non diabetic controls. ELISA detected serum levels of CCL5. The Gap-PCR method was applied to analyze the δ32 mutation in the CCR5 gene, and demographic data (eg, age, sex, occupation, socio-economic status) were collected using a questionnaire. **Results:** Our results showed that the serum levels of CCL5 were significantly increased in NT2D patients, while, it was not differ in nephropathic complication in compare to healthy controls. Our findings indicated the absence of CCR5-Δ32 in type 2 diabetic patients with and without DN, while only two controls showed a heterozygotic pattern of mutation. **Conclusion:** According to these findings, it can be concluded that the serum levels of CCL5 are associated with T2D without nephropathy. It also that the CCR5-Δ32 mutation is not associated with T2D and its appears related nephropathic complications.

Keywords: Chemokine, CCL5, T2D, Nephropathy

2116P

A comparative evaluation of rehydration and cup-loading sample application for modified two-dimensional gel electrophoresis of human serum proteins using immobilized pH gradient

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Background: Proteomics is a powerful technique for the large-scale study of proteome that extracted from biological sources. Proteome analysis is possible with two-dimensional electrophoresis (2D-E) for protein separation and mass spectrometry (MS) for the protein identification. The serum protein analysis is a useful diagnostic agent that can use as indicator of the physiological or clinical status of a patient. One of the central and critical steps is sample application, therefore this method should be considered and optimized for 2-DE with immobilized pH gradient. The aim of this study is focused on comparative application of in-gel (rehydration loading) and in-cup (cup loading) sample application for 2-DE of human serum analysis. Also in this work, in order to obtain the best gels with high resolution, a 2-DE procedure was optimized. **Methods:** In this study, we applied the IPG strip that rehydrated for overnight at 37°C for isoelectric focusing with immobilized pH gradients. After the isoelectric focusing, the gel was incubated twice with equilibration buffers. Equilibrated IPG strip was transferred to the SDS-PAGE for second dimension electrophoresis. Protein spots were visualized by silver staining. **Results and conclusion:** In conclusion, we compared the in-gel and in-cup sample application for 2-DE and this method was optimized for plasma proteins analysis. In addition, we introduced modified 2-DE with immobilize pH gradient for serum proteome analysis. Resolution of pattern profiles of this study is better than those reported by other researchers. These data can be considered as improved set of conditions and basis for future investigation.

Keywords: Proteomics, 2D Electrophoresis, Proteome, Sample application

2684P

Clinical impacts of HLA-G Expression in Cancers

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Background: Because detection and treatment at an early stage can significantly improve patient survival, there has been great interest in developing diagnostic and prognostic biomarkers. For pathological diagnosis and prognosis, the TNM system is commonly used. However, as the number of clinical documents has shown, the prognosis could be significantly different between patients with the same disease stage. Therefore, finding biomarkers that can indicate biological characteristics and predict clinical outcome for patients with carcinoma is greatly in demand. Remarkably, the detection of HLA-G was reported to be correlated with certain clinicopathological parameters in some malignancy. These studies all indicate that HLA-G might serve as a clinical marker for the diagnosis or prediction of clinical outcomes for those diseases. HLA-G expression In non-pathological situations, is largely restricted to extra-villous cytotrophoblastic cells, placental chorionic endothelium, activated monocytes, thymic epithelial cells, nail matrix , cornea and erythropoietic lineage cells from the bone marrow , and is not found in other healthy tissues expressing MHC class Ia antigens. However, HLA-G has been found to express aberrantly in a number of common carcinoma types such as melanoma, renal cell carcinoma, carcinoma of the lung, breast carcinoma, lymphomas, ovarian carcinoma, endometrial adenocarcinoma, and various gastrointestinal cancers including pancreatic ductal adenocarcinoma, ampullary cancer, biliary cancer, colorectal cancer, and gastric carcinoma. HLA-G expression in cancer cells have been hypothesized to play a role in the evasion of immunosurveillance by host T-lymphocytes and NK cells. Remarkably, the detection of HLA-G was reported to be correlated with certain clinicopathological parameters in some malignancy. Many studies indicate that HLA-G might serve as a clinical marker for the diagnosis or prediction of clinical outcomes for those diseases.

Keywords: HLA-G Expression, malignancy, prognosis

2825P

Comparison of Serological testsiELISA with Wright for Detection of Brucellosis

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Background: Brucellosis is a zoonotic chronic and infection diseases globally that its control is dependent on the prevalence of diseases in animal populations. Brucellosis is endemic in Iran. The purpose of this study was tocompare the sensitivity and specificity of serological tests ELISA with Wright for detection of brucellosis. **Methods:** This cross-sectional study was done in Tehran, Iran in 2013.Theblood specimens were collected from 92 cattle (vaccinated and unvaccinated). Blood samples of 10 ml were obtained using a sterile vacationer tube from the jugular veins of the cows and were divided into two tubes, the first containing the anticoagulant EDTA, the other without anticoagulant for serum separation. Subsequently, Blood in plain tube was centrifuged at 6000 rpm for 5 min to obtained serum samples. The

serum samples were aliquoted and stored at −20°C until tested. The brucellosis diagnosis was established by indirect ELISA and wright methods. Analysis of data was performed by using SPSS software. **Results:** In this investigation frequency of brucellosis by using an indirect ELISA and Wright methods was 82.6 % (76) and 50 % (46) respectively. The sensitivity ELISA and wright tests was 95.83%, 83.89% ELISA and wright tests respectively. Specificity was 65% and 100% .The positive predictive value of ELISA tests and wright was 90.79% and 100%, respectively and the negative predictive value of ELISA and wright was 81.25% and 43.48%. **Conclusion:** The findings of this study showed regarding the high sensitivity of i ELISA and high specificity of wright test. So, brucellosis diagnosis by using of these two tests is recommended.

Keywords: Cattle, Brucellosis, Indirect ELISA, Wright method

3394P

Assessment of latent tuberculosis and allergy in patients with chronic lower respiratory tract symptoms

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Background: Chronic lower respiratory tract symptoms, especially persistent coughing could be a clinical sign of latent tuberculosis. Respiratory hypersensitivities may have some similar manifestations and they could also deteriorate these symptoms. In this study, we assessed the correlation of latent tuberculosis and respiratory hypersensitivities in elderly patients complaining from chronic respiratory symptoms. **Methods:** In this study, 333 elderly participants were assessed for latent tuberculosis by a commercial IFN-γ release assay (IGRA) using two recombinant specific antigens (ESAT-6 and CFP-10). Moreover, serum total and specific IgE levels were measured by sandwich and disc ELISA methods, respectively. **Results:** The IGRA test revealed that about 39.9% of the patients suffered from latent tuberculosis. There was not any significant difference between the total IgE levels of IGRA positive or IGRA negative patients (p=0.56). We also did not find any significant correlation between IgE reactivity to specific respiratory allergens including mites, tobacco dust or pollen extracts from weeds, grasses or trees with IGRA results. **Conclusion:** We concluded that there is no correlation between latent tuberculosis and hypersensitivity to respiratory allergens.

Keywords: IGRA, Latent tuberculosis, Respiratory allergy

Immunology & Immunotherapy in Reproductive Medicine

Oral Presentations:

2476O

The therapeutic efficiency of *invitro* generated regulatory T cells for prevention of abortion in abortion-prone mice

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Background: Pregnancy constitutes a major challenge to the maternal immune system. $CD4^+CD25^+$ regulatory Tcells (Tregs) play a major role in tolerating conceptus antigens and therefore contribute to the maintenance of pregnancy. Transforming growth factor beta (TGF-β)plays a critical role in the induction of Foxp3 expression and reproductive. Here, we investigated the therapeutic efficiency of invitro generated Tregs in a mouse model of abortion. **Methods**: Mouse spleen mononuclear cells were isolated and $CD4^+$ T cells were purified on the MACS Cell Separator. $CD4^+$ cells were stimulated with plate-bound anti-mouse CD3 and anti-CD28 in the presence of TGF-β1, retinoic acid and IL-2. The cells were incubated for 4 days. Induction of Treg cells was surveyed by flowcytometry, then CD4+CD25+Tregs were isolated for further experiments. CBA/J female mated with DBA/2J male mice (abortion model) and with BALB/c male (control mice). Pregnant CBA/J mice from abortion group were injected intravenously with the freshly isolated or in vitro generated Tregs (2×10^5cell per mouse) on day 1, 3 or 4 of pregnancy. **Result:** This study showed that TGF-b can induce Tregs from $CD4^+$ T cells and increased the expression foxp3 up to 70% after 4 days of incubation. CD4+CD25+ Tcells were isolated and the purity of these cells was around 98%. Adoptive transfer of invitro generated Tregs in day 1, 3 and 4 of gestation resulted in 78%, 89% and 78% pregnancy rate, respectively. Fetal rejection completely prevented by adoptive transfer of Tregs from normal pregnant mice and the fetal resorption rates were 0%. **Conclusion:** We propose that invitro generated Tregs could be seen as therapeutic method in prevention of abortion.

Keywords: Regulatory T cells, TGF-$b_{1,}$ Abortion-prone mice, Abortion

2424O

Analysis of vitamin D3 metabolism in women with recurrent spontaneous abortion and fertile controls

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Background: Regulation of immune system is essential for pregnancy maintenance. Vitamin D3 levels appear to be connected to the pregnancy success through its immunomodulatory functions. We hypothesized that impaired vitamin D3 metabolism could be associated with miscarriage in women with recurrent spontaneous abortion (RSA). **Methods:** Blood and endometrial samples were obtained from unexplained RSA patients and fertile women. Serum levels of 25-hydroxyvitamin D, parathyroid hormone and calcium were measured. Expression of transcripts for molecules essential in vitamin D3 action including vitamin D receptor (VDR), 1α-hydroxylase and 24-hydroxylase was investigated by real time RT-PCR. Protein levels of aforesaid molecules were quantified in endometrial samples by immunohistochemistry and western blot using antibodies generated against specific peptides. **Results:** There was no significant difference in VDR, 1α-hydroxylase and 24-hydroxylase gene and protein expressions in endometrial tissues of RSA patients compared to normal subjects. While both groups had comparable levels of serum 25-hydroxyvitamin D, RSA patients had significantly higher levels of PTH and lower levels of calcium compared to fertile controls. **Conclusion:** Comparable levels of endometrial VDR and enzymes involved in vitamin D metabolism in both groups reflect insignificant connection of inherent aberrations in vitamin D metabolism and RSA. Instead, extracellular calcium levels may be more relevant determinant in the course of recurrent spontaneous abortion.

Keywords: Recurrent spontaneous abortion, Vitamin D, Vitamin D receptor (VDR), 1α-hydroxylase, Calcium.

2857O

Comparative analysis of NK cell subsets in menstrual and peripheral blood of patients with unexplained recurrent spontaneous abortion and fertile subjects

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Background: Natural killer (NK) cells play fundamental function in maintaining pregnancy. Based on the availability and non-invasive method of collection of menstrual blood (MB), here we investigated for the first time comparative analysis of NK cell subsets in MB and peripheral blood (PB) of recurrent spontaneous abortion (RSA) and fertile women. **Methods:** PB and MB of healthy fertile (n=15) and RSA women (n=15) were sampled simultaneously in the second day of menstrual cycle. Frequency of CD56+CD3-CD16+/-, CD56+CD3-CCR7+/-, and CD56+CD3-CD45RO+/- cells was analyzed by flowcytometry. **Results:** Menstrual blood CD16+ and CD45RO- NK cells were significantly lower compared to PB in both normal subjects (p=0.011 and p=0.038) and RSA patients, (p= 0.002 and p=0.023). In parallel, CD56+CD16+CCR7- and CCR7+ cells were present at significantly lower frequencies in MB than PB in fertile women (p=0.005 and p=0.008) and women with RSA (p=0.003 and p=0.013). However, the frequencies of CD56+CD16-CCR7- and CCR7+ cells were higher in MB. In comparison to fertile group, percentage of MB CD45RO+ NK cells was significantly lower and frequencies of PB CD16-, CD45RO- and CD56+CD16+CCR7+ subsets were significantly higher in RSA patients (p=0.039, p=0.016, p=0.023, p=0.010 respectively). **Conclusion:** Different subsets of NK cells are differentially distributed in MB in comparison with PB in RSA and fertile subjects. Population differences of NK cell subsets in RSA patients and normal controls were more reflected at the systemic level.

Keywords: Fertile women, Menstrual blood, NK cell subsets, Peripheral blood, Recurrent spontaneous abortion

2056O

The effect of Vitamin D3 on Th17/Treg ratio in recurrent miscarriage (RM) patients candidate for paternal lymphocyte therapy: a double-blind placebo-controlled study

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Background: Recurrent Miscarriage (RM) defined as 3 or more consecutive abortion in 2-5% of women desiring child. Previous studies indicated the importance of tolerance retention in successful pregnancy. One of the most important mechanisms for tolerance is the balance of regulatory T cells (Treg) and Th17 cell which is disturbed in RM patients. New therapeutic strategies have introduced for RM patients due to contradictory outcomes of paternal lymphocyte therapy as a common treatment. In this study, the effects of combinational therapy with paternal lymphocyte and vitamin D3 as an immunomodulatory agent on the balance of Th17/Treg were further evaluated in RM women. **Methods:** The expressions level of CD4 and FOXP3 for Treg and CD4 and IL-17 for Th17 were analyzed using Flowcytometry before and 3 months after immunotherapy in RM women treated with lymphocytes immune therapy and vitamin D3 (treatment group) compared with RM women receiving lymphocyte therapy alone (control group). The Th17/Treg ration in RM women was also compared before and after intervention in each group. **Results:** Vitamin D3 therapy increased Treg cells frequency while

reduced Th17 fraction and Th17/Treg ratio in peripheral blood of RM patients in treatment group compared with control group (P<0.05).**Conclusion**: In addition to paternal lymphocyte therapy, Vitamin D3 might be considered as an effective therapeutic approach for women with abortion due to reduction of Th17/Treg ratio in RM women in treatment group compared with control group.

Keywords: Recurrent miscarriage, Regulatory T cell, T helper 17, Th17/Treg ratio, Paternal lymphocyte therapy, Vitamin D3

1899O

Evaluation of Th1 and Th17 cells cytokines in women with recurrent spontaneous abortion

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Background: Various immunological abnormalities have been reported in women with recurrent spontaneous abortion of unknown etiologies including autoimmune abnormalities and increased cellular immunity such as elevated natural killer cells, Th (T helper)1 and Th17 cell levels. Th17 and Th1 cells play a central role during inflammation. Th1 cells product cytokines IFNγ, IL-2 and Th17 cells mainly cytokines IL-17A, F, IL-22. The aim of this study was to evaluate of Th1 and Th17 levels in women with recurrent spontaneous abortion. **Methods**: In this case-control study, 30 women with history of two or more recurrent abortion who at least 3 months past after last abortion considered as case and 30 normal fertile healthy women with at least one delivery as control group. We determined the levels of IL-17A, F and IFNγ in cell culture supernatant of peripheral blood mononuclear cells stimulated with the Phytohemagglutinin by ELISA method and compared in the two groups. **Results**: The level of IFNγ in case group was significantly higher than control group (186.53±30.41 Vs. 88.06±21.44 pg/ml, P= 0.004). Also the level of IL-17 A, F in case group was significantly higher than control group (84.74±21.26 Vs. 28.41±8 pg/ml, P = 0.01). IFNγ concentration showed positive correlation with IL-17 A, F in case group (P=0.015, r= 0.455).**Conclusion**: In this study the increased levels of cytokines IFNγ and IL-17 A, F in women with recurrent spontaneous abortion shows a propensity of pro inflammation via Th17 and Th1 immunity and may be these cells play a pivotal role in rejecting fetus antigens.

Keywords: Recurrent Spontaneous Abortion, Cytokine, Th1, Th17

1648O

Evaluation of the expression of HSP70 and LOX-1 molecules in placental tissues of pre-eclampsia and normal pregnancies

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Background: Pre-eclampsia, a hypertensive disorder of human pregnancy is a main cause of fetal and maternal morbidity and mortality.Growing evidences suggest that placentaloxidative stress is involved in the pathogenesis of pre-eclampsia and HSP70 is a novel marker of oxidative stress which can bind with high avidity to Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1). Therefore, the aim was to evaluate co-expression of HSP70 and LOX-1 in the placental tissues of normotensive and pre-eclamptic pregnancies.**Methods:** The expression of HSP70 and LOX-1 on the placental tissues of normal pregnancies (n=35) and pre-eclampsia patients (n=33) were examined by using immunohistochemistry technique. The intensity of the molecules expression graded by using a semiquantitative scale as as+(few positive), 2+(mild), 3+ (moderate) and 4+ (many positive).**Results:** The rates of 1+ and 2+ HSP70 expression were significantly higher in healthy women in comparison to pre-eclamptic subjects ($P<0.0002$ and $P<0.001$, respectively). However, the rates of 3+ and 4+ HSP70 expression were significantly higher in patients compared to healthy women ($P<0.0001$ and $P<0.03$, respectively). Similarly, the percentages of 1+ and 2+ LOX-1 expression were significantly higher in healthy women compared to pre-eclamptic group ($P<0.0001$ and $P<0.0002$, respectively). But the percentages of 3+ and 4+ LOX-1 expression were significantly higher in pre-eclamptic women in comparison to normal women ($P<0.0001$ and $P<0.008$, respectively). The frequencies of 1+ and 2+ HSP70 and LOX-1 co-expression were also significantly higher in healthy women than patients ($P<0.006$ and $P<0.0003$, respectively), whereas, the percentages of 3+ and 4+ HSP70 and LOX-1 co-expression were significantly higher in pre-eclamptic group than that in healthy women ($P<0.0009$ and $P<0.001$, respectively).**Conclusion:** These results showed higher expression of the HSP70 and LOX-1 molecules in the placental tissues of preeclampsia patients which may contribute the disease pathogenesis. Further studies need to clarify their role in the pathogenesis of this hypertensive disorder.
Keywords: Preeclampsia, HSP-70, LOX-1, Syncytiotrophoblasts.

2177O
Profiling of vitamin D receptor and 1α-hydroxylase expression in male mouse reproductive tract

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Background: In recent years, the potential immunomodulatoryroles of vitamin D3 (VD3) in the female and male reproduction have been the focus of many researches. Vitamin D has been introduced as one of the main regulators of spermatogenesis. **Methods**: Here, for the first time, we evaluated the expression of vitamin D receptor (VDR) and 1α-hydroxylase, the

enzyme responsible for the synthesis of biologically active form of vitamin D3, in all organs of male mice reproductive tract by immunohistochemistry and Western blotting. **Results**: Epithelial cells of epididymis, seminal vesicle, coagulating gland, ductus deferens, preputial gland, and prostate were the prominent cell types that concomitantly expressed VDR and 1α-hydroxylase. Nearly all cell types in testis expressed both proteins. Interestingly, VDR intensity in epididymis epithelial cells was reduced toward cauda, in which only strong staining of stereocilia was observed. Although in the caput epididymis spermatocytes considerably expressed VDR, they lost VDR expression in cauda region. In all organs, sperms failed to express 1α-hydroxylase. Specific bands of the VDR and 1α-hydroxylase were determined in all tissues, except testis in which novel unprecedented isoforms of 1a-hydroxylase were observed. **Conclusion**: Our findings provide further compelling evidence for pivotal role of vitamin D in male reproductive biology.

Keywords: Mice, Reproductive tract, Vitamin D receptor, 1α-hydroxylase, Testis

1654O

Association between polymorphisms of Foxp3 gene and unexplained recurrent spontaneous abortion in an Iranian population

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Background: Unexplained recurrent spontaneous abortion (URSA) has been suggested to be associated with the failure of fetal-maternal immunologic tolerance in which the regulatory T lymphocytes (Treg) play a curtail role. Tregs have been suggested to be involved in the pathogenesis of some autoimmune diseases. These cells express the fork head/winged helix transcription factor, FOXP3, which appears to be of key importance in the development and function of Tregs .Recent Studies have reported that single-nucleotide polymorphisms (SNPs) in the FOXP3 gene could contribute to susceptibility to some autoimmune disorders. However, reports on polymorphism of FOXP3 gene in URSA are limited. This study evaluated the association between FOXP3 gene SNPs and URSA in an Iranian population. **Methods**: In a case–control study, 196 patients with histories of at least three consecutive miscarriages with unexplained etiology before 20th week of gestation and 104 healthy women with at least two normal pregnancies were included as case and control groups, respectively. We genotyped four SNPs in the FOXP3 gene (-924A/G, -3279C/A, -20G/A, 459A/G), using polymerase chain reaction- -restriction fragment length polymorphism (PCR-RFLP) analysis. **Results**: Our results revealed that -924A/G ($p<0.001$), -20G/A ($p<0.01$), 459A/G ($p<0.01$) polymorphisms were significantly associated with URSA. Additionally, no associations were found between -3279C/A polymorphism and URSA. **Conclusion:** These results suggest that polymorphisms of the FOXP3 gene may confer susceptibility to URSA, probably by altering Foxp3 function and/or its expression.

Keywords: Recurrent spontaneous abortion, Regulatory T lymphocytes, FOXP3, Polymorphism

1678O

The effect of HLA-DRB1 sharing between the couples with recurrent pregnancy loss on the pregnancy outcome after leukocyte therapy

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Background: Miscarriage is a common phenomenon complicating more than half of pregnancies. Recurrent Pregnancy Loss (RPL) is defined as three or more pregnancies lost before the twentieth week of gestation. In spite of the fact that the etiology is unknown in the majority of the cases, it is thought that the immune reaction disorders may contribute to the mechanism of RPL. It is widely believed that abnormality in maternal immune reaction to fetus is the main reason for RPL. In this respect, it was suggested that sharing of HLA antigens might be associated with RPL. One of the methods for treatment of RPL is paternal leukocyte therapy. There is still controversy about the effectiveness of this method and heterogeneity in the inclusion criteria in case selection is one of the major sources for divergent results. The present study aimed to investigate the effect of HLA-DRB1 sharing between the couples with recurrent pregnancy loss on the pregnancy outcome after leukocyte therapy. **Methods:** Sixty primary RPL women who were immunized and followed after therapy (30 successful and 30 unsuccessful) and their husbands formed the cases of this study. In addition, one hundred healthy women were considered as the controls. HLA-DRB1 genotypes of all the cases and controls were checked by PCR-SSP method. **Results:** HLA typing indicated that the prevalence of HLA-DRB1 sharing (defined as at least one allele sharing) between the couples with unsuccessful outcomes was significantly higher compared to those with successful outcomes (63.3% vs. 23.3%, $P<0.004$). Moreover HLA DRB1*07:01 allelic group was significantly more frequent in the patients with unsuccessful outcome compared to the controls (18.3% vs. 8%, $P<0.04$).**Conclusion:** The results of this study confirmed the role of HLA sharing in RPL and revealed HLA-DRB1 typing as a valuable prognostic factor for the leukocyte therapy outcome.

Keywords: HLA-DRB1, Leukocyte therapy, RPL

2557O

Inhibitory impacts of menstrual blood-derived stromal stem cells on phenotypic and functional features of monocyte-derived dendritic cells

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Background: There is growing interest in applying stem cell therapy to the modulation of immune-associated adverse reactions following allogenic transplantation. Menstrual blood

stromal stem cells (MenSCs) possess unique features rendering them advantageous over other types of stem cells for such purposes. Nevertheless, the potential impacts of MensSCs on different arms of the immune system remain unclear. Accordingly, we sought to explore whether Men SCs affected the generation and function of dendritic cells (DCs) from monocytes. **Methods**: MenSCs were obtained from menstrual blood of normal women and characterized. Blood monocytes from unrelated donors were differentiated towards immature DCs (iDCs) and mature DCs (mDCs) in the presence or absence of MenSCs. Men SC impact on phenotypic and functional features of monocyte-derived iDCs and mDCs was evaluated. **Results**: DCs generated in the presence of MenSCs were shown to acquire regulatory properties as judged by the expression level of surface markers (CD14, CD1a, CD40, CD80, CD86), the amount of certain immunostimulatory and immuneinhibitory cytokines (IL-6, IL-10, IL-12, and TNF-α), and the ability to induce $CD4^+CD25^+FOXP3^+$ regulatory T cells. To our results, MenSCs exerted their inhibitory effect on the first step of DC generation (*i.e.* monocyte to iDC differentiation), rather than the second step (*i.e.* iDC to mDC development).**Conclusion:** This is the first study to report the inhibitory impact of MensSC on DC generation and function. Considering the pivotal role DCs play in the regulation of immune responses, MenSC could be regarded as a future candidate to be used in the clinic.

Keywords: Transplantation, Menstrual blood, Stromal stem cell, Dendritic cell, Treg

2797O

Decidual-secreted factors of abortion prone mice decrease dendritic cells capacity to induce regulatory T cell and to skew T cell differentiation toward Th2

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Background: The state of local immune regulation in pregnancy which is the result of complex interactions between cells of the immune system and microenvironmental factors is started to become clarified gradually. The aim of this study was to evaluate the immunomedulatory effects of the decidual microenvironment of abortion prone and non-abortion prone mice on dendritic cells (DCs) regulatory functions. **Methods:** The decidual cell supernatants (DS) were obtained from abortion prone and non-abortion prone mice on gestation day 13.5. Splenic DCs were treated with various concentrations of DS and conalbumine as candidate antigen and injected into mice palms. After 5 days, the mononuclear cells of the regional lymph nodes were isolated from immunized mice and cultured in the presence and absence of antigen. The frequency of CD4+CD25+ Foxp3+Treg cells and production of cytokines such as IL-4 and IFN-γ were measured by flow cytometry and ELISA, respectively. **Results:** Our results showed that DS from abortion prone mice compared with DS from non-abortion prone mice significantly decrease DCs capacity to induce Treg cells ($P < 0.05$) and to stimulate IL-4 production ($P < 0.001$) by primed lymphocytes. We also showed that DS from non-abortion prone mice more efficiently inhibits the DCs capacity to stimulate IFN-γ production ($P < 0.01$) by primed lymphocytes. **Conclusion:** Taken together, these findings suggest that in a poor pregnancy outcome associated with maternal immune responses to fetal alloantigens, soluble factors within decidua display altered immunomodulatory effect on DCs capacity to induce

Treg cells and to skew T cell differentiation toward Th2.

Keywords: Abortion, Decidua, Dendritic cell, Regulatory T cell

2742O

The effects of vitamin D_3 on pre-cancerous state of endometrioma

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Background: Endometriosis is a benign chronic inflammatory disease characterized by the growth of endometrial tissue outside the uterine cavity. The process of endometriosis development and progression including adhesion and invasion of endometrial cells to the ectopic regions followed by proliferation, induction of angiogenesis and resistance to apoptosis mimics the processes usually observed in the course of malignant diseases. Regarding its anti-tumor and anti-inflammatory action, the effect of vitamin D_3 on pre-cancerous state of endometrioma cells were investigated in this study. **Methods:** Stromal cells were isolated from eutopic (EuESCs) and ectopic (EESCs) endometrial tissues from 10 endometriotic patients and from endometrium of 10 non-endometriotic patients (CESCs) as control. ESCs were characterized by immunocytochemisty and flowcytometry using a panel of 14 antibodies and cultured in the presence or absence of the active form of vitamin D_3. Cultured cells were analyzed for adhesion to extracellular matrix, invasion to matrigel, proliferation, apoptosis and angiogenesis. Additionally, the levels of IL-6 ، IL-8، IL-17 ،TGF-β ، TNF-α and IFN-γ in culture supernatants was determined. **Results:** In all groups, vitamin D_3 caused a significant increase of attachment ($p<0.05$), while decreased invasion to matrigel ($p<0.05$) and proliferation ($p<0.01$) of EuESCs and EESCs. Such treatment also resulted in a significant decrease in IL-6 production by EESCs ($p<0.05$), but had no significant effect on the IL-8 production. Stromal cells from all groups, showed no detectable secretion of other cytokines. This vitamin also caused a significant decrease in Bcl-2 gene expression by EuESCs ($p<0.05$) and Bcl-xL by EESCs ($p<0.05$), but had no significant effects on Bcl-2 gene expression and caspase-3 gene and protein expression. In addition, vitamin D_3 treatment reduced VEGF-A gene expression by EESCs ($p<0.01$). **Conclusion**: Based on our results, it seems that this hormone exerts beneficial effect over disease course and can be effectively used for inhibition of disease progression.

Keywords: Endometrioma, Endometrial stromal cells, Gene expression, Pre-cancerous state, Vitamin D_3

Poster Presentations:

2982P

Frequency of Interleukin-1β, Interleukin-6 and Interleukin-10 Gene Polymorphisms in Iranian Infertile Women

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Background: The women infertility could be caused by several factors such as cytokine dysfunctions. It seems there is no data regarding the polymorphisms of cytokine genes such as IL-1β, IL-6 and IL-10 as risk factors for infertility. **Methods:** The study group comprised 185 infertile women who referred to Avicenna Infertility Clinic (Tehran-Iran). 103 healthy fertile women were also considered as a control group. Genomic DNA from blood was extracted using salting out method. Genotype and allele frequency of IL-6 (-174 C/G), IL-1β (-511C/T, -31 T/C and +3954 C/T) and IL-10 (-592 A/C and -819 C/T) polymorphisms were compared between infertile patients and healthy controls using Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Statistical analysis was performed using SPSS 13.0 software. **Results:** The data showed significant differences in IL10- promoter gene polymorphism (-592 A/C) frequencies between case and controls groups (P- value < 0.05). However the distribution of other polymorphisms and allele frequencies of the in IL-1β, IL-6 and IL-10 (-819 C/T) genes in the infertile groups did not differ from control group. **Conclusions**: Our findings imply that polymorphisms of IL-10 (-592 A/C) can affect the susceptibility to infertility, whereas polymorphisms of IL-1β and IL-6 are not important. Further study using a large sample size will be required to confirm our findings.

Keywords: Cytokine, Infertility, PCR-RFLP, Polymorphism

2460P

HLA-G gene expression pattern in placental tissue in recurrent miscarriage

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Background: Recurrent miscarriage (RM) is a disorder that deprives many families of the blessings of having children. Despite several studies conducted in the field, mechanisms of the RM are not discovered entirely. However, it has been proved that the main cause of recurrent miscarriages of unknown origins, disregarding the genetic reasons such as genetic abnormalities and thrombophilic disorders, is immunological factors like altered expression of maternal and fetal HLA- G and resulted incompatibility. The aim of this study was to find a significant correlation between the expression level of this regulatory gene and recurrent

miscarriages. **Methods:** RNA extraction was carried out from the collected samples. After cDNA synthesis, Real-Time PCR was performed using specific primers and Beta- globulin gene as reference. **Results:** A significant decrease was observed in the HLA-G expression in decidua tissues of women with recurrent miscarriage compared to control tissues. **Conclusion:** The findings of the present study are consistent with the previous studies about the reduced rate of HLA-G gene expression in placentas, which are aborted by unknown causes. The reduced rate of HLA-G expression in the patients' could be considered as a marker and also a target for treatment of miscarriage.
Keywords: Recurrent miscarriage, HLA-G expression, Real time PCR, Placenta,

2264P
Relation of HLA-G*0105N null allele frequency with recurrent miscarriage patients in East Azarbayjan

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Background: Human leukocyte antigen–G (HLA-G) is a non-classical class I molecule that extra villous cytotrophoblast cells highly expresses it. The HLA-G gene contains 15 alleles including HLA-G*0105N null allele. HLA-G*0105N presents a single base pair deletion, which rules out translation of both membrane bound (HLA-G1) and full length soluble isoform (HLA-G5),but other isoforms such as HLA-G2 can compensate their roles. The aim of this study was to demonstrate the frequency of HLA-G*0105N null allele in East Azarbayjan patients with recurrent miscarriage. **Methods**: For investigating the frequency of HLA-G *0105N null allele in recurrent miscarriage patients, the method of PCR-RFLP was used. Genomic DNA was extracted from the whole blood of 60randomly selected patients using salting-out technique. Then, PCR amplification of the exon 3 of HLA-G gene was done. The work was continued with the digestion of products of PCR with PpuM-I enzyme. Afterward, the analysis resulted fragments was done by using gel electrophoresis technique. In order to understanding the accuracy of done RFLP method, six samples were selected and were sent for sequencing. **Results:** In this research, digestion of the restriction enzyme showed heterozygous HLA-G*0105N null allele in 10% of the patients population. **Conclusion:** The ultimate analysis of data demonstrated that the frequency of heterozygous HLA-G*0105 N null allele was partly high in patients with recurrent miscarriage.
Keywords: Miscarriage, HLA-G

2769P

Evaluatoin of relationship between ABO blood groups and Anti sperm antiibody (ASA) in men infertility

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Background: The ABO blood group is recognised as major and clinically significant blood group. Blood groups are not only important in relation to blood transfusion and organ transplantation, but also have been utilized in genetic, fertility and infertility researches. Anti sperm antibody (ASA) has different effects on human fertility. Objective of the present study is to evaluate a possible relationship between ABO blood groups and men infertility and also we want to evaluate ASA existence in men infertile. **Methods:** This is a retrospective, cross sectional study. Our study was carried out in fertility and infertility center of Yazd city. Blood group of 100infertile males and 100 fertile males, ASA and sperm analyses were evaluated. Data were analyzed with SPSS 16 software using T-Test and chi-square test. **Results:** Our results indicated that there is a significant relationship between male infertility and O blood group (P value :0.04).Where as there is no significant relationship between O blood group and fertility in control group(P value:0.07).There is significant difference between case and control group due to ASA concentration (P value:0.03) while there is no significant relationship between type of blood group and ASA existence in men infertile(P value:0.12).In addition to our results showed that there is no significant relationship between ASA and sperm quality(sperm count and mobility) (P value:0.1,0.07)**. Conclusion**: The present study revealed that frequency of blood group O is more than other blood groups in infertile men and also concentration of ASA is higher in infertile males.

Keywords: ABO blood group, ASA, Men, Infertility

2767P

Association between functional A/G rs1264457In HLA-E gene variant (rs11209026) and Recurrent Spontaneous Abortion(RSA)

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Background: Recurrent spontaneous abortion (RSA) is defined as three or more consecutive abortions before the 20th week of gestation. There is increasing evidence to support an immunological mechanism for the occurrence of RSA. The purpose of our study was to investigate the association between a functional single nucleotide polymorphism (SNP):A/G rs1264457In HLA-E gene in patients with RSA. **Methods**: This is a case-control study. We recruited 200 patients with RSA (case group) using established diagnostic criteria and 200, normal individuals (control group) at the fertility and infertility center in Yazd city and Isfahan city during the period from 2012 to 2013. By PCR-RFLP method we screened the A/G rs1264457 variant in HLA-E gene in patients and controls, and we performed an association analysis between A/G rs1264457 variant In HLA-E gene and RSA. The data was analyzed by

spss 16 software using Chi-square test. **Results:** Significant differences in the genotype and allele frequencies of the SNP:A/G rs1264457 variant In HLA-E gene were identified between patients with RSA and healthy controls. **Conclusion**: Our results showed that we can use of results in RSA prognosis.

Keywords: Recurrent spontaneous abortion, A/G rs1264457 variant , HLA-E gene

1429P

Evaluation of inhibitory effect of royal jelly on stanozolol-induced apoptosis in mouse sperm using fluorescent labeled annexin V

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Background: Apoptosis as a programmed, physiological mode of cell death plays a crucial role in many normal biological processes as well as in pathological states. Phosphatidylserine externalization following lipid remodeling of the sperm plasma membrane is a primary feature of apoptosis. The aim of this study was to analyze the possible anti-apoptotic effect of royal jelly (RJ) in stanozolol (ST)-induced apoptosis of mouse spermatozoa. **Methods:** Adult male NMRI mice were assigned into four treatment groups. Two groups of mice received ST (4.6 mg/kg/day) via gavage for 35 days. RJ was given orally to one of these groups at the dose level of 100 mg/kg body weight per day synchronously. Corresponding control groups were also included. Apoptotic sperms were detected by fluorescently-labeled annexin V binding assay. **Results:** The incidence of apoptosis in spermatozoa was significantly higher in ST-exposed mice than those of control, whilst RJ co-administration substantially reduced sperm apoptosis in comparison with ST-only treated group. **Conclusion:** Data from the current study suggest that RJ prevents the development of ST-induced spermatotoxicity by a mechanism related, at least in part, to its ability to decrease apoptosis in sperm cells with the consequent improvement infertility outcomes.

Keywords: Royal jelly, Stanozolol, Sperm, Apoptosis, Annexin V

2343P

Increased secretion of TGF-β in adipose derived mesenchymal stem cells in preeclampsia

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Background: Mesenchymal stem cells (MSCs) in addition to be involve in tissue repair, are

involved in immunomodulation by secretion of several cytokines such as TGF-β. In order to better understand the potential role of mesenchymal stem cells in changes of TGF-β levels in preeclampsia status, in this study was compared TGF-β secreted by adipose derived mesenchymal stem cells of healthy pregnant and with preeclampsia. **Methods:** Subcutaneous adipose of 10 preeclamptic and 10 healthy pregnant women, during cesarean section, was sampled. After isolation and expansion of mesenchymal stem cells, their differentiation and their immunophenotyping characteristics were assessed. Then, the release of TGF-β was evaluated by Using ELISA sandwich method. **Results:** Stem cells isolated from adipose in both groups were differentiated into osteocyte and adipocytes. Flowcytometric analysis showed that the expression of CD90, CD73 CD44 and CD105 markers and lack expression of CD-14, CD34, CD45 markers and HLA-DR in both groups. Significant increase in the levels of TGF-β secretion in preeclamptic women compared to control group was observed. **Conclusion:** This study suggests that TGF-β secreted by MSCs may have an effective role in the control of immune responses or complications of disease. More studies are necessary to clarified the role of MSCs in preeclampsia.

Keywords: TGF-β, Mesenchymal stem cells, Adipose tissue, Preeclampsia

2342P

Comparison of nitric oxide secretion by human adipose derived mesenchymal stem cells from healthy pregnant and with preeclampsia

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Backgrounds: Preeclampsia, a pregnancy specific syndrome characterized by hypertension and proteinuria that occurs after 20th weeks of pregnancy in women with normal blood pressure. The pathophysiology of this disease is unknown. Due to changes in serum levels of nitric oxide in preeclampsia women and also the role of mesenchymal stem cells in the secretion of nitric oxide as an immunoregulator, in this study we aimed to be evaluated the levels of nitric oxide secretion by adipose derived mesenchymal stem cells in normal pregnancies and patients with pre-eclampsia. Methods: Subcutaneous adipose of 10 preeclamptic and 10 healthy pregnant women, during cesarean section, was sampled. After isolation and expansion of mesenchymal stem cells, their differentiation and their immunophenotyping characteristics were assessed. Then via Using Griess method was evaluated the release of nitric oxide by them. **Results:** Stem cells isolated from adipose in both groups were differentiated in to osteocyte and adipocytes. Flowcytometric analysis showed that the expression ofCD90, CD73 CD44andCD105markers and lack expression ofCD-14, CD34, CD45markersandHLA-DRin both groups. Significant change in the levels of nitric oxide secretion in both groups was not observed. **Conclusion:** This study suggest that nitric oxide secreted by mesenchymal stem cells don´t significantly contribute in variation of sera level of this factor and maybe don´t play any role in pathology of preeclampsia.It is necessary to test this study in more cases.

Keywords: Nitric oxide, Mesenchymal stem cells, Adipose tissue, Preeclampsia

2773P

The effect of ovarian induction on uterus natural killer (uNK) cell population on day 7 of mouse pregnancy.

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Background: The cells of immune system especially NK cells are present in an important role in pregnancy maintenance. Any change in their frequency and distribution can affect the pregnancy outcome. Sex hormones such as progesterone and estrogen are the main mediators that orchestrate the recruitment of NK cells to the uterus. Since ovarian induction is widely used in IVF clinics, it seems that the high concentration of sex hormones following ovarian hyperstimulation can affect the uterine NK cells frequency and distribution as well as the pregnancy rate after embryo transfer. This investigation was done to clarify the effect of hyperstimulation on uNK cells population in early pregnancy. **Methods:** Female NMRI mice were mated following ovarian hyperstimulation. On day 7 of pregnancy the concentration of estrogen and progesterone were measured in the serum of pregnant mice by ELISA and the frequency and localization of uterine NK cells were studied through immunohistochemistry and morphometric techniques, using DBA lectin (Dolichos biflorus agglutinin) as uNK cells marker. The pregnant mice without hyperstimulation were used as controls. **Results:** The blood concentration of estrogen and progesterone in hyperstimulated mice increases significantly compared with the control group. The frequency of uNK cells was significantly decreased in hyperstimulated group compared with the control ones. Moreover in the control group uNK cells were located mostly in mesometrial site of deciduas while they were distributed in all parts of deciduas in hyperstimulated group. **Conclusion:** Considering the increase in serum progesterone and estrogen after ovarian induction and the presence of receptors for these hormones on uNK cells, the changes in frequency and distrib the uterus and play ution of uNK cells could be explained. Since uNK cells are key players to development and maintenance of normal pregnancy, their changes can affect the rate of IVF success.

Keywords: Ovarian induction, uNK cell, Deciduas, IVF

3114P

Human sperm lacks TNF-R1 and cannot express it in varicocele condition

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Background: Infertility is considered as one of the main public health issues, because it affects about 15% of the couples of reproductive age. The male factor is involved in 40-50% of infertility cases. Varicocele is an abnormal dilation of the pampiniform venous plexus in

the scrotum that develops during puberty; it can affect testicular growth and semen parameters (Especially count and motility), and is considered to be a major cause of male infertility. It is suggested that the spermatogenic dysfunction in varicocele testis may be related partly to an abnormal control of sperm death and apoptosis. The presence and possible function of TNF-R1 in the semen have not yet been clarified. Therefore, the purpose of the present study was to determine the extent to which of TNF-R1 is present in the semen, by measuring its expression on the sperm cells of patients with varicocele and without varicocele. **Methods:** In a case/control study, semen samples were obtained following 3–5 days of ejaculatory abstinence, from 45 adolescents (Mean age 28.3 ± 7.8 years, age matched) with varicocele of grades II and III (study group), and 45 adolescents without varicocele (control group). Semen analysis was done according to World Health Organization. The TNF-R1 expression on Sperm cells was performed using Flow cytometry. The demographic characteristics were collected through a questionnaire by interview. The obtained data were analyzed by using t-test and through SPSS version 17. **Results:** Presence of TNF-R1 on the surface of sperm ejaculation in patients with varicocele and control groups was not detected. **Conclusion:** The present findings do not support the importance of Immunologic factors in apoptosis of sperm cells in patients with varicocele. Based on our results, the effects of TNF-induced apoptosis are not responsible for alteration in sperm parameters (count and motility) compared to control group. Decrease in sperm count and sperm motility in varicocele seems not to be attributed to TNF-related mechanisms and probably may occur through other pathways and needs farther studies to be elucidated.

Keywords: Varicocele, Apoptosis, TNF-R1, Flow cytometry

2697P

Prevalence and clinical significance of antinuclear antibodies in Iranian women with unexplained recurrent miscarriage

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Background: Antinuclear antibodies (ANAs) in women with recurrent miscarriage (RM) have been reported. The presence of moderate to high titers of these antibodies represents an autoimmune condition that can endanger the health of the fetus in pregnant women. In this study, we evaluated the prevalence of ANAs in Iranian women with a history of two or more unexplained abortion. **Methods:** 560 women with unexplained recurrent miscarriage and 560 healthy controls accounted for this study over a period of 13 months. ANAs were detected by indirect immunofluorescence (IIF) technique. **Results:** ANAs were detected in 74 of 560 (13.21%) patient with RM, and in only 5 of 560 (0.9 %) controls ($p < 0.001$). ANA positivity was generally found with low-positive results (1/40 – 1/80) in about 38% of positive cases, whereas moderate titers (1/160 – 1/320) and high titers (≥ 1/640) were seen in about 46% and 16% of cases respectively. Finally evaluating of microscopic ANA patterns revealed that about half of positive cases had antibodies against DNA- histone complex, associated with SLE disease. **Conclusion:** Antinuclear antibodies are not uncommon in women with unexplained recurrent miscarriage, suggesting the possible role of an autoimmune disorder on abortion, at least in a subgroup of patients.

Keywords: Recurrent miscarriage, Antinuclear antibodies, Indirect immunofluorescence

2481P

HLA-G 14-bp polymorphism in couples with recurrent spontaneous abortions

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Background: Human leukocyte antigen (HLA)-G plays an important role for maintaining an immunotolerant of the semiallogrnic fetus. 14-bp insertion/ deletion polymorphism in exon 8 of HLA-G is in related with the stability of mRNA of this gene, therefore influence the function of HLA-g in pregnancy. This study was conducted to investigate, the association between 14-bp polymorphism of HLA-G gene and recurrent spontaneous abortion (RSA) in women with RSA. **Methods:** Blood was taken from 50 women with RSA who had experienced 3 or more spontaneous abortions and 50 unrelated fertile control women who had normal pregnancies. Genomic DNA was extracted from peripheral blood samples. Amplification of 8th exon of this gene was performed by PCR method and analysis of genotype for this polymorphism was performed by using 10% poly acrylamide gel electrophoresis. The results were analyzed using x^2 test. **Results:** It was observed that more patients with recurrent spontaneous abortions had homozygous genotype (60%),14bp insertion was obtained in 24% of the samples and 36% of patients showed 14bp deletion. Most participants in the control group (66%) had heterozygous genotype +14 /-14 bp. **Conclusion:** To the best of our knowledge this is the first study in Iran which investigated this polymorphism between RSAs and further studies must be performed by using a larger sample size and in different racial and ethnic groups in order to investigate the relationship of this polymorphism as a predisposing factor for recurrent spontaneous abortion.

Keywords: HLA-G gene, 14bp polymorphism, Recurrent spontaneous abortion, Iran

3311P

Evaluation of uterine macrophages on days 4.5 and 7 of NMRI mouse pregnancy

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Background: Along with other uterine leukocytes, macrophages play a crucial role in appropriate implantation of fetus and successful pregnancy. These cells are under the control of sex hormones and the uterine microenvironment. So any change of them can affect the frequency and localization of uterine macrophages. **Methods:** To investigate this issue, blood was collected from NMRI pregnant mice on days 4.5 and 7 of pregnancy. Serum 17- β estradiol and progesterone concentrations were measured using the ELISA method. The frequency and localization of macrophages in decidua were also investigated by immunohistochemistry and morphometric studies. **Results:** The results of this study showed that macrophages are distributed throughout the whole uterus on day 4.5 of pregnancy while their distribution was restricted to myometrium on day 7 of pregnancy. The frequency of uterine macrophages on day 7 was also less than day 4.5 of gestation. The concentrations of serum 17-β estradiol and progesterone increased significantly on day 7 of pregnancy compared to day 4.5. **Conclusion:** Considering the changes in progesterone and estradiol concentrations and their indirect

effects on macrophages recruitment through the factors such as granulocyte monocyte colony stimulating factor (GM-CSF), the differences in frequency and distribution of macrophages in different stages of pregnancy could be explained. Regarding the role of macrophages in embryo implantation and regulation of feto-maternal immune responses, it seems that the changes in their frequency and localization can be helpful for a successful pregnancy.

Keywords: Macrophage, Estradiol, Progesterone, Uterus

2480P

TNF-alpha as a promising therapeutic target in polycystic ovary

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Background: Pro-inflammatory cytokines like tumor necrosis factor (TNF)-alpha seem to play a central role in the pathogenesis of polycystic ovarian syndrome (PCOS). Several studies have shown that TNF-α levels are increased in the women with PCOS. It is also likely that elevation of ovarian TNF-alpha levels may play a role in the pathogenesis of subfertility associated with PCOS. In this regard, use of drugs which inhibit TNF-α activity, showed in animal studies to reduce the severity of the hyperandrogenism complications and the ovarian cystogenesis. **Results and conclusion:** Our recent studies support the role of TNF-*α* and oxidative/nitrosative stresses in the pathogenesis of PCO indicating that development of cysts involves changes in ovarian function and an imbalance in the oxidant–antioxidant equilibrium. Also TNF-α blockers like IMOD and Pentoxifylline are able, directly or indirectly, cope the histopathological, endocrine and biochemical alterations produced when rats are hyperandrogenized with letrozole. These results may introduce TNF-alpha as a promising therapeutic target in patients with polycystic ovary.

Keywords: Tumor necrosis factor -alpha, Polycystic ovary

2204P

The value of Cytomegalovirus IgG avidity tests in early pregnant women

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Background: Cytomegalovirus can occur viral infection during pregnancy that can impose significant risks to the mother and fetus. These infections can be caused by primary and secondary transmission of infection that can cause clinical symptoms likes microcephaly, anemia and hepatitis at birth. Sensitive markers for early detection of infection are measurement of increasing CMV IgG levels and CMV IgM, but using these methods have some problems. So the method that should be used is IgG Avidity. Avidity is the definition of binding power

of IgG to the antigen. In the early phases of infection, the levels of IgG Avidity are low but during the months that immune response takes place, the rate increases. Methods: Totally 147 pregnant women who were in the 2 to 4 initial months of their pregnancy period were collected from October 2008 to October 2012. The levels of anti-CMV IgG and IgM were measured and Modify avidity test was performed by Dr. Bonyadi. **Results:** The results characterized in 3 groups. Group one contains 38.09% that IgG were positive and IgM were negative. Group two contains 18.37% that IgG were negative and IgM were positive and Group three contains 43.54% that both IgG and IgM were positive. Among this group 0.68% showed low avidity rate witch show the presence of active CMV infection. **Conclusion:** It can be concluded that the avidity test of CMV is important because early detection and timely and appropriate treatment of infection during pregnancy can be prevented from causing fetal abnormalities.

Key word: Cytomegalovirus, IgG avidity, Pregnant women

3014P

Evaluation of epididymal necrospermia following experimental unilateral iatrogenic vas deferens injury in mouse

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Background: Inguinal surgical operations such as inguinal herniorrhaphyentail the risk of involuntarily ipsilateral vas deferens damages. Evidence exists that the incidence of unilateral vasal injury in infertile men with histories of herniorrhaphyis as high as27.8%.The main objective of this experimental study was to evaluate the epididymal necrospermia following unilateral iatrogenic vas deferens injury (UIVI) in mouse. **Methods:** Male adult NMRI mice (n=16) were randomized into control and test group. Following anesthesia with ketamine (5 mg/100 g body weight; IP), UIVI was induced by clamping left vas deferens with a mosquito clamp in fully locked fashion for 2 minutes in anesthetized mice. Control mice were not exposed to any type of injury or medication. Acridine orange (AO) staining was used for the morphological detection of necrotic sperm cells at 5 weeks postoperatively. **Results:** The results obtained from the AO staining showed that the rate of necrospermia in mice with UIVI is significantly increased when compared with control group ($p<0.05$). **Conclusion:** The findings suggest that a non-recognized UIVI may have necrotic effect on spermatozoa leading to infertility.It is, therefore, recommended thatthe surgeon who operates in the inguinal region, especially in children, is careful to avoid vasdeferens injury.

Keywords: Necrospermia, Vas deferens, Injury, Acridine orange

2880P

Unilateral Iatrogenic Vas Deferens Injury Induces Apoptosis inMouse Spermatozoa

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Background: Inguinal hernia repair is one of the most common operations performed worldwide by general and paediatric surgeons. Intraoperative involuntarily manipulation of vas deferens is one of the complications of this surgery, regardless of the surgical technique used. The aim of this study was to determine the effect of unilateral iatrogenic vas deferens injury (UIVI) on sperm apoptosis in an experimental mouse model. **Methods:** Experiments were performed on two equal groups each comprising eight mice. UIVI was induced by clamping left vas deferens with a mosquito clamp in fully locked fashion for 2 minutes under intraperitoneal ketamine (5 mg/100 g body weight) anesthesia. Corresponding control group was also included. The extent of apoptosis in spermatozoa was quantitated based on their annexin V-affinity, resulting from phosphatidylserine (PS) externalization at 5 weeks postoperatively. **Results:** The comparison of annexin-V binding assay results for both groups revealed that percentage of spermatozoa with PS externalization is significantly higher in mice with UIVI compared to controls. **Conclusion:** It appears that delayed detection of iatrogenic vas deferens injuries can lead to increased apoptosis in sperm cells, which in turn affects fertility.

Keywords: Vas deferens, Injury, Sperm, Apoptosis, Annexin V

2437P

Association study of CX3CR1 and CCR5A>G polymorphisms with recurrent miscarriages among Iranian women from east Azerbaijan province

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Background: Recurrent miscarriage (RM), defined as three or more consecutive losses before the 20th week of gestation, affects 0.5–2% of pregnant women. The aim of this study was to investigate the association between polymorphisms of chemokine receptors CCR5A>G and CX3CR1 Val249Ile with recurrent miscarriage in women from east-Azerbaijan province. **Methods:** Genomic DNA was extracted from peripheral blood samples from 100 patients and 100 controls. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to identify the polymorphisms. **Results:** Our finding showed that CCR5A>G and CX3CR1 Val249Ile variant alleles had not significant difference between women with recurrent miscarriage and controls. The Heterozygous of CCR5A>G (OR =2.842; % CI =1.432-5.677; p- value=0.002) was found significant association between RM cases when compared with the controls. The combination of the VV/AA (OR =0.478; % CI =0.230-0.986; p value =0.045) and VV/AG (OR =2.417; % CI =1.043-5.683; p-value =0.037) and VI/AG (OR =4.235; % CI =1.250-15.773; p-value =0.014) genotypes observed significant association with RM cases and control groups.

Keywords: CX3CR1, CCR5A>G, Polymorphism, Recurrent miscarriage

2436P

Association of CCR5-Δ32 polymorphism with recurrent miscarriages among Iranian women from east Azerbaijan province

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Background: Recurrent miscarriage (RM) is defined as three or more consecutive, spontaneous pregnancy losses before 20 weeks of gestation. The genes of immune system are one of the factors contributing in recurrent miscarriage. CCR5 as a chemokine receptor has been reported to be one of the immunemodulators which may determine pregnancy outcome. In this study, we have analyzed genetic association between CCR5-32bp deletion polymorphism (CCR5-Δ32) with recurrent miscarriage in women from east-Azerbaijan provinces. **Methods:** 100 RM cases and 100 age-sex and ethnically matched healthy individuals were genotyped for CCR5-Δ32 polymorphism by using polymerase chain reaction. **Results:** Alleles and genotypes of CCR5-Δ32 showed statistically significant associations with RM cases when compared with the controls. Allele frequency among cases and controls was 5% and 1% respectively, and the difference were statistically significant (P-value = 0.036, OR = 5.21; 95% CI=1.056–34.901). RM patients had a five times higher (10% vs. 2%) frequency of heterozygote genotype compared with the controls (P-value = 0.033, OR = 5.44; 95% CI =1.078-37.44). **Conclusions:** Our data support a possible susceptibility trend that CCR5-Δ32 may be a potential genetic risk factor for RM, but future studies are required to establish the exact role of this deletion for susceptibility to RM.

Keywords: CCR5, Polymorphism, Recurrent miscarriage

2420P

Effect of Conjugated Linoleic Acid (CLA) on the Amount of Stem Cells and Primary Spermatocytes in Male Mice

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Background: Conjugated linoleic acid (CLA) is a fatty acid found in dairy products, beef and lamb. Some cell culture and animal studies have reported that conjugated linoleic acids (CLAs) have several health related benefits. CLAs have been shown to have anticancer, antiadipogenic, anti-osteoporosis and anti-inflammatory properties. The aim of this study was determine the effects of different amounts of conjugated linoleic acid (CLA) in diet on the amount of stem cells and primary spermatocytes in male mice. **Methods:** Fifty matured male mice were divided into five groups including control, placebo and three treatment groups. Therefore mice were fed either control diet with 0 gr/kg of CLA or treatments diet with 0.1, 0.3 and 0.5 g/kg of conjugated linoleic acid (CLA) respectively for 30 days and placebo group received sunflower oil containing oleic acid. Then there were anesthetized and testes for preparation tissue sections stained with hematoxylin - eosin were dissected. Stem cell and primary spermatocytes were counted. The data were analyzed by SPSS software using

ANOVA and Duncan's test. P-value <0.05 was considered significant. **Results:** Ressults of CLA-treated mice showed that amounts of 0.1, 0.3 and 0.5 mg/kg, conjugated linoleic acid (CLA) significantly decreased the number of stem cell and primary spermatocytes compared with the control (P<0/05). **Conclusion:** According to these results, we can say that conjugated linoleic acid have negative effect on reproductive system of male rats.

Keywords: Conjugated linoleic acid (CLA), Stem cell, Primary spermatocyte, Mice

2496P

Immunochemical assessment of 8-hydroxydeoxyguanosine, as an oxidative DNA biomarker, in seminal plasma of smoking and non-smoking infertile men

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Background: A common byproduct of DNA oxidation, 8-hydroxydeoxyguanosine (8-OHdG) caused by high levels of reactive oxygen species (ROS). Cigarette is a rich source of oxidants and free radicals that can have detrimental effects on survival and function of spermatozoa. The aim of this study was to investigate effect of smoking on 8-OHdG levels in seminal plasma. **Methods**: This study was conducted on a total of 37 infertile men, who attended at the infertility clinic of the Fatemehzahra Hospital in Babol, Iran. Routine semen analysis was performed within 1h according to WHO guidelines (1999). Then subjects were classified in two groups: 18 non-smoking and 19 smoking infertile (IF.ns and IF.s, respectively) patients who had a history of primary infertility and an abnormal semen analysis. Smokers had consumed 10–20 cigarettes per a day for at least 2 years. The level of 8-OHdG in 50 µL of seminal plasma was measured using an ELISA kit by ELISA reader at 450 nm. Final results expressed as ng/mL. **Results**: Standard semen parameters (volume, count, total count, motility and morphology) and age were matched in both groups, as results were shown no any differences between groups. The mean 8-OHdG in the seminal plasma was significantly higher in the IF.s than those in the IF.ns (p= 0.03). **Conclusions**: From the results, it could be concluded that, smoking is associated with the increased levels of 8-OHdG in seminal plasma and this can lead to increase oxidative DNA damage of spermatozoa and decrease of fertility in men.

Keywords: Oxidative stress, 8-hydroxydeoxyguanosine, Smoking, Male Infertility

2645P

Effect of BMI on follicular fluid levels of vitamin D and vitamin D receptor in patients with PCOS

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Background: Polycystic ovary syndrome (PCOS) is the most common cause of oligo anovulation, infertility and hyperandrogenism in women. Many PCOS patients exhibit obesity

in varying degrees. Obesity may compromise the success of fertility treatment in PCOS. Low vitamin D levels were found to be associated with the development of obesity and insulin resistance in women with PCOS, also Function of vitamin D is associated with vitamin D receptor. The aim of this study was to investigate vitamin D and VDR levels in PCOS and assessment of correlation these with obesity. **Methods**: The descriptive study was taken on 80 women with average ages of 20-35 years who referred for in vitro fertilization. The patients were divided into four groups. On the day of oocyte retrieval, follicular fluids were collected in separate tubes. Total RNAs were extracted from GCs. Reverse transcription was performed and quantification of gene expression levels was achieved by real-time quantitative PCR. Also, we measured follicular fluid levels of vitamin D and serum levels of insulin. **Results:** vitamin D and VDR levels were significantly lower in PCOS patients than control group. There was a negative correlation between BMI with vitamin D levels ($r = -512$, $P<0.05$) and VDR levels ($r = - 397$, $P<0.05$). A positive correlation between vitamin D and VDR($r = 576$, $P<0.05$). There was a negative correlation between insulin resistance and VDR($r = -435$, $P<0.02$). **Conclusion**: In PCOS patients, BMI induces increasing insulin resistance. It also decreases vitamin D, VDR levels. However, all these factors are importantly effective on the infertility. Therefore, obesity can affect the severity of PCOS symptoms.

Keywords: PCOS, BMI, VDR, Vitamin D, Infertility

2646P

Obesity and its effect on Follicular fluid Ghrelin levels in women with and without PCOS

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Background:, obesity is present in varying degrees (30-70%) in women with PCOS and the central type is the commonest one. Central obesity is a prominent feature of the syndrome that is called metabolic syndrome. Ghrelin is an important factor involved in most of the metabolic and hormonal signals which adapt the reproductive functions in conditions of altered energy balance. Moreover Ghrelin's role in pcos and reproduction was first suggested by its wide ex-pression in many human reproductive tissues including its immuno-histochemical expression in the human ovary aim of this study the value of measuring ghrelin in follicular fluid of polycystic ovarian syndrome patients**. Method:** The study was conducted in Laboratory of Islamic Azad University, Science & Research, Tehran, Iran. 80 patients were divided into four groups according to their body mass index(BMI) and PCOS diseases. Follicular fluid was obtained from patients. Ghrelin levels were determined by Immunoassay method, also serum of levels insulin was determined and HOMA-IR was calculated. Result: Ghrelin levels were negatively correlated with BMI ($r = 0.744$), Ghrelin levels did not correlate to any of the parameters of fasting insulin and glucose concentrations ($P>0.05$). Significant differences weren't found in ghrelin levels in women with and without PCOS ($P>0.05$). Conclusions: Our findings indicate that ghrelin may not be considered risk factors for pathogenesis of PCOS. But it can be a marker for obesity.

Keywords: PCOS, Ghrelin, BMI, Reproductive.

2780P

Correlation of MTHFD1 and eNOS gene polymorphisms with recurrent abortion in Ahwaz

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Background: The aim of this study was to evaluate the frequency of G894T in eNOS and G1958A in MTHFD1 in a group of women with 2 or more abortion in pregnancy. **Methods**: The study involved 100 women with recurrent abortion (mean age = 30 years). The control group consisted of 50 healthy women with the same average age (with no history of abortion and at least one normal full-term delivery). The frequency of genotypes and alleles of the investigated polymorphisms was evaluated by polymerase chain reaction/restriction fragments length polymorphism method (PCR/RFLP). **Results**: Despite of 1958G>A polymorphism in MTHFD1 gene (p value <0.32), over representation of 894GT genotype in eNOS gene have been observed in the group of patient women vs. control group(p value <0.015). **Conclusion**: Our findings suggest lack of 1958G>A MTHFD1 gene polymorphism but much correlation of 894G>T eNOS gene polymorphism with recurrent abortion. Further studies, concerning other genetic variations conditioning inherited thrombophilia and environmental factors influencing the risk of recurrent abortions, are needed.

Keywords: Genetic, Polymorphism, Recurrent abortion, Ahwaz

3202P

Mitigating effect of cyclosporine on impairment of contralateral testicular germ cell maturation in a mouse model of unilateral vas deferens obstruction

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Background: Pathologies that damage the ipsilateral testis are known to induce contralateral testicular deterioration through cell-mediated autoimmune response. The present study seeks to determine whether cyclosporine (Cs) with potent immunosuppressive activity could serve as a protective agent against hazardous effects of unilateral vas deferens obstruction (UVO) in the contralateral testis of mice. **Methods:** Adult male mice were randomly allocated into four equal experimental groups. Two groups of mice were undergone to UVO under anesthesia with a mixture of ketamine (45 mg/kg; IP) and xylazine (35 mg/kg; IP) using a sterile technique. UVO was induced via left vas deferens ligation by a 4/0 silk suture 2 cm from the epididymis. One of these groups received Cs (10 mg/kg per day) orally for 7 days starting from the day of induction of experimental UVO. Corresponding control groups were also included. Johnsen's criteria and annexin V binding assay were used to evaluate spermatogenesis and sperm cell apoptosis in the contralateral testes at 5 weeks following operation, respectively. **Results:** UVO in mice led to spermatogenesis impairment and increased sperm apoptosis in the contralateral testis. Notably, Cs treatment minimized the adverse effects of UVO on germ cell maturation and sperm apoptosis in the contralateral testis. **Conclusion:** These results suggest that Cs treatment could exert valuable cytoprotective effects on contralateral testicular injury following UVO.

Keywords: Cyclosporine, Vas deferens, Spermatogenesis, Apoptosis

3116P

Effect of unilateral blunt testicular trauma on mononuclear immune cells infiltration in the contralateral testis in mice

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Background: In children, blunt testicular trauma is often a sports-related injury and can result in contusions, rupture, and, in rare cases, torsion of the testicle. Though, effects of unilateral blunt testicular trauma (UBTT) on the contralateral testis are still a matter of debate. This study was thus designed to examine the effect of UBTT on contralateral testicular histology in pre-pubertal mice. **Methods:** Male NMRI mice aged 20 days were randomized into two equal groups. Following anesthesia with ketamine (5 mg/100 g body weight; IP), abdomens of group I (control) were sham operated without disturbing either testis. In group II (UBTT), the abdomen was opened and the right testis was placed on a sterile firm surface and 5 g sterile weight was dropped on to the testis from a height of 10 cm. At around 70 days of age the left testis of all animals was harvested and microscopic evaluation of MNICs infiltration determined by using the unbiased counting frame. **Results:** Histological studies revealed that UBBT in mice led to marked elevation of MNICs infiltration in the contralateral testis. **Conclusion:** The results suggest that UBTT produces an immunological response in the contralateral testis, which may have the potential to adversely affect fertility in cases of unilateral testicular trauma.

Keywords: Blunt testicular trauma, Mononuclear immune cell, Testis, Mouse

3265P

Lymphoid and myeloid cell populations in the non-pregnant human Fallopian tube and in ectopic pregnancy

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Top of FormBottom of Form

Background: Lymphoid and myeloid cell populations in human endometrium are well-documented and are known to play important roles in providing immune tolerance, controlling trophoblast invasion, and mediating vascular remodeling. Immune cell populations in the Fallopian tube have not been comprehensively studied. The aim of this study was to characterize lymphoid and myeloid cell populations in non-pregnant Fallopian tube and determine whether they are altered in Fallopian tube from women with ectopic pregnancy. **Methods:** Fallopian tube was analyzed by flow cytometry and immunohistochemistry. **Results:** Populations of CD3+ (CD4+ and CD8+) lymphocytes, LIN1−HLADR+ (CD123+ and CD11c+) dendritic cells, monocytes, neutrophils, and $CD56^{dim}CD16-$ natural killer (NK) cells were demonstrated to be present in non-pregnant Fallopian tube. CD123+ dendritic cells were predominant over CD11c+ dendritic cells. Numbers of CD11c+ cells were significantly higher in the progesterone-dominant mid-luteal phase of the menstrual cycle compared with the follicular phase. Numbers of CD45+ leukocytes, CD68+ cells, and CD11c+ cells were

higher in Fallopian tube from women with ectopic pregnancy compared with mid-luteal phase Fallopian tube. **Conclusion:** These data will advance our understanding of normal human Fallopian tube physiology and disorders of Fallopian tube function, such as ectopic pregnancy.
Keywords: Ectopic pregnancy, Fallopian tube, Lymphocytes, Dendritic cells, Natural killer cells

3267P

Ovarian follicular cells have innate immune capabilities that modulate their endocrine function

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Backgrounds: Oestrogens are pivotal in ovarian follicular growth, development and function, with fundamental roles in steroidogenesis, nurturing the oocyte and ovulation. Infections with bacteria such as Escherichia coli cause infertility in mammals at least in part by perturbing ovarian follicle function, characterised by suppression of oestradiol production. Ovarian follicle granulosa cells produce oestradiol by aromatisation of androstenedione from the theca cells, under the regulation of gonadotrophins such as FSH. Many of the effects of E. coli are mediated by its surface molecule lipopolysaccharide (LPS) binding to the Toll-like receptor-4 (TLR4), CD14, MD-2 receptor complex on immune cells, but immune cells are not present inside ovarian follicles. The present study tested the hypothesis that granulosa cells express the TLR4 complex and LPS directly perturbs their secretion of oestradiol. **Methods:** Granulosa cells from recruited or dominant follicles are exposed to LPS in vivo and when they were cultured in the absence of immune cell contamination in vitro. **Results:** They produced less oestradiol when challenged with LPS, although theca cell androstenedione production was unchanged. The suppression of oestradiol production by LPS was associated with down-regulation of transcripts for aromatase in granulosa cells, and did not affect cell survival. Furthermore, these cells expressed TLR4, CD14 and MD-2 transcripts throughout the key stages of follicle growth and development. **Conclusion:** It appears that granulosa cells have an immune capability to detect bacterial infection, which perturbs follicle steroidogenesis, and this is a likely mechanism by which ovarian follicle growth and function is perturbed during bacterial infection.
Keywords: Oestrogens, Ovarian follicular, Gonadotropins

3322P

Investigation of the Relationship between Allergy and Microbiology Findings in Infertile Women going to Infertility Center of Qom Jahad Daneshgahi

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Background: Allergy is a hygiene problem in different countries and the most common kind is Anaphylaxis outside the hospital. This study investigates the relationship between allergy and

microbiology findings in infertile women going to infertility center of Qom Jahad Daneshgahi in 1392. **Methods:** This research is a cross-sectional descriptive study done on 100 infertile women with allergy symptoms suffering from secondary infertility, without abortion and tobacco consumption, going to Infertility Center of Qom Jahad Daneshgahi in the second half of 1392. Data are analyzed through SPSS. **Results:** Individual's average age has been 34.73. Most of the infertile women have been housewives and their educations were less than high school and (85.3%) of infertile ones have suffered from allergy. ESR/1hr of 35.3% has been normal in infertile ones suffering from allergy and 14.7% been abnormal. ESR/2hr of 35.3% has been reported normal, 19.1% abnormal, 20.6% VDRI negative and 3.1% positive. TB/ Test of 10.3% has been determined positive and 32.4% negative; also Rubella IgG in 48.5% was positive and 27.9% negative. Toxoplasmosis IGM in 17.6% has been reported positive and in 73/5% negative. **Conclusion:** The average year of the ones suffering from allergy has been more than those infertile ones not suffering from this disease; besides, in this study an increase in age has contributed to allergy. There is also a meaningful relationship between positive Rubella IgG and positive Toxoplasmosis IGM with allergy. IG overproduction leads to increased susceptibility, and effective cells such as mast cells and basophiles have used that for applying their operations; in other words, the individual gets sensitive and susceptible.
Keywords: Infertility, Allergy, Microbiological findings.

3325P
Serum anti-cardiolipin antibodies in women with a history of unsuccessful pregnancies compared with normal women

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Background: Abortion with incidence of approximately 15% of clinically recognized pregnancies has been the most common complication of pregnancy. In our country due to specific culture and a high tendency of couples to have babies this issue is of special importance. Despite all the researches the causes of abortions remain unknown. Some researchers have the idea that presence of anti-cardiolipin autoantibodies is caused abortions. This is the main objective of this study. **Methods:** In this cross sectional study, 120 women were studied in two groups of 60 each. The first group of women who had abortions with unknown etiology and the group of normal women with no abortions. 5 ml of peripheral vein blood was taken from each and cardiolipin antibodies were determined with ELISA method on their sera. Finally, descriptive statistics and SPSS, Z-test and t-test were used to evaluate and analyze obtained data. **Result:** In 26.7% of women with abortion, anti-cardiolipin antibody levels were <50 IU/ml= Normal. Antibody levels of 26.7% of cases were 75-50IU/ml= Borderline and 46.6% of them were > 75 IU / ml= Elevated. These values in healthy women, were, 86.7 percent, 14.3 percent and zero percent respectively. Between the two groups, there were significant differences in the levels of cardiolipin antibodies. (P <0/05). **Conclusion:** The results of this study confirmed the results of other studies that level of anti-cardiolipin antibody in women with recurrent miscarriage is higher than normal women Therefore, it is suggested that the

levels of these autoantibodies in women with abortion must be measured as a causing factor of abortion.

Keyword: Anti cardiolipin antibodies, Abortion, Autoimmunity

1635P

HLA-G6 and reproductive immunology

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Background: The definite cause of several pathologies of pregnancy including reproductive failure is still unknown. HLA-G6, a nonclassical class IbHLA, is novel molecule in the tolerance between fetus and mother. The purpose of this study was evaluation of HLA-G6 soluble isoforms and also difference in total expression of these antigens in threatened miscarriage pregnant women termination to reproductive failure in comparison with control group. **Methods:** The study sample enrolled 101 threatened miscarriage pregnant women in age of 20-32Y in first trimester without any physiological or genetic disorders related to reproductive failure as cases and 101 women with normal pregnancy by history of term pregnancy and no miscarriage as controls. We evaluated expression of HLA-G6 soluble isoforms on PBMCs by Real-time PCR. **Results:** The results showed the significant decrease in expression level of HLA-G6 isofrms in threatened miscarriage pregnant women in comparison with controls ($p < 0.05$).**Conclusion:** The different concentration of HLA-G6 molecule is necessary for saving of pregnancy. Therefore, It is concluded that HLA-G6 is an important molecule as predictive marker in early stages of reproductive failure for setting a clinical test.

Keywords: HLA-G6, Reproductive, Real-time PCR , HLA

1589P

Th17-related cytokine profile in preeclampsia patients: the role of pro-inflammatory cytokines in abortion or as predictor?

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Background: Preeclampsia (PE) is a medical condition characterized by high blood pressure and significant proteinuria of pregnant women. Although the etiology of preeclampsia remains unknown, there are many proposed theories regarding the pathogenesis of the preeclamptic disease processes: including defective immunological adaptation to pregnancy. Several lines of evidence also support the concept that preeclampsia is an excessive maternal inflammatory response to normal pregnancy. We therefore examined IL-17 and TGF-β expression in peripheral blood of patients with PE. **Methods:** For this reason, peripheral blood was collected from patients with PE (30 patients), normal pregnant controls (30 volunteers) and non-pregnant normal control (30 healthy people) as control from hospitals of Jahrom University of Medical

Sciences. Then serums were isolated and assessed for them using by ELISA. **Results**: The cytokine profile in preeclampsia shows that the production of Th17 cytokines, which induce inflammation. Maternal cytokine levels IL-17 and TGF-β are increased during preeclampsia in compare to normal pregnancy and non-pregnancy ($P<0.0001$). **Conclusions:** Cytokines have major roles in the pathogenesis of preeclampsia. It is seen circulating placental microvesicles that shed from placenta influence on immune cells to increase inflammatory cytokines such as IL-6, IL-17, TGF-β, IFN-γ and TNF-α. Consistent with elevated innate cytokine levels include TGF-β and IL-6 in the maternal circulation, placental tissue and blood cytokine levels from Th17 are also altered, which implies Th17 inflammatory responses that may occur both in the maternal and the placental compartments. Therefore, increased inflammatory factors may lead to abortion. On the other hand, we can use inflammatory biomarkers as noninvasive predictors in starting of diseases.

Keywords: Preeclampsia, Inflammation, IL-17, TGF-β

1844P

Expression of TLR6 in endometrium of women with history of unexplained recurrent spontaneous abortion

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Background: Pattern recognition receptors (PRRs) as one of main components of innate immune system recognize ligands derived from various pathogens which are called as pathogen associated molecular patterns (PAMPs). One of the main PRRs which are found in female reproductive tract is Toll like receptors (TLRs). TLRs family consists of 10 functional receptors in human that recognize different PAMPs. On the other hand, unexplained recurrent spontaneous abortion (RSA) is usually defined as three or more consecutive pregnancy losses before 20th week of gestation without any known cause. Immunological factors are suggested as one of etiologies of unexplained RSA. TLR6 forms heterodimer with TLR2 and recognizes components of gram positive bacteria. The objective of current study was to investigate TLR6 gene expression in endometrium of patients with unexplained RSA in compare to normal fertile women. **Methods:** Endometrial biopsies were obtained between day 19th and 24th of menstrual cycle from 10 women with unexplained RSA and 6 fertile women (having at least one successful pregnancy). TLR6 gene expression was studied by RT-PCR and then quantified by real time PCR. **Results:** TLR6 gene expression was detected in endometrium of women with unexplained RSA. The mean relative expression of TLR6 gene was higher in women with RSA in compare to normal women. **Conclusions:** Higher expression of TLR6 gene might have a role in the pathogenesis of unexplained RSA because TLR6 signaling could result in inflammatory cytokine production. It has been proposed that inappropriate inflammation has detrimental effect on pregnancy.

Keywords: Toll like receptors, Recurrent spontaneous abortion, Innate immunity; PCR, Gene expression

2062P

HLA-G 14 bp ins/del polymorphism in threatened- abortion women

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Background: abortion is one of the productivity decreasing agents that immunological factors are most important in this process. HLA-G is a non-classical molecule of MHC Ib that has limitary polymorphism. It has been postulated that 14 bp ins/del polymorphism in exon 8 has a prominent role in developing pregnancy loss. In this study, we evaluated relation between HLA-G 14 bp ins/del polymorphism with threatened- abortion women in comparison with control. **Methods:** In a case-control research executed on patient that had referred to tooba special clinic in 2012-2013.case group was 101 threatened -abortion women that registered in this study along with same control group. After DNA extracting with PCR method, we evaluated association between 14 bp ins/del polymorphism and abortion. **Results:** In this study, we found no significant difference between 14bp Ins/Del polymorphism (allele and genotype) and threatened- abortion women ($p>0.05$). Although heterozygote group was incrassated in case group in comparison with control. In addition, age and pregnancy period wasn't any significant different ($p>0.05$).**Conclusion:** This study show that 14bp Ins/Del polymorphism has not related to threatened -abortion women. Thus, it is concluded that further study necessary for completing this results that should be survey in another population.

Keywords: HLA-G, Threatened abortion, Polymorphism

2109P

Soluble Fas and Fas Ligand in pregnancy complicated by Preeclampsia

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Background: Preeclampsia is multisystemic diseases with high perinatal and maternal mortality and morbidity in 5-10% of pregnancies. Recent data suggest a dominant role of the abnormal activation of the adaptive immune system in pregnancy related immunoregulation and preeclampsia by affecting on placentation. A main cause for this may be irregularities in the critical relationship between Fas and its ligand (FasL), the two important members of extrinsic apoptotic pathway. The Fas and FasL exist in two forms of soluble (sFas/sFasL) and membrane (mFas/mFasL) and induce apoptosis of activated lymphocytes in tissues with immune privilege such as placenta. Some studies suggested that abnormal levels of soluble form of these proteins are related to preeclampsia pathogenesis mechanism. To date, the results of investigations related to this hypothesis on patients with preeclampsia are controversial. To test this hypothesis and found better understanding, we measured Fas and FasL in the serum of patients with preeclampsia compared with healthy pregnant women. **Methods**: The case-

control study included a group of 100 pregnant women with preeclampsia and 108 healthy pregnant women. Samples of serum were collected and concentration of sFas(pg/dl) and sFasL(pg/dl) was measured by ELISA. **Results**: We determined higher sFas (*P*-value=0.027) and lower FasL (*P*-value=0.033) in patient group compared with controls. **Conclusion**: Elevated levels of sFas with inhibitory role for mFas can prohibit of TH apoptosis and maybe decrease the expression of Fas. Another way decreased amount of FasL-secreted by trophoblas -diminish the protective environment of placenta against activated leukocyte.

Keywords: sFas, sFasL, Preeclampsia, Apoptosis

1812P

The relationship between risperidone-induced hyperprolactinemia and IVF rate and early embryonic development in NMRI male mice

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Background: Understanding of the ways in which medications affect reproductive efficiency is of great importance. Risperidone as atypical antipsychotic agent causes prolactin secretion to increase. The clinical consequences of hyperprolactinemia include hypogonadotropic hypogonadism, the latter manifesting as loss of libido and infertility in both sexes. The aim of this study was to investigate the effects of risperidone- induced hyperprolactinemia on male reproductive efficiency by assessment of fertility potential. **Methods:** 8-week old male NMRI mice were randomly assigned to two groups as control-sham and test groups. Risperidone was administered by gavage once daily for 45 consecutive days in dose of 3.2 mg/kg. The control group just received vehicle. At the end of the study period the animals were euthanized by decapitation. Sperms were removed from cauda epididymis and the rate of fertilization, two cell embryos, blastocysts and arrested embryos was examined using zygotes cultured in HTF-BSA medium. The intact, fragmented and/or lysed embryos that did not develop were recorded as "arrested embryos". **Results:** The data showed that fertilization rate and two cell embryos rate in the group of mice treated with risperidone were lower than of the control group. Percentage of embryos in blastocyst stage after culturing for 120h in the control group was higher than that of risperidone treated group. There was a marked increase in percentage of arrested embryos in risperidone treated mice in comparison with the control group. **Conclusion:** It was concluded that chronic exposure of mice to the therapeutic dose of risperidone had toxic effect on fertility potential and early embryonic development which may be due to hyperprolactinemia induced by risperidone.

Keywords: Blastocysts, Hyperprolactinemia, Mice, Risperidone

2078P

Evaluation of serum zinc levels in women with recurrent spontaneous abortion

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Background: Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy failure before the 20th week of gestation, affecting approximately 1 in 300 pregnant women. Various causes including genetic, anatomical, endocrinological disorders, environmental agents such as infection and immunological factors have been implicated in the etiology of RSA. In addition, maternal zinc deficiency has been related to adverse effects on fetal outcome and pregnancy failure. Regarding the critical role of this trace element in the immune system and these effects on pregnancy, in this study, we aimed to investigate serum zinc levels in patients with RSA and in normal pregnancy. **Methods:** Serum was obtained from 243 non-pregnant women with at least three RSA and 73 non-pregnant control women. Then, serums were stored at -20°C. Zinc concentrations were evaluated by using CBG Atomic Absorption Spectrophotometer Systems. **Results:** Serum zinc levels in women with a history of RSA were 68.13±11.43 µg/dL, which was significantly lower than those in normal controls with the zinc concentrations of 82.90±12.36 µg/dL ($p<0.05$).**Conclusion:** According to statistically significant decreases in the serum zinc in RSA Patients compared with age-matched controls, we conclude that zinc deficiency may be one cause that contributes to miscarriage. More research is required to assess the benefit of zinc supplementation in infant health and survival.

Keywords: Zinc, Pregnancy, Recurrent Spontaneous Abortion

1412P

Tumor necrosis factor alpha and oxidative/nitrosative stress may trigger ovarian cystogenesis following experimentally induced hyperandrogenism

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Background: Polycystic ovarian syndrome (PCOS) as an inflammatory condition is the leading cause of anovulatory infertility in reproductive-aged women. This study aimed to investigate the possible relationship between ovarian functionality and the oxidative/inflammatory response during cystogenesis induced by hyperandrogenization in poly cystic ovary (PCO). **Methods:** Ovarian cysts were induced by oral administration of letrozol (1 mg/kg/day) for 21 consecutive days in the female rats. Serum estradiol (E), progesterone (P), testosterone (T), and the ovarian prostaglandin E (PGE) as biomarkers of ovarian function, were analyzed. To determine the role of oxidative stress (OS) in PCO, the level of cellular lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and peroxynitrite

(ONOO), and TNF-α as a marker of inflammation and apoptosis were measured in serum and the ovaries. **Results:** Hyperandrogenism-induced PCO in rats exhibited a significant increase in LPO and ONOO in serum and ovary while significantly decreased serum and ovarian SOD, CAT, and GPx. Also, serum T and TNF-α levels, and ovarian PGE were increased in PCO rats compared with healthy controls, while E and P diminished. When compared to control group, letrozole-treated group showed irregular sexual cycles, polycystic ovaries characterized by high incidence of sub-capsular ovarian cyst with diminished or scant granulosa cell layer, increased number of atretic pre-antral and antral follicles and absence of corpus luteum. There were almost no primary, secondary and tertiary follicles in PCO rats. **Conclusion**: The results further support the role of oxidative/nitrosative stresses and inflammatory responses in the pathogenesis of letrozole-induced hyperandrogenic PCO rats.

Keywords*:* Polycystic Ovary, Letrozole, Hyperandrogenism, Oxidative Stress

1461P

Rehabilitation of cryopreservation-induced damaged sperm by mesenchymal stem cell-derived microvesicles

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Background: Diminishing sperm quality during cryopreservation process ends up in a complete or partial loss ofsperm's fertilizing potential. Rehabilitation of such affected sperm is crucial to improve their fertiliziing potential. A variety of evidence has indicated that secreted microvesicles (MVs) from mesenchymalstem cells (MSCs) are involved in regeneration of injured endogenous cells via shuttling MSC trophicmolecules. **Methods:** Sperm obtained from caudaepididymides of adult male wistar rats were equally assigned to four separate groups. Following suspension in cryoprotectant extender, sperm were untreated or treatedwith increasing concentrations of MSC-derived MVs (25, 50 and 100µg). After incubation in successive steps, sperm were cryopreserved. The frozen–thawed sperm were assessed for viability, motility andantioxidant capacity parameters. Consequently, expression levels of surface adhesion molecules (CD29,CD44, ICAM-I and VCAM-I) involved in sperm fusogenic and signaling properties, were assessed by flowcytometry. **Results:** Results showed an enhanced quality parameters and adhesive properties of cryopreservedsperm following treatment with MSC-derived MVs.

Keywords: Cryopreservation, Sperm, Mesenchymal stem cell-derived microvesicles, quality parameter

1736P

Evaluation of sFas and sFas-L concentrations in seminal fluid and expression of proapoptptic receptors Fas and Fas-L on sperm cells from male Patients with and without varicocele

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Background: Infertility is considered as one of the main public health issues, because it affects about 15% of the couples of reproductive age. The male factor is involved in 40-50% of infertility cases. Varicocele is an abnormal dilation of the pampiniform venous plexus in the scrotum that develops during puberty; it can affect testicular growth and semen parameters (Especially count and motility), and is considered to be a major cause of male infertility. It is suggested that the spermatogenic dysfunction in varicocele testis may be related partly to an abnormal control of sperm death and apoptosis. The results of studies about the presence of Fas system in semen is a matter of controversy. In this study, we measured the presence on sperm cells of Fas/Fas-L and levels in seminal plasma of their soluble forms as the major triggers of apoptosis in the semen of patients with and without varicocele. **Methods:** A case/control study, semen samples were obtained following 3–5 days of ejaculatory abstinence, from 45 adolescents (Mean age 28.3 ± 7.85 years, age matched) with varicocele grades II and III (study group), and 45 adolescents without varicocele (control group). Semen analysis was done according to World Health Organization. The Fas and Fas ligand (Fas-L) expression on Sperm cells was performed using Flow cytometry. Also levels of soluble Fas (sFas) and soluble Fas-L (sFas-L) in seminal fluid was measured by ELISA. The demographic characteristics were taken by a data collection form. Data were analyzed by using SPSS version 17.**Results:** Based on the results of the study, Fas and Fas-L proteins on the surface of sperm ejaculation in patients with varicocele and control groups were not observed. However, seminal concentrations of sFas in case group (3.32 ± 2.04 ng/mL) were significantly lower than the control group ($p \leq 0.0001$). sFas-L was detected at pg/mL but no significant difference was observed between groups. **Conclusion:** According to our results, the effects of apoptosis via this system on main sperm parameters (count and motility) were not demonstrated. Decrease in sperm count and motility in varicocele may occur thru other mechanisms.

Keywords: Varicocele, Apoptosis, Fas/Fas-L system, Flow cytometry

1726P

The effect of ovarian induction on uterine macrophage population of pregnant mouse on day 4.5 of pregnancy

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Background: Ovarian induction is widely used in IVF clinics. The main purpose of this

method is to stimulate folliculogenesis so the number of oocytes in each ovarian cycle will be increased. Following ovarian induction, ovarian hormones, particularly estradiol and progesterone dramatically increase. Immune cells especially macrophages are located in uterus enviroment and play a crucial role in an appropriate implantation and successful pregnancy. Uterine macrophages have receptors for estradiol and progesterone. The increase in estradiol and progesterone concentrations due to ovarian stimulation can affect the recruitment and frequency of leukocytes particularly macrophages. **Methods:** To investigate this issue, blood was collected from two groups of pregnant mice (with and without ovarian stimulation) on the day of 4.5 of pregnancy. Serum estradiol and progesterone concentration were measured using the ELISA method,. The frequency and localization of macrophages in decidua were also studied by immunohistochemistry. **Results:** The results of this study showed that the frequency of macrophages decreased in hyper-stimulated group compared to the control ones, significantly. The pattern of their distribution was also different between the test and control groups. In addition, an increase in progesterone and estradiol concentration was seen. **Conclusion:** Considering the increase in progesterone and estradiol concentrations after ovarian induction and the presence of receptors for these hormones on macrophages, the changes in frequency and distribution of macrophages could be explained. Regarding the role of macrophages in embryo implantation and regulation of feto-maternal immune responses, it seems that their changes may affect the rate of pregnancy success after IVF.

Keywords: Ovrian induction, Macrophage, Implantation, Uterus, IVF

1677P

Anti-GRP78 antibody and pre-eclampsia

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Background: Preeclampsia (PE) is a pregnancy specific syndrome that is associated with high maternal and fetal morbidity and mortality. Although the exact etiology of PE is not well known, there is no doubt that PE is a placentation disorder. Glucose regulated protein78 (GRP78) is an Endoplasmic Reticulum (ER) protein which is expressed on the cell surfaces of trophoblast cells under stress or hypoxic condition. GRP78 has a role in aggressive behavior of the invasive cells and may play a role in normal placentation. The presence of antibody against GRP78 has been reported within sera of pregnant women and anti-GRP78 antibody may influence the invasive behavior of trophoblast cells. The present study aimed to investigate the autoantibody against GRP78 in sera of the patients with PE. The correlation between antibody and severity of the disease was assessed as well. **Methods:** In the present study, we evaluated the anti-GRP78 antibody within the sera of fifty pre-eclamptics (12 severe and 38 mild PE) and fifty healthy pregnant women using a Lab. made ELISA technique. Furthermore, western blot technique was used to assess the expression of GRP78 and presence of anti-GRP78 antibody in the placental and sera samples from pre-eclamptics and healthy women respectively. **Results:** The results indicated that GRP78 was expressed by the third trimester placenta and both healthy and pre-eclamptic women produce anti-GRP78 antibody. Although no significant

differences was found between the pre-eclamptics and healthy women regarding the mean level of anti-GRP78 antibody, the difference between severe-pre-eclamptics and healthy control women regarding the level of anti-GRP78 antibody was statistically significant (0.80 and 1.11, respectively, P<0.003). **Conclusion:** The findings of the present study indicated that measurement of anti-GRP78 antibody may account as a new marker for severe pre-eclampsia. Yet, future studies are required to be conducted to the issue.

Key-words: Anti-GRP78, GRP78, Pre-eclamspsia, Pregnancy

1682P

Anti-sperm protein targets in azoospermia men

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Background: The number of couples which meet the definition of infertility at reproductive ages is increasing worldwide. One of the most known conditions of infertility in male individuals is azoospermia, defined as complete absence of spermatozoa in the semen. Azoospermia manifests in two forms, named obstructive and non-obstructive azoospermia. Obstructive azoospermia (OA) accounts for around 40 percent of azoospermia cases. Although the presence of the anti sperm antibody (ASA) is reported in 88 percent of patients with OA, but interestingly there is no data regarding ASA targets in OA individuals. The aim of the present study was identification of sperm antibody targets in a group of obstructive azoospermic men. **Methods:** The sperm proteome was separated using two dimensional gel electrophoresis (2-DE) technique, transferred onto the PVDF membrane and blotted with sera from a group of obstructive azoospermic men and compared with membranes blotted with a group of healthy fertile men sera. MALDI TOF/TOF mass spectrometry was used to identify the different blotted spots and finally the results of mass analysis were confirmed using RT-PCR method. **Results:** The results indicated that OA patients may produce antibody against two sperm proteins, Tektin-2 and Isoform 2 of triose phosphate isomerase(TPI1P1) . Moreover the expressions of two targeted proteins were confirmed at RNA level. **Conclusion:** The data of the present study candidate two functionally important sperm proteins as antibody targets in azoospermic men.

Keywords: Azoospermia, Tektin 2, TPI1P1, 2D-PAGE, Western blot

1680P

Investigation of the association between IL-17A & IL-17F gene polymorphisms and susceptibility to pre-eclampsia (PE) in Iranian women

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Background: Pre-eclampsia (PE) is one of the most important and life-threatening pregnancy disorders that affect at least 3-5% of all pregnancies. Several lines of evidences emphasizedon the role of imbalance in helper T cell functions in predisposing to PE or severity of the disease. Placental inflammation and oxidative stress are hallmark of pre-eclampsia. Recently elevated level of Th17 cells in the peripheral blood of PE patients is reported. Several single nucleotide polymorphisms (SNP) within IL-17 gene have been identified that may affect the IL-17 production. Interestingly the relation between IL-17 gene polymorphisms and PE has been not investigated yet. Theaim of the present study was to investigate the association between IL-17A (197A/G) and IL-17F (7488A/G) gene polymorphisms and susceptibility to PE in group of Iranian women.**Methods:**250 PE patients and the equal number of age matched healthy women with at least two previous normal pregnancy formed the cases and control of this study. IL-17A (197A/G) and IL-17F (7488A/G) polymorphisms were genotyped using PCR-RFLP method. The genotype, allele and haplotype frequencies were compared between cases and controls using chi-square test. **Results**: Statistical analysis indicated that there are no differences in genotype, allele or haplotype frequencies regarding the studied SNPs between cases and controls. **Conclusions**: This study for the first time tested the association between IL-17A and IL-17F polymorphisms and susceptibility to PE. Although the results of the present study failed to find any association between the tested SNPs withinIL-17 gene and susceptibility to PE, it's not clear whether other SNPs within IL-17 gene are associated with PE.Further studies with a larger sample size orother SNPs are suggested.

Keywords: IL-17, Pre-eclampsia, Polymorphism

1679P

Different expression of TLR-2 and TLR-4 in placentas from women complicated with pre-eclampsia compared with healthy pregnant women

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Background: Pre-eclampsia (PE) is one of the most complex and life-threatening pregnancy disorders and is a major cause of mortality among mothers and fetuses worldwide. However, the exact etiology of PE is not well known but several lines of evidence support an immunological etiology for PE. It is well known that innate immune system has a major role in pregnancy. Toll like receptors (TLR) are a large family of the innate immune receptors that play several important roles in the human pregnancy from implantation to delivery. The present study aimed to investigate the expression of TLR2 and TLR4 in placentas from PE and healthy pregnant women in third trimester of pregnancy. **Methods:** In the present study real time PCR (RT-PCR) technique was used to determine the expression of TLR2 and TLR4 at RNA level

in maternal and fetal parts of the placenta from 15 PE and 15 healthy women at third trimester of pregnancy. **Results:** Although the expression of both receptors were detected at RNA level in placental from PE and healthy women, statistical analysis revealed that TLR-2 is up-regulated in the fetal parts of the placenta from PE patients ($p<0.002$). Moreover TLR-4 was significantly over expressed in both maternal and fetal parts of the placentas from PE patients ($p<0.001$ and $p<0.001$ respectively) when compared with the healthy women. **Conclusions:** In conclusion the results of the present study confirmed the role of innate immune receptors in pre-eclampsia.

Keywords: Pre-eclampsia, RT-PCR, TLR

1681P

Follicle stimulating hormone receptor (FSHR) gene polymorphisms and susceptibility to azoospermia in Iranian infertile men

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Background: Azoospermia is the medical condition of a man not having any measurable level of sperm in his semen. Azoospermia manifests in two forms, named obstructive and non-obstructive azoospermia. Follicle-stimulating hormone (FSH) is a member of the glycoprotein hormone family that plays an important role in human reproduction because it's essential role for normal spermatogenesis. The FSH acts through the FSH receptor. FSH receptor in men is expressed only in Sertoli cells of the testis and is consists of 10 exons and 9 introns that are required to support developing spermatozoa. Various single nucleotide polymorphisms (SNP) are reported within FSHR gene and may affect the receptor function. Interestingly only a few studies have been published regarding the relation between FSHR gene polymorphisms and susceptibility to azoospermia. The aim of the present study was investigation of the correlation between two FSHR SNPs at positions A919G & A2039G and susceptibility to azoospermia in Iranian infertile men. **Methods:** This Case -control study was performed on 212 (126 non-obstructive & 86 obstructive) azoospermia and 200 healthy Iranian men. Two FSHR gene SNPs were genotyped using PCR-RFLP method and chi-square test was used for analysis the results. **Results:** Statistical analysis indicated that at A919G position, AA genotype and also A allele are more frequent in obstructive azoospermia cases as compared with normal men ($P=0.048$ and $P=0.015$ respectively). Regarding A2039G polymorphisms a significant difference between both azoospermia groups and controls was also observed. The AA genotype in cases was less, while AG genotype was more frequent as compared with controls ($P=0.047$). **Conclusion:** The results of the present study indicated that the genetic polymorphisms in FSHR gene might increase the susceptibility to azoospermia in Iranian men.

Keywords: FSH receptor, Male infertility, Polymorphism, Azoospermia

1803P

Association of IL-17A and IL-17F gene polymorphisms with recurrent pregnancy loss in Iranian women

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Background: Recurrent pregnancy loss is defined as missing two or more fetuses before the 20th week of pregnancy. Th17 cells are novel subset of T cells, which secrete IL-17.Th17 cells play an important role in host defense to protect against extra cellular pathogens and fungi. We investigated the role of IL-17A and IL-17F gene polymorphism in susceptibility of unexplained recurrent pregnancy loss. **Methods:** Two groups consisted of 85 normal healthy women with at least one delivery and 85 women with the history of two or more RPL. The polymorphism of rs2275913 in IL-17A and the polymorphism of rs763780 were carried out by PCR-RFLP.**Results:** In the case group, the genotypes frequencies of polymorphism of rs2275913 were GG (8.2%), AG (30.6%), and AA (61.2%) and in the control group, they were GG (3.5%), AG (42.4%) and AA (54.1%). Also, the genotypes frequencies of polymorphism of rs763780 in the case group were TT (43.5%), TC (49.4%) and CC (7.1%). Moreover, they were TT (25.9%), TC (70.6%) and CC (3.5%) in the control group. The comparison between the population of the case and the control groups in the three genotypes of AA, AG and GG has shown no significant difference (P= 0.1). In rs763780 the comparison between the case and the control group has shown a significant difference in the three genotypes of TT, TC and CC (P=0.01).**Conclusion:** This study showed that the rs763780 (IL-17F) is associated with the high risk of infertility in Iranian women ($p<0.01$).

Keywords: IL-17, Polymorphism, Infertility, Pregnancy

2169P

Starvation is an efficient technique for purification of rat sertoli cells

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Background: Sertoli cells (SCs) are one of the most important components of seminiferous tubules and are vital for normal spermatogenesis and male fertility. In recent years many studies have shown their unique immunological properties in vivo and in vitro. It has been known that, having pure SCs are a necessity for in vitro studies. In this study we have evaluated the efficiency of starvation method for purification of SCs. **Methods:** The cells isolated from rat testis seminiferous tubules, underwent two different techniques for purification of SCs. For the

first group (washing group), medium was changed every 3-4 days together with washing the cells twice with PBS^- before adding the fresh medium, and for the second group (starvation), the medium was changed every 7-8 days. Primary culture (P0), passage 1 (P1) and P2 cells were analyzed for the expression of SC-specific genes, vimentin, Wilm's tumore 1 (WT1), germ cell gene, vasa and a marker of peritubular myoid cells, alpha smooth muscle actin (αSma), by RT-PCR and real-time RT-PCR. **Results:** Gene expression analysis showed that, starvation caused significant downregulation of vasa and αSma expression, in P0, P1 and P2 cells. While vimentin and WT1 were upregulated. In contrast, washing had much lower impacts than starvation to remove germ cells and pretubular myoid cells ($p<0.001$).**Conclusion:** Finally, our results revealed that, although washing is the only common technique for elimination of contaminant cells in the culture of SCs, starvation has a stronger effect and is a suitable and affordable technique for purification of SCs.

Keywords: Starvation, Efficient technique, Purification, Sertoli cells

1426P

Royal jelly alleviates stanozolol-induced embryotoxicity and improves the blastocyst development rate

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Background: Stanozolol (ST) is a synthetic androgenic steroid with anabolic properties intensively used as a performance enhancer in athletics. It has been shown that reprotoxicity is one of the serious consequences of ST abuses. The aim of this study was to explore the hypothesis that royal jelly (RJ) may be protective against ST-induced embryotoxicity through antioxidant-mediated mechanisms. **Methods:** Experiments were performed on four groups each consisting of five mice. ST reproductive toxicity was induced by oral administration of ST at a dose of 4.6 mg/kg body weight daily for 35 days. ST plus RJ group received RJ (100 mg/kg/day, p.o.) concurrently during ST treatment. Corresponding control groups were also used. **Results:** Daily administration of ST caused a significant decrease in fertilization rate along with poor blastocyst formation in ST-treated animals. A concomitant administration of RJ, to ST receiving mice, markedly attenuated ST-induced embryotoxicity and ameliorated all the negative changes observed in the above-mentioned parameters. **Conclusion:** Findings from this study point out that RJ has a potential repro-protective action against ST-induced embryotoxicity in mice. However, clinical studies are warranted to investigate such an effect in human subjects.

Keywords: Royal jelly, Stanozolol, Embryo, Blastocyst development, Mouse

1403P

Royal jelly inhibits stanozolol-induced spermatotoxicity

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Background: Stanozolol (ST) is an active nutritional anabolic-androgenic steroid often abused by athletes and bodybuilders. An increasing body of evidence points towards the role of ST misuses in the pathogenesis of a wide range of adverse effects including spermatotoxicity. The current study was designed to investigate the efficacy of royal jelly (RJ) as an efficient antioxidant in inhibiting the spermatotoxic effect of ST in a mouse model. **Methods:** 20 adult male mice were divided into four groups of five animals each. Two groups of mice were administered ST (4.6 mg/kg/day, p.o.) for 35 days. One of these groups received RJ (100 mg/kg/day, p.o.) concurrently. A vehicle-treated control group and a RJ control group were also included. **Results:** ST treatment caused a significant decrease in sperm count and motility with an increase in DNA damage of the sperm cells. Moreover, the proportion of spermatozoa that retained their cytoplasmic droplet was markedly elevated in ST-treated mice. The above-noted parameters were restored to near normal level by RJ co-administration. **Conclusion:** Our findings revealed an inhibition of ST-induced spermatotoxicity by RJ supplementation.

Keywords: Royal jelly, Stanozolol, Sperm, Mouse

1810P

Success rate of Low-dose intravenous immunoglobulin treatment of immunologic abortion

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Background: Immunological abortion is a recurrent spontaneous abortion that is associated with immunological disorders. One of the proposed treatments is the use of intravenous immunoglobulin (IVIG) during pregnancy. The aim of this study was to determine the success rate of low-dose IVIG for treatment of recurrent miscarriage. **Methods**: This interventional case-control study involved fifty women with recurrent spontaneous abortion and exclude the other cause of recurrent miscarriage and positive antibody test at least one of Immunological disorder. All of women randomly divided to two groups (case and control).Case group were received 5gr IVIG monthly till 32 weeks of gestational versus 80mg aspirin daily in control group. **Results**: The mean age of the patients was 27 years old (17-37 year old) and mean number of previous miscarriage was 2.4times. Between abnormal immunological tests, anti-cardiolipin antibody 38% and anti-sperm antibody16% were highest and lowest level of antibodies respectively. Also 8% of control group and 76% of the cases had reached to full term pregnancy ($P<0.001$). Neither of those tow group did not experience the side effects of drugs. **Conclusion**: The use of low-dose IVIG during pregnancy improves pregnancy outcome and related immunologic factors in recurrent miscarriage and should be considered as one of

the therapies in recurrent miscarriage.
Keywords: Abortion, intravenous immunoglobulin, Aspirin, Recurrent abortion

1895P
Isolation and partial characterization of human amniotic epithelial cells:the effect of trypsin

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Background: Despite the extensive information available in the literature, cell surface marker signature of human Amniotic Epithelial Cells (hAECs) remained controversial. The aim of the present study was to characterize immunophenotypic features, proliferative capacity and immunogenicity of hAECs. We also tested whether expression of some cell surface markers is influenced by the type of trypsin used for tissue digestion. **Methods:** Single cell suspensions of amniotic membranes from four human placentas were isolated by enzymatic digestion and expression of CD9, CD10, CD29, CD34, CD38, CD44, CD45, CD73, CD105, CD133, MHC-I, HLA-DR, HLA-G, SSEA-4, STRO-1 and OCT-4 was then evaluated by flow cytometry. The differential impact of four trypsin types on the yield and expression of CD105 and HLA-I was also determined. The proliferative capacity of cultured hAECs was assessed and compared in the presence and absence of Epidermal Growth Factor (EGF). To test their immunogenicity, hAECs were injected into Balb/c mice and the reactivity of hyperimmunized sera were examined by immunofluorescence staining. **Results:** Nearly all purified cells expressed mesenchymal markers, CD9, CD10, CD29, and CD73 and embryonic marker, SSEA-4. A large proportion of the cells also expressed STRO-1 and OCT-4. The purified cells also expressed HLA-G and MHC-I. A very small proportion of hAECs expressed CD34, CD38, CD44, CD133 and HLA-DR. The type of trypsin used for enzymatic digestion affected both the percentage and expression of HLA-I and CD105. Proliferative assessment of hAECs revealed substantial proliferative capacity only when these cells were cultured in the medium supplemented with EGF. hAECs were showed to be capable of inducing high amounts of anti-donor antibodies. **Conclusion:** The results of the present study highlighted the impact of isolation procedure on immunophenotype of hACEs. Low immunogenicity attributed to these cells might not stem from the absence of HLA-I expression.

Keywords: Cell proliferation, Epithelial cells, Immunophenotyping, Placenta, Stem cell, Trypsin

1532P

The HLA-G 14-bp insertion/ deletion polymorphism in recurrent spontaneous abortion among iranian women

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Background: HLA-G is a non-classical HLA class Ib molecule with limited protein variability generated by alternative splicing. HLA-G displays immunotolerant properties and hence plays important roles in the maintenance of a successful pregnancy and maternal tolerance of the semiallogenic fetus. Polymorphism of the HLA-G gene may potentially affect the biological properties of the protein, and a 14-bp insertion/deletion polymorphism in exon 8 of the 3′ untranslated region (3′ UTR) of the HLA-G gene is thought to influence HLA-G expression. **Methods:** To study the association of the 14-bp insertion/deletion (INDEL) polymorphism with the risk of recurrent spontaneous abortion (RSA) we used polymerase chain reaction (PCR) amplification, and genotyped 85 women each for the case (women who have had two or more unexplained RSA) and control (women who have had at least one normal pregnancy) groups. **Results:** Our results showed that the frequencies of the−14 bp/−14 bp and +14 bp/+14 bp genotypes were reduced in women with RSA, while that of the +14 bp/−14 bp genotype was significantly increased in RSA compared with the control group of normal fertile women; no significant differences in the allele frequencies of the HLA-G 14-bp polymorphism were observed. **Conclusion:** These results suggest a possible significance of the HLA-G 14-bp INDEL polymorphism in the outcomeof pregnancy. However, further studies on other polymorphic sites in the 3 UTR and 5′ UTR regions, as well as monitoring the serum HLA-G concentration in order to determine the potential implications of this marker in our population.

Keywords: Abortion, HLA-G, PCR, Polymorphism, 14-bp INDEL, 3′ UTR

1981P

Th17 cells and related cytokines in unexplained recurrent spontaneous miscarriage

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Background: Unexplained recurrent spontaneous miscarriage (URSM) is presumed to be

caused by a mother's immunological rejection of her fetus. This study has evaluated the frequency of T helper 17 (Th17) and T regulatory (Treg) cells and contribution of Th17 related cytokines during the luteal phase in the window of implantation in peripheral blood lymphocytes (PBL) of 20 women with URSM compared to 20 normal non-pregnant women (NNP).**Methods:** Flow cytometry analysis was used to measure the frequencies of Th17 and Treg cells. Quantitative real-time PCR (qRT-PCR) was performed for expressions of IL-21, IL-23, IL-6, IL-17 and transforming growth factor-beta (TGF-β) cytokines. **Results**: There were 5.66±0.85% Treg cells in URSM subjects which were lower than normal NNP (9.5±1.48%). The frequency of Th17 cells in the URSM group (2.8±0.51%) was higher than the NNP group (1.82±0.41%). Expressions of IL-23, IL-17, and IL-6 and IL-21 cytokines in URSM subjects were higher than those in NNP. However, there was less expression of TGF-β and FoxP3 in URSM subjects compared to NNP subjects. All results were statistically significant except for IL-21 expression. Significant correlations were found between the frequency of Th17 cells to IL-23, IL-6 and IL-17. IL-23 showed significant correlations with IL-6 and IL-17. IL-6 showed a significant correlation with IL-17.**Conclusion:** We concluded that evaluation of cytokines related to Th17 cells as well as the frequency of Treg and Th17 cells could be used as prognostic factors in PBLs of patients with URSM.

Keywords: Unexplained recurrent spontaneous miscarriage, Th17, Treg

2976P

Evaluation of Immunologic factors (TNF-a/TNF-R, Fas/Fas-L) in apoptosis of sperm cells in patients with varicocele

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Background: Infertility is considered as one of the main public health issues, because as it affects about 15% of the couples of reproductive age. The male factor is involved in 40-50% of infertility cases. Varicocele is an abnormal dilation of the pampiniform venous plexus in the scrotum that develops during puberty; it can affect testicular growth and semen parameters, and is considered to be a major cause of male infertility. Varicocele is found in approximately 15% of the general population, in 35% of men with primary infertility, and in 70% to 81% of men with secondary infertility. The exact mechanism by which varicocele affects male fertility and spermatogenesis is unknown. Clearly, the factors contributing to abnormal sperm function caused by varicocele that lead to infertility are ambiguous. It is suggested that the spermatogenic dysfunction in varicocele testis may be related partly to an abnormal control of apoptosis. There has not been done any study about the role of TNF-Ra /TNF-R and sTNF-R simultaneously so far. **Results:** This study shows that a number of factors including autoimmunity, oxidative stress, leukocytes, cytokines and apoptosis are associated with varicocele. **Conclusion:** Although some studies was shown the presence of Fas on ejaculated sperm, by the abortive apoptosis" theory. But our research showed that Fas protein was not detected on the ejaculated sperm of normozoospermic and non-normozoospermic men. In order to determine the relationship between apoptosis and varicocele, we explored the main factors of apoptosis (TNF-a/TNF-R and sTNF-R, Fas/Fas-L) during varicocele.

Keywords: varicocele, infertility, apoptosis, spermatogenesis

3088P

The role of Leydig Cells in Regulating Macrophage Numbers, Differentiation, and Function: a review

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Background: The purpose of this study is to present one of the paracrine interactions that exists between macrophages of the testes and neighboring Leydig cells. Macrophages primarily reside in the connective tissue of most organs. Cells possessing markers for monocyte/macrophages appear in the testicular interstitium near the time of birth in rats and, subsequently, grow in size and number as they differentiate and populate the testis. When new born rats are given human chorionic gonadotropin for 8 to 10 days to precociously stimulate neighboring Leydig cells, the macrophage population doubles while those of the liver remain unchanged. This effect is not due to the release of testosterone by Leydig cells in response to human chorionic gonadotropin (hCG) because Casodex, an antiandrogen, did not block this action, nor did testosterone injections mimic the effects of hCG. It has also been shown that the effects of Leydig cells on the number of macrophages are not indirectly mediated by way of the seminiferous epithelium. Because macrophages are closely associated with Leydig cells and are known to secrete a wide variety of substances that signal other cell types at multiple anatomic locations, initially proposed the hypothesis that they may secrete a factor that also influences testosterone secretion by Leydig cells. Within the testis, macrophages play important roles during immune activation and normal physiologic interactions with Leydig cells (when development is stimulated and the production of testosterone is promoted by the secretion of 25-hydroxycholesterol). This study may provide valuable insight for the treatment of infertility.

Keywords: macrophage, Leydig cell, human chorionic gonadotropin, infertility

2827P

Protective effects of ethylpyrovate in histomorphometric the testis in cyclophosphamide treated adult mice

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Background: Increases in the survival rate of men treated with chemotherapeutic drugs and their desire to have children increased concerns about the effects of these drugs on germ cells. This study performed to evaluate the protective effects of Ethylpyrovate in oxidative stress induced by cyclophosphamide (CP) on testis. **Methods:** Three groups (6 mice in each) of adult mice were used. Control group treated with normal saline.,ip, and group 2 treated with CP 15 mg/kg/week.,ip, and group 3 treated with CP along with Ethylpyrovate 40 mg/kg/day.,ip. After 35 days samples were taken and fixed in 10% formal saline and paraffin sections were prepared and stained by H&E method. The thickness of epithelium and diameter of the seminifrous tubules were mesured in 5 regions of each slides by using grated were abjective divice lens and tubular differentiation index(TDI) and spermiogensis index(SI) were counted with latticed objective divice in 1mm^2 field in region of each slides. All obtained data were analyzed by SPSS software in ANOVA and Duncan test. **Results:** Results showed that The

diameter of seminiferous tubules and its epithelial thickness,tubular differentiation index(TDI) and spermiogensis index(SI) in Ethylpyrovate treated group were significantly more than the groups that only received CP($P<0.05$). Whereas control group were significantly different with two other groups ($P<0.05$). **Conclusion:** This study showed that Ethylpyrovate ameliorate the oxidative stress effects of CP on male reproductive organ.

Keywords: Ethylpyrovate, Testis, Cyclophosphamide, Mice

2638P

Study of leptin, insulin and testosterone hormones in infertile male and their association with the lipid composition of sperm

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Background: More than 15% of couples are incapable to reach pregnancy. At least 40% to 50% of infertility cases are due to abnormality in male factor. Infertility is multifactorial disorder and leptin, insulin and testosterone plasma leveles play an important role in lipid composition of sperm and seem to be important role in these abnormalities. **Methods:** 60 subjects (30 fertile and 30 infertile male) serum levels of leptin, insulin, and testosterone were measured using a sandwich immunoassay. sperm phospholipids assayed by the Bartlett method, and cholesterol by the Liebermann–Burchard test. Statistical analysis was performed using SPSS version 10.0 software, and to compare the mean values of insulin, testosterone, and leptin between fertile and infertile men was used the t-test .**Result:** serum levels of leptin, and testosterone were significantly associated with sperm fertility and infertility. Between the amount of leptin and insulin in serum and cholesterol, phospholipids in the sperm cells of infertile patients was not significant correlation. testosterone serum level was significantly associated with cholesterol levels in the sperm cells of infertile patients in fractions 1(sperms have three fraction 1,2 3), but significant correlation between cholesterol and phospholipids was not observed in other fractions. Significant and direct correlation was observed between leptin serum level with morphology of sperm cells in infertile men However, no significant correlation between the motility and sperm count in infertile men were found. an inverse significant relationship was observed between serum testosterone levels in infertile men class D with mobility of sperm cells. The number of sperm in infertile men has a direct significant correlation with serum testosterone levels in infertile men. Significant relationship between testosterone levels in infertile male with sperm morphology were not observed. Between serum insulin levels with morphology, motility and sperm count in infertile patients werenot observed correlation. **Conclusion:** serum levels of leptin and testosterone have role in sperm lipid composition(cholesterol, phospholipids) and sperm fertility.

Keywords: Male Infertility, Leptin, Insulin, Testosterone, Sperm morphology

2270P

Analysis of 508 infertile male patients in Iranian –Azari patients in 1999–2010: hormonal status and factors predisposing to immunological infertility

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Background: To analyse the factors predisposing to male immunological infertility from the hospital records of 508 patients that had been treated for infertility in the Alzahra Hospital (Tabriz) from 1999 to 2010. In addition, the hormonal status was investigated at the beginning of treatment. **Results**: Patients with a history of mumps, or either a fresh varicocele or a history of varicocele had statistically significant lower levels of MAR antisperm antibodies (ASAs) than patients with no such conditions. Repair of varicocele (either surgical or embolisation), showed a statistically significant enhancement of the total sperm cell counts in ejaculates, but it appeared not to have any influence on other parameters of the semen analysis (mobility and morphology). Of all male infertility patients, 66.3% had normal hormonal status at the beginning of treatment, 12.6% of patients had hypotestosteronemia and 22.1% had subclinical hypogonadism. Patients with subclinical hypogonadism had lower total sperm cell count in ejaculates than patients with normal hormonal status although they had statistically significant more offspring. In addition, it appeared that mumps orchitis as well as smoking and alcohol abuse are risk factors for subclinical hypogonadism. **Conclusion**: No clear predisposing factor for male immunological infertility could be found. However, patients with subclinical hypogonadism differed from other male infertility patients and thus may form a special group among the male infertility patients.

Key word: Infertility, Anti sperm antibody

2277P

Oxytocin: an unexpected risk for cardiologic and broncho-obstructive effects, and allergic reactions in susceptible delivering women

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Background: Oxytocin (Sintocynon) is considered an uncommon cause of severe allergic reactions during delivery. We have recently shown that allergic sensitization to latex might constitute an important predisposing risk factor for anaphylaxis after the first infusion of oxytocin during delivery. Some oxytocin cardiovascular activities such as lowering blood pressure, negative cardiac inotropy and cronotropy, parasympathetic neuromodulation, vasodilatation etc. can induce significant side effects mimicking cardiac anaphylaxand constitute an additional differential diagnostic problem in delivering women with suspected or real allergic background. Finally, some ex vivo models have shown that oxytocin, under pro-inflammatory cytokines stimulation, such as those occurring in asthma, may induce contraction of smooth muscle and airway narrowing. This background suggests that allergic sensitization to latex allergens constitutes a significant but underestimated risk factor for triggering severe systemic reactions after the infusion of oxytocin and, consequently, there is a need of particular attention in managing delivering women suffering from latex allergy and bronchial asthma. An accurate anamnestic, clinical and diagnostic evaluation, latex-free anesthesiological setting, use of oxytocin-alternative agents and, if necessary, a drug premedication are likely to reduce

the risk of anaphylactic/broncho-obstructive reactions in these women
Keywords: Broncho-obstructive effects, Oxytocin, Allergic reactions

2210P

The presence and role of Fas/Fas-L system in normal and abnormal human semen

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Background: Over the past few years, several studies have investigated system Fas/Fas-L and apoptosis in ejaculated sperm. It is suggested that the spermatogenic dysfunction in testis may be related partly to an abnormal control of apoptosis. Sperm apoptosis have been considered as potentially useful indices of male fertility. Apoptosis, or signal-induced cell death, is a process in which a genetic mechanism, responsible for a series of events related to morphologic and biochemical changes, initiates by certain stimuli and culminates in the death of a cell. It is an important mechanism through which altered or excessive cells are removed from a population, maintaining tissue integrity, differentiation, and characteristics. There are two major pathways that lead to apoptosis: an intrinsic, mitochondria-initiated pathway and an extrinsic pathway which initiates upon the activation of membrane death receptors. Fas (CD95, APO-1) is a 45-kDa type I transmembrane receptor protein, which belongs to the tumor necrosis factor (TNF) /nerve growth factor (NGF) receptor family. The natural binding partner of Fas is its ligand (Fas-L or CD95-L/CD178) which is a 37 kDa type II membrane protein that belongs to the TNF family. The Fas signaling system is a widely recognized apoptosis initiating pathway. As for human reproduction, the expression and function of Fas and FasL are a matter of debate. However, recently, in several reports, it has been shown that FasL is expressed in spermatogenic cells, not in Sertoli cells. Fas and Fas ligand are membrane proteins that exist in both transmembrane and soluble forms. **Results:** Sakkas et al. first described the presence of Fas on ejaculated sperm and proposed the “abortive apoptosis” theory states that an apoptotic process begins in germ cells but fails to be completed and deleted, can end up as Fas positive sperm in the semen. Sakkas etal. showed that 10–50% Fas expression in oligoasthenoteratozoospermia and oligoteratozoospermia samples were significantly higher than the percentage of Fas positive sperm in men with normal semen parameters. Fujisawa et al. indicated that apoptosis was decreased in the testes of patients with varicocele compared with those of controls. Contrarily, Fujisawa and Ishikawa demonstrated increased apoptosis in oligozoospermic patients with varicocele having lower seminal sFas than patients without varicocele or fertile men. **Conclusion:** Some studies have reported that Fas protein do not have in normal and abnormal sperm samples. In order to become clear, correct and resolve conflicting results regarding the role of Fas/Fas-L in semen, Dr.mohammadi et al. developed carful research results that could explain the differences in results obtained in previous studies. Some results will be published soon.

Keywords: Apoptosis, Fas/Fas-L system, Semen

Immunology & Nutrition

Oral Presentations:

2822O

Whether vitamin A supplementation is effective in T-bet and IFN-γ gene expression reduction?

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Background: The aim of present study is evaluation of vitamin A supplementation efficacy on IFN-γ and T-bet gene expression in atherosclerotic patients. **Methods:** 31 patients and 15 healthy controls were participated in this study. Healthy control and patients in Vitamin A group received 25000 IU retinylpalmitate daily for 4 months. Control patients, also received 1 pearl of placebo per day up to 4 months. Gene expression levels were assessed by real-time PCR using SYBR green detection method. **Results:** IFN-γ gene expression in fresh cells of patients taking vitamin A has fallen slightly (0.85 fold, p=0.068), But the expression of this gene in patients taking placebo, and in healthy control subjects 1.2 fold (p=0.267) and 1.7 fold (p=0.580); respectively, was increased. Between three groups in terms of IFN-γ gene expression in cells stimulated with PHA, there were no significant difference (p=0.159). In order to determine whether PHA stimulation of PBMCs in vitro had an effect on T-bet expression, we measured the difference between three groups of studied. The results showed significant differences between groups studied (p=0.046). IFN-γ gene expression in cells activated with ox-LDL in healthy control subjects and patients taking vitamin A, was reduced 0.43 (p=0.0001) and 0.41 (p=0.001) respectively, but in placebo patients was increased 2.2 fold (p=0.959).**Conclusion:** Considering role of vitamin A on suppression of Th1 cells in atherosclerotic patients, it can be concluded that vitamin A supplement may be advantageous for these patients.

Keywords: Vitamin A, T-bet, IFN-γ

1802O

Impact of omega-3 fatty acids supplementation on fatty acid composition of cultured human airway epithelial cells

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Background: Inflammatory behavior of the airway epithelial cells can be changed due to the manipulation of their fatty acid content, which has a critical importance in asthma. The objective of the present study was to determine the fatty acid composition of human airway epithelial cells after co-culturing with Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). **Methods:** Airway epithelial cells (Calu-3, Passage 40–43, from ATCC, USA) were incubated with different concentrations (0, 10, 200, 400µM) of EPA and DHA for 24h at 37C in the presence of 5% CO_2 and the incorporation of fatty acids was analyzed using gas chromatography (GC). Cellular viability of cultured Calu-3 cells was checked after each step of supplementation. **Results:** Findings showed that there was a significant decreasing trend in the concentration of n-6 polyunsaturated fatty acids (PUFAs) and non-significant increasing trend in total n-3 PUFAs due to EPA and DHA supplementation. As a result of EPA incorporation, the levels of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and DHA declined significantly. On the contrary, docosapentaenoic acid (DPA) content elevated markedly due to EPA supplementation. The EPA concentration increased significantly as a consequence of DHA incorporation. Additionally, the n-3/n-6 ratio elevated notably in both DHA and EPA supplemented groups. **Conclusion:** Incorporation of DHA and EPA can alter the fatty acid content of airway epithelial cells in a way which has a lower inflammatory characteristic.

Keywords: Airway epithelial cells, Docosahexaenoic acid, Eicosapentaenoic acid, Fatty acid composition

2122O

The effect of ginger consumption on prostaglandin E2, TNFα and CRP in patients with type 2 diabetes mellitus

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Background: To assess the effect of ginger consumption on prostaglandin E2, TNFα and CRP in patients with type 2 diabetes mellitus. **Methods:** This is a double- blind, placebo controlled clinical trial. Seventy type 2 diabetic patients were enrolled. They allocated randomly into ginger group (n=35) and control group (n=35). They consumed 1600 mg powdered ginger versus 1600 mg wheat flour placebo (one 800 mg capsule before lunch and one 800 mg capsule

before dinner) daily for 12 weeks. Serum C-reactive protein, prostaglandin E2 and tumor necrosis factor α were measured before and after intervention. **Results:** Sixty three patients were analyzed: Ginger group (n=33) and control group (n=30). Ginger reduced C-reactive protein and prostaglandin E_2 significantly compared with placebo group ($p<0.05$). However there were no significant differences in tumor necrosis factor α between two groups ($p>0.05$). **Conclusion:** Ginger improved inflammation by reducing C-reactive protein and prostaglandin E_2 in type 2 diabetic patients. Therefore ginger can be considered as an effective treatment for prevention of diabetes complications.

Keywords: Ginger, Type 2 diabetes,C-reactive protein, Prostaglandin E2,Tumor necrosis factor α, Inflammation

2699O

Impact of Vitamin A Supplementation on RAR Gene Expression in Multiple Sclerosis Patients

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Background: Vitamin A and its derivatives have been shown to modulate the immune system via retinoic acid receptor (RAR). This study explored the impact of retinylpalmitate supplementation on RAR subtype gene expression in peripheral blood mononuclear cells (PBMCs) in multiple sclerosis (MS) patients. **Methods:** The study designed as a double-blind randomized clinical trial in which relapsing remitting multiple sclerosis patients were evaluated. Both groups received one capsule 50,000 IU vitamin D3per 2 weeks and one intramuscular injection interferon beta-1aper week. The intervention group received one 25,000 IU retinylpalmitate capsule daily for 6 months and the placebo group received one placebo capsule daily. The PBMCs were isolated from participants and the expression level changes of RAR-α and RAR-γ genes were determined by real-time PCR. **Results:** After supplementation, in the intervention group, the RAR-α gene expression level was significantly decreased compared to theplacebo group ($p =0.03$); however, the expression of RAR-γ gene did not significantly change ($p =0.10$). **Conclusion:** These results show that vitamin A supplementation can significantly downregulate the expression of RAR-α gene in PBMCs of MS patients that suggest the presence of in vivo regulatory mechanisms for the action of vitamin A on the immune system.

Keywords: Multiple sclerosis, Retinoic acid receptor, Retinylpalmitate, Gene expression

2597O

The effect of vitamin A supplementation on retinoic acid-related orphan receptor (ROR) γt interleukin 17 gene expression in Avonex treated multiple sclerotic patients

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Background: Multiple sclerosis (MS) is an inflammatory autoimmune disease determined by consecutive relapsing episodes dependent on demyelination and axonal lesion mediated by CD4+ T cells with a proinflammatory phenotype such as Th17 and its hallmark cytokine eg. IL-17. The immunopathological aspects of MS are not completely clear, but it is believed to have a gene–environment interactions base. Studies have shown transcription factors such as RORγt have a key role in the differentiation of Th17 cells. Over expression of ROR-α and ROR-γt, can induce the differentiation of Th17 synergistically. **Methods:** Thirty-nine relapsing-remitting MS (RRMS) patients (including 11 males and 28 females, aged 21-44 years old; mean age 31 years) enrolled in this survey. Three patients withdrew from the study because of drug alternation. All participants were divided into two randomly allocated groups. Patients in the vitamin A group received 25,000 IU retinylpalmitate per day, while patients in the placebo group took one capsule of placebo per day for 6 months. Gene expression was measured by real time PCR at the first and end of the study. **Results:** The results of this study show that Vitamin A down-regulates IL17 and ROR-γt gene expression. **Conclusion:** To our knowledge, this is the first clinical trial of the effects of vitamin A supplementation on IL17 and ROR-γt gene expression in MS patients.

Keywords: interleukin 17, retinoic acid-related orphan receptor (ROR)γt, Multiple Sclerosis

Poster Presentations:

1554P

Nutritional therapy in infants with food allergy

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Background: In recent years, allergic disorders have reached epidemic proportions in children and adults. Today, 1 in every 13 children suffers from food allergies with nearly 12 million affected across the country. As food allergies continue to increase in prevalence, research centers are working together to achieve what seems like an impossible dream. **Methods:** We examined 45 formulae fed infants 1-12 months old with food allergy. The examination included a collection of allergy history, clinical evaluation and specific methods – the measurement of the total IgE and allergen-specific IgE to the cow milk protein and casein. The level of total IgE, cow and goat Milk proteins IgE allergen-specific antibodies in coprofiltrates were measured by immunoenzyme. **Results:** Use of adapted formula based on the goat milk in the complex anti-allergic therapy resulted in positive clinical effect in 37/50 (74%) infants. The remission of atopic dermatitis and gastrointestinal allergy was observed on 10th -20th day of the treatment and was accompanied by Decrease of the serum levels of total IgE and allergen specific IgE to the cow milk proteins. **Conclusion:** The beneficial clinical effect was observed in 74% infants with skin and gastrointestinal forms of the food allergy during the use of adapted formula based on the goat Milk. The detection of general IgE and allergen-specific IgE antibodies to cow's milk protein and goat's milk protein in coprofiltrates makes it possible to optimize the diet therapy for infants with cow milk protein allergy.

Keywords: Allergy, Food, Treatment

1392P

Serum Level of Trace Elements (Zinc, Lead, and Copper), Albumin and Immunoglobulins in Asthmatic Children

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Background: Bronchial asthma (BA) is a chronic inflammatory disease and it is a major health problem. Trace elements such as copper and zinc are essential components of anti-oxidant enzymes and optimal function of the immune response. Changes in the levels of these elements may lead to increase the risk of asthma. **Methods:** The study group consisted of 175 asthmatic children and 165 control group of healthy general population who attend the outpatient allergic clinic (Bou Ali Hospital) in Sari, Mazandaran, Iran between August 2010 and March 2011. Complete blood count, eosinophil count and serum total IgE level and Serum trace element levels (Zinc, lead and copper) were measured in both groups. **Results:** There was a significant difference in serum levels of copper, lead, IgE (increased), and decreased IgA, between two

groups (p=0.001). There was no significant difference in blood zinc levels and eosinophilia between two groups (p=0.732 and 0.068, respectively)6T. **Conclusion:** Increased serum levels of copper and lead may be associated with asthma6T.

Keywords: Children,Asthma,Zinc,Lead,Copper

2172P

The effect of Zinc consumption on Cell Immunity in healthy 6 years old Children

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Background: The aim of study was to determine effect of zinc consumption on cell immunity in healthy 6 years old children. **Methods:** In a double blind clinical trial after license of parents 40 children 6-7 years old were enrolled. In case group (N=20) twenty mg zinc sulfat syrup orally has been prescribed daily for 6 months. The control group (N=20) received placebo as the same as case group. Serum zinc level and cellular culture were measured before intervention and 12 hour after the last dose of zinc sulfate. No parents and no laboratory staff knew about intervention. Zinc serum was measured by manual colorimetric method technique. Zinc level less than 65 ug/dl considered zinc deficiency. The lymphocyte response before and after zinc treatment have been compared by paired t test analysis. **Results:** The mean weight of children in case group was 20.37±2.21kg (17.1 minimum and 24.6 maximum) and in control group was 20.92±1.98 kg (17.2 minimum and 27 maximum). There were no significant difference between two groups in weight (P= >0.05). Serum zinc level was in normal limit and did not differ between two groups before and after intervention (P=0.86). After supplementation of 20 mg zinc sulfate per day for 6 month, there was not a significant increase of lymphocytes culture (with / without PHA). **Conclusion:** This study indicates that moderate supplementation of zinc for six months cannot efficiently improve lymphocytes culture (with/without PHA) in healthy children.

Keywords: Zinc consumption, Cell Immunity, Children

1643P

Nutritional interactions in insect-microbial symbioses

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Background: The aim of this paper is to discuss the recent findings in nutritional research in the context of immunological studies. It has long been recognized that the immune response is modulated not only by host (and parasite) genetics, but also by host nutrition. **Method**: To generate distinct immune challenges on different nutritional states, fed or starved worker bees were injected with lipopolysaccharides or micro-latex beads to simulate bacterial presence and activate a combination of immune processes such as antimicrobial peptide production and phagocytosis, without the confounding effects of a growing parasite population. The survival of challenged and control bees was then followed. Survival time was reduced for challenged workers that were starved, but not when they were well-fed. **Results:** Insects and their pathogens show great promise as model systems in the study of the relationships between nutrition, innate immunity and gut microbiota. Opportunities now exist to explore the interaction between nutrients and gene expression and their products to determine the mechanism behind disease development. This will provide significant insights into nutritional regulation of the innate immune system, the gut microbiota and pathogenesis. **Conclusion:**In particular, insect models have the advantage of lacking confounding effects due to individual differences in adaptive immune responses. Insects also possess relatively simple microbial communities, which aids the quantification and manipulation of microbiota. In addition, recent findings concerning Drosophila melanogaster intestinal pathology suggest that this organism might be well suited as a model for the study of intestinal physiology during ageing, stress and infection.

Keywords: Immunity, Microbes, Nutrition

1880P

The impact of Retinylpalmitate on TGF-beta/IL-17 and IL-2/IL-4 ratios in patients with multiple sclerosis: arandomized double blind clinical trial

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Background: Multiple sclerosis (MS) symptoms are associated with increased production of inflammatory cytokines, and decreases in some anti-inflammatory cytokines. Vitamin A plays an essential role in immunological function. Vitamin A can increase anti-inflammatory cytokines and decrease inflammatory cytokines, so we studied the vitamin A supplement (retinylpalmitate) effect on cytokine production in MS patients. **Methods:**Thirty-five MS patients participated in this study. Retinyl palmitate (25000 IU per day) and placebo, were taken for six months. Blood samples were taken before and after supplementation period. Peripheral blood mononuclear cells (PBMCs) were isolated. PBMCs were cultured and Phytohemagglutinin (PHA) ormyelin oligodendrocyte glycoprotein (MOG) was added. After

96h incubation period, supernatant was isolated and cytokines were determined by ELISA kits. Retinol binding protein (RBP) assay kit was used to measure plasma RBP levels. **Results:** TGF-beta/IL-17 ratio in cells stimulated with PHA was increased significantly in patients who received vitamin A supplement after six months period ($P<0.05$). TGF-beta/IL-17 ratio showed a significant correlation with RBP ($r= 0.539$, $P<0.001$). This ratio decreased in placebo group but was not significant. The ratio of TGF-beta/IL-17 in cells stimulated with MOG and IL-2/IL-4 ratio in cells stimulated with PHA or MOG was not significant too. **Conclusion:** The result showed no influence on T-helper 1/T-helper 2 ratio changes (according to IL-2/IL-4 ratio). TGF-beta/IL-17 ratio has also revealed that vitamin A can affect IL-17 or TGF-beta secretion, and it is important for increasing the anti-inflammatory/pro-inflammatory cytokines ratio in MS patients.

Keywords: Vitamin A, Multiple sclerosis, Transforming growth factor-beta, Interleukin-17

1879P

The effect of vitamin A supplement on C-reactive protein in patients with multiple sclerosis: a randomized double blind clinical trial

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Background: Multiple sclerosis (MS) is an autoimmune disease with more incidence in females and young people. Vitamin A (retinoids) can play a role as an immune regulator. This vitamin decreases T-helper 1 (Th1) cytokines secretion, so on the basis of this theory that Th1 is a responsible factor for MS progression, vitamin A supplement (retinyl palmitate) was givento MS patients. We investigate the effect of vitamin A on high sensitivity C-reactive protein (hs-CRP) as a chronic inflammatory marker. **Methods**: Thirty-five MS patients, participated in this study and were divided in two groups, the vitamin A group (25000 IU retinyl palmitate per day), and the placebo. Blood samples were taken at the first and the end of supplementation period (six months). Immunoturbidimetric assay was performed to evaluate hs-CRP in plasma. **Results**: Hs-CRP had no significant difference between females and males. Hs-CRP in plasma had a significant correlation with age before supplementation period ($\rho = 0.336$, $P= 0.048$), but this correlation was not significant after six months. Hs-CRP increased in vitamin A group after six months and a significant differencewas seen between two vitamin A and placebo groups after supplementation period ($P< 0.001$). **Conclusion:** It is an interesting question that why hs-CRP increased in patients who received retinyl palmitate. Maybe just vitamin A precursors (carotenoids) can decrease CRP levels for the antioxidant functions of carotenoids. For the lack of studies about the action mechanism of vitamin A on CRP levels, more studies should be designed to explain it.

Keywords: Vitamin A, Multiple sclerosis, C-reactive protein

2121P

Effect of Conjugated Linoleic Acids, with and without Vitamin E on some immunity and Inflammatory factors in adults with active Rheumatoid Arthritis: a randomized controlled trial

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Background: There is little information about the effects of Conjugated Linoleic acids (CLAs) on inflammation and immune function in humans. This study investigated the effects of CLAs, with and without vitamin E on the inflammatory mediators and Immunity factors in adults with active rheumatoid arthritis (RA).**Methods:** In a randomized, double-blind, placebo controlled clinical trial 66 RA patients were randomly divided into 3 groups, each group receiving one of the following daily supplement for 3 months; Group C: 2.5 gr CLAs, Group CE: CLAs plus vitamin E, Group P: placebo. Cytokines, Matrix metalloproteinase 3(MMP-3) and Citrolinated Antibody (CCP-A) were measured by ELISA method, and Vitamin E by HPLC. **Results**: Consider statistical methods were no significant differences between groups in cytokines IL2, IL4, TNFα, IL1β, Il2/ IL4, Citrolinated Antibody, WBC and Neutrophils, Lymphocyte, Monocytes, and Eosinophils numbers. TNFα decreased in all groups, but its reduction was significant in group CE. IL1β increased in group P but decreased in group CE significantly. IL4 decreased in groups C, CE and E (P=0.03, P=0.03, P = 0.07 respectively). IL2 increased in group P but decreased in other groups. IL2/ IL4 increased in all groups. CCP-A increased in groups P and E while decreased in group CE significantly. CCP-A decrease was significant between groups P and CE.MMP-3 reduction was significant in group CE, and the differences between group C and E was significant (P<0.05). **Conclusion:** Co-supplementation CLAs and Vitamin E may be effective in rheumatoid arthritis patient's improvement.

KeyWords: Conjugated linoleic acids, Vitamin E, Immunity, Inflammatory factors, Rheumatoid Arthritis.

1901P

The evaluation effect of Caraways extract on Neutrophil function

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Background: The Caraway is a plant that cultured in Kerman, this plant is used for many years in Iran as a food additive. **Methods:** In this study, Caraways extracts should be prepared in different concentrations. Then encounter different concentrations of Caraways extracts to isolated neutrophils which are obtained from 5 ml blood heparin-coated of who is based on entry criteria and is not suffering from any disease or has not received any drug. The result is liquid form and should maintain on 37c for 60 min. Next step is to add 100 ml NBT/PMA and

maintain for 30 min more on the same temperature. **Result:** Study by the Microscope shows that in a dilution of 1/8, neutrophils activity was not only inhibited but the NBT was perfectly reduced. In addition Caraways stock is able to inhibit neutrophils activity and neutrophils and NBT was used as positive control.

Keywords: Caraway, Neutrophil function, NBT

3237P

CompareThe Role ofCasein-Genisteinwith Casein Diet on Oxidative Stress Indices in Experimental Adriamycin Induced Nephrotic Syndrome

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Background: Nowadays, many people, especially children are affected with Nephrotic syndrome in different community. Free radicals and Reactive Oxygen Species (ROS) play an important role in creating the pathogens of this syndrome. Usage of Genisteinas one of the main isoflavones of soy will lead to reduction and help control of inflammation due to its antioxidant property. The purpose of this study is to investigate the effect of isoflavone of soy along with casein diet on progress of oxidative stress indices in experimental model of Nephrotic syndrome. **Methods**: This study was done on 40 adult male Sprague- Dawely. The rats were 12-15 weeks old and weighed 300+/-50 g on average. To induce inflammatory reaction by single dose of 8 mg/kg Adriamycin was done. The rats were divided into four groups, include 10 rats in each group, such as: Normal group (N), Patient group (P), Casein group (C) and Casein-Genistein group (CG). Treatment protocol was performed with daily gavage of 40 mg/kg Genistein for CG group and 40 mg/kg CMC for Ca group along with casein diet for 8 weeks. At the end, urine protein to creatinin ratio and oxidative stress in kidney tissue were measured. **Results**: Treatment with Genistein along with casein diet (CG) compared with Patient group could meaningfully increase the activity level of Catalase enzyme ($p = 0.009$). On the other hand, significant difference was observed between two groups in Malondialdehydes ($p = 0.031$) index and Carbolynated proteins ($p = 0.014$) index. In addition, index level of Total antioxidant capacity ($P = 0.001$) was increased between Ca and P. Also, in multiple comparisons between Patients with Casein ($p<0.001$) and Casein-Genistein ($p = 0.025$), Casein with Casein-Genistein ($p<0.001$) there were a meaningful difference in urine protein to creatinin ratio. **Conclusion**: The results of this study show that treatment with main isoflavone of soy protein (Genistein) due to antioxidant and anti-inflammatory property not only decreases the Proteinuria, but also can reduce oxidative indices and prevents the progression of destructive glomerular lesions in experimental model of the disease.

Keywords: Nephrotic syndrome, Adult male (Sprague Dawely), Adriamycin, Casein-Genistein, Oxidative stress indices

3104P

Relationship between Leptin Concentration, Body Fat Mass and Fat Percentage withPBMCs Cytokines among Obese and Overweight Iranian Adults

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Background: Overweight and obesity has been suggested to be well correlated with altered levels of pro-inflammatory cytokines and adipokine. **Methods:** A total of 83 healthy overweight and obese men and women, who were the staff members of Tehran University of Medical Science, participated in the present cross-sectional study that was conducted in Tehran, Iran between August 2011 and June 2012. Individuals were divided into two BMI-category groups (BMI <30 Kg/m^{2}and BMI≥ 30 Kg/m^2). Fasting blood samples (FBS) were collected; peripheral blood mononuclear cells (PBMCs) were purified, cultured and stimulated and the concentrations of TNF-α, TGF-β, IFN-γ, IL-17, IL-4, IL-10 and leptin levels were measured by ELISA method, finally. **Results:** The mean values of weight, hip, WC, MUAC, body fat percentage, body fat mass and leptin levels (all P<0.001) and more so the concentrations of TNF-α(P=0.028) and IFN- ɣ(P=0.029) were significantly higher among individuals with BMI≥ 30 Kg/m^2. No significant differences were observed for the levels of IL-10, IL-4, TGF-$_\beta$, IL-17 between the BMI categories. Body fat percentage has a significant positive correlation with leptin (P=0.032), IL-17 (0.001), IFN-ɣ (0.01) and TNFα (p<0.001) and significant negative relationship with IL10 (p=0.03), TGF-β (p=0.02) and IL-9 (P=0.032). The body fat mass also was significantly and positively correlated with leptin (p<0.001), IL-17 (p=0.001) and TNF-α (p=0.02). Leptin levels had a positive significant association with IL-17 (p=0.01) and a negative significant correlation with IL-10 (p=0.001) and TGF- β (p<0.001).**Conclusion:** The results suggest that the body fat mass and the body fat percentage are positively associated with leptin levels, and leptin levels are negatively associated with IL-10 and TGF- β. Focusing on such strategies may lead to promises for alleviating obesity and its co-morbidities which are contributed to the altered pro inflammatory cytokines.

Keywords: Leptin , BMI, Cytokine, PBMCs

2515P

Alpha-lipoic acid supplementation in rheumatoid arthritis patients:Effectson serum biomarkers ofinflammationand joint erosion

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Background: Rheumatoid arthritis (RA) is a common autoimmune inflammatory disease in which immune cells develop systemic and articular inflammation and oxidative stress through excess production of molecules such as reactive oxygen species and cytokines. Alpha-lipoic acid (ALA) has been considered as a potent antioxidant with anti-inflammatory functions. However, few studies have evaluated its efficacy in RA. Therefore, we aimed to examine the effects of ALA on serum biomarkers of joint damage and inflammation in women with RA. **Methods:** In this randomized, double-blinded, placebo-controlled clinical trial RA patients (n=70) aged 20–50 years were randomly assigned 1:1 to receive either ALA (1200 mg/day) or placebo for 8 weeks. Fasting blood samples were taken to analyze serum high sensitive C-reactive protein (hs-CRP), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and serum matrix metalloproteinase-3 (MMP-3) before and after the study. Moreover, three-day dietary records and international physical activity questionnaire were used at baseline and the end of the study. **Results:** Sixty-five RA patients completed the trial. No statistically significant differences were observed in hs-CRP, TNF-α, IL-6 and MMP-3 within and between the ALA and placebo groups (P>0.05). Initial and final assessments of dietary intakes and physical activity level as confounding factors showed no significant inter- and intra-group changes (P> 0.05). **Conclusion:** In the present study inflammatory biomarkers and MMP-3 were not significantly affected by 8 weeks of ALA supplementation. It seems further clinical trials with longer duration are needed to affect inflammatory mediators produced by immune cells involved in pathogenesis of RA.

Keywords: Alpha-lipoic acid, Autoimmune disease, Rheumatoid arthritis, Inflammation, Matrix Metalloproteinase-3

2618P

Determining Serum globulin levels following the use of green tea in rats

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Background: Today, the indiscriminate use of antibiotics has led to a high incidence of resistance in pathogenic bacteria; so, the necessity ofusing alternative methods to be felt. The use of herbs and extracts is proposed as an effective alternative since, in addition to antimicrobial effect, sometimes contribute to the healing process to the positive effect on the immune system. **Methods**: In this study, conducted experiments on rats, the effects of green tea on several factors including the number of white blood cells and immune serum globulin levels were examined. **Results**: finally it was proved that the amount of white blood cells, the level of serum total protein and globulin levels had a relative increase in the treatment groups compared with the control group and albumin level was significantly higher in treated groups than the control group. **Conclusion**: It was concluded that green tea had a relative effect on increasing the immune system efficiency.

Keywords: Serum globulin, Green tea, Rat

2271P

Changes of Inflammatory Cytokines and Cortisol Hormone Following by an Exhaustive Exercise: Effect of Olive Oil on Active Girls

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Background: Immunity system is affected by physical activities. Exercise may be influenced on inflammatory markers secretion as IL-6, TNF-α, CRP. But the role of olive oil supplementation unknown. **Methods**: 24 young healthy women (age 23.5±4.1) were randomly assigned in control and exercise groups, participated a session exhaustive exercise including the sports Ellestadprotocol, was investigated for the presence of IL-6, TNF-α, CRP and cortisol hormone by ELISA. Blood samples were taken in a week before of exercise test, immediately before of exercise and 1hour after the end of the exercise. **Results**: The results showed that the amounts of IL-6, TNF-α, CRP and cortisol hormone in all of the stages in both of groups a week before of exercise test and before exercise test was increased($P\leq 0{\cdot}05$) and this increasing in supplemental group after the exercise test and 1 hour exercise test was less in comparison with the control group ($P\leq 0{\cdot}05$).However the level of cortisol immediately and 1 hour after exercise test was increased ($P\leq 0{\cdot}001$). **Conclusion:** Data suggest that an exhaustive exercise increased some inflammatory cytokines and cortisol hormone but olive oil prevent from increasing inflammatory markers followed by an exhaustive exercise in active girls.

Keywords: IL-6, TNF-α, CRP, olive oil, exhaustive exercise, active girls

2351P

The effect of vitamin A supplementation on IL-4 and GATA-3 gene expression in atherosclerotic patients

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Background: Since T helper cells type 2 (Th2) are considered as anti-inflammatory agents, this study was designed to investigate the possible role of vitamin A as a regulator of immune function in atherosclerotic patients. **Methods:** vitamin A treated groups (patients and healthy controls) and placebo group (control patients) received 25,000 IU of retinylpalmitate and one pearl of placebo per day for 4 months, respectively. Peripheral blood mononuclear cells were isolated and divided into 3 groups; fresh cells, activated with PHA and activated with ox-LDL. Gene expressions of Th2 cells were studied by real-time PCR. **Results:** IL-4 gene expression in fresh cells significantly increased in vitamin A treated patients compared with two other groups. IL-4 gene expression in PHA-activated cells also showed a significant increase in vitamin A treated patients compared with placebo group (p=0.027), however, there were

no significant differences in IL-4 gene expression in ox-LDL activated cells among the 3 groups (p=0.737) although all groups had an increase of the gene expression level. There were no significant differences in GATA-3 gene expression in fresh cells, among the 3 groups (p=0.084), while the mean of GATA-3 gene expression in PHA and ox-LDL-activated cells in vitamin A treated patients showed a significant difference in compare with healthy controls (p=0.016) and placebo group (p=0.019) respectively. **Conclusion:** It can be concluded that vitamin A supplementation leads to increase of the gene expression of Th2 which in turn may reduce the progression of atherosclerosis.

Keywords: IL-4, GATA-3, Atherosclerosis, Gene expression

2352P

Vitamin A supplementation reduces IL-17 and RORc gene expression in atherosclerotic patients

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Background: Vitamin A is a potential mediator of T helper (Th) cells in atherosclerosis. The purpose of the present study was to evaluate effect of vitamin A supplementation on expression of Th 17 cells related IL-17 and RORc genes in atherosclerotic patients. **Methods:** 31 atherosclerotic patients and 15 healthy controls were studied for 4 months. Atherosclerotic patients were randomly divided into vitamin A or placebo groups. Healthy controls and patients in vitamin A group received 25000 IU retinylpalmitate per day. Peripheral blood mononuclear cells were isolated, cultured and divided into three groups including fresh cells, phytohemagglutinin (PHA) activated T cells and ox-LDL activated T cells. Gene expressions of T cells were studied by real-time PCR. **Results:** In atherosclerotic patients, vitamin A supplementation resulted in significant decrease in IL-17 gene expression by 0.63 fold in fresh cell, 0.82 fold in PHA activated cells and 0.65 fold in ox-LDL activated cells ($p<0.05$ for all). RORc gene expression in fresh cells as well as ox-LDL activated cells decreased significantly after vitamin A supplementation in atherosclerotic patients ($p=0.0001$ for both). In PHA activated cells vitamin A supplementation significantly decreased RORc gene in both atherosclerotic patients and healthy subjects by 0.87 fold and 0.72, respectively, while in placebo group the RORc gene expression significantly increased by 1.17 fold ($p<0.05$ for all). **Conclusion:** Findings of the present study suggest that vitamin a supplementation may be an effective approach to slow progression of atherosclerosis.

Keywords: Atherosclerosis, Vitamin A supplementation, IL-17, RORc, Gene expression

3037P

Effect of 8 weeks soccer training and L-carnitin supplementation on salivary IgA and cortisol in adolescent soccer players

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Background: Despite an abundance of literature, describing the basic mechanisms of action of L-carnitine, there remains some uncertainty regarding the effects of supplementation on immunity system index in healthy subjects. The purpose of this investigation was to determine the effects of L-carnitine supplementation on salivary Igor and cortisol in adolescent soccerplayers. **Methods:** This semi experimental study is done double-blind and includes pre-test, test and post-test. Twenty adolescent soccer player boys with a verge age,weight,height and body mass index of 15±1.4,66±7.6,172±8.5 and 21±2.1 respectively are divided randomly into two groups: supplementation (n=10) and placebo (n=10).The supplementation group consumed 1000 mg L-carnitin per day for 8 weeks and the placebo group consumed 1000 mg Vitamin B1. Saliva samples of subjects were collected before and after exercise sessions. Data was analyzed by paired sample T test, Univariate Analysis of Variance and Tukey tests by SPSS software (version 19). **Results:** Results showed that S-IgA was increased significantly in supplementation-received group (P ≤0.014) and cortisol was decreased significantly in supplementation-received group (P ≤0.001). **Conclusion:** It can be concluded that L-carnitin supplementation has been effecting on immunity system in adolescent soccer players.

Keywords: adolescent ، Immunity Indexes، Supplementation، Soccer

3015P

Effects of Chlorella vulgaris supplementation on viability of lymphocyte sand body weight ofhealthy Balb/c Mice

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Background: Chlorella vulgaris (CV) is a green microalga that has been claimed to have immunomodulatory and apoptosis-inducing role. However, effects of oral supplementation of its powder on viability of immune cells have not been elucidated. We evaluated effects of three doses of CV powder on viability of splenocytes and also body weight (BW)in healthy BALB/c mice. **Methods:** Twenty-four6-8 week-old mice were randomly divided into four groups (n=6); Water (W) group which by gavages received Distilled water (control), and CV1, CV2, and CV3groups which received100, 200 and 300 mgCV/kg, respectively, for six weeks, and then were sacrificed. BW was measured during supplementation. Splenocytes from all groups were obtained (using ficoll), were cultured either alone or with PHA and/or with SMMT Antigens for 48 hours. Then, viability of the cells was evaluated by MTT test. Comparisons of data among and within all groups were done by ANOVA and Post Hoc Tests. **Results:** Viability of splenocytes from CV2 and CV3 groups (but not CV1) was significantly higher than viability of splenocytes from W group, after either of three types of culture (P<0.05). There were no changes in BW of W and CV1 groups; however, an insignificant increase was

observed in BW of CV2 and CV3 groups(P>0.05). **Conclusion:** Our results indicate that CV powder may dose dependently increasethe viability of immune cells in healthy BALB/c mice. Moreover, CV may increase BW in a dose dependent manner. However, these findings needs to be further investigated.

Keywords: Chlorella vulgaris, Lymphocytes, Splenocytes, Viability

3123P

Effects of Resveratrol supplementation on inflammation in male professional basketball players

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Background: Exercise can lead to acute oxidative stress, which can result in oxidative damage and induce inflammation. Resveratrol may reduce the levels of inflammatory cytokines. Thus, we investigated the effects of this compound on the plasma levels of tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) in male professional basketball players. **Methods:** Twenty healthy male professional basketball players were randomized into two groups (10 each). For 6 weeks, they received daily either 200 mg of polygonumcuspidatum extract (PCE) standardized to contain 20% trans-resveratrol equivalent to 40 mg trans-resveratrol or placebo. Indices of inflammation were measured before and after 6 weeks of supplementation. **Results:** There was a significant reduction in plasma levels of TNF-α and IL-6 after 6 weeks of supplementation (P<0.05); while no significant change was observed in these markers in the control group. **Conclusions:** Present study shows that 6 weeks of PCE containing resveratrol supplementation reduces the inflammation in male professional basketball players.

Keywords: Cytokines, interleukin-6, inflammation, resveratrol, tumor necrosis factor-α.

3239P

Effects of PolygonumCuspidatum containing Resveratrol on oxidative stress in male professional basketball players; a randomized, double blind, placebo-controlled trial

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Background: The increased consumption of oxygen during strenuous exercise can lead to an imbalance between productions of reactive oxygen species (ROS) and antioxidants which is involved in inflammation and muscle damage. Resveratrol is a polyphenolic compound with wide variety of pharmacological properties such as antioxidant activity. The aim of this study was to investigate the effects of this compound on the plasma levels of F2-isoprostane in male professional basketball players. **Methods:** The subjects were randomly assigned into two groups (10 each) participating in a 6 weeks intervention with receiving daily either 200 mg of polygonumcuspidaum extract (PCE) standardized to contain 40 mg trans-resveratrol or placebo. The plasma levels of F2 iso-prostane as a biomarker of oxidative stress were measured at the baseline and end of the study. **Results:** After a 6 weeks supplementation with resveratrol, the plasma levels of F2-isoprostane significantly decreased in resveratrol group ($P<0.05$) with no significant change in placebo group. **Conclusions:** The findings of this study indicate that resveratrol as a natural antioxidant compound has suppressive effects on oxidative stress.

Keywords: Resveratrol, Polygonum Cuspidatum, F2-isoprotane, Oxidative stress

3158P

Effects of Probiotics on Quality of Life in Children with Cystic Fibrosis; A Randomized Controlled Trial

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Background: Patients with cystic fibrosis (CF) usually have abnormal intestinal microbiota and deregulated immune mediators due to massive exposure to antibiotics. Probiotics as immunomodulatory and anti-inflammatory substances are considered to improve both clinical and biochemical intestinal and pulmonary function in CF patients. We decided to investigate the effects of probiotics on quality of life and pulmonary exacerbations in children with cystic fibrosis. **Methods:** In a prospective, randomized, controlled clinical trial, 37 CF patients (2-12 years old) were randomly divided into two groups. 20 patients of probiotic group took probiotics (2×10CFU/d) for one month while 17 patients of control group took placebo capsules. Quality of life was determined usingPedsQL4.0 questionnaire at the beginning, then three and six months after completing the treatment period. Rate of pulmonary exacerbation in probiotic group patients was also evaluated during three months after intervention and compared to the same three months of the previous year. Results were analyzed using SPSS(11.5). $P<0.05$ was considered statistically significant. **Results:** Significant improvement was observed in the mean total score of parent reported quality of life among probiotic group patients in comparison with placebo group at 3month ($P=0.01$), but this was no significant at 6thmonth of probiotic treatment. Rate of pulmonary exacerbation was significantly reduced among probiotic group ($P<0.01$). **Conclusion:** Probiotics are considered as useful nutritional supplements on reducing number of pulmonary exacerbations and improving quality of life in patients with

cystic fibrosis. Effects of probiotics seem to be temporary and probably continuous ingestion might have more stable improving effects on quality of life.
Keywords: Cystic Fibrosis, Probiotics, Quality of Life, Pulmonary Exacerbation

3323P

A study on some medicinal essential oils on humeral immunity against viral infections in broilers

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Background: Feed additives are used inpoultry feed in order to improve their performance, health and the quality of their products. Since the use of chemical compounds has many side effects and residues in animal production, finding an appropriate alternative has become of great performance. Essential oils are a group of natural compounds, derived from different plants, which have useful effects and low side effect and residues in poultry. The aim of this study was to study the effect of natural essential oils on health and immunity of broilers in order to find the natural composition as a useful alternative for chemical medicine. **Methods:** In this study 120 one day-old male broilers from Ross 308breed were randomly divided into 5 treatment groups with 3 replicates each containing 10. Chicks were kept under separated commercial farms, fed on commercial ration based on corn-soyebean meal and vaccinated routinely. The essential oils included Thyme and Rosmary, Garlic, Mint. The control group received the basal diet.In addition to basal diet, plus 0.5% thyme, 1% of Mint, 1% of rosemary essential oil and 1% of garlic added for related treatment groups. Blood samples were collected from wing vein at days 28 and 42 of age. Complete blood count was done and antibody titers against Newcastle, IB and IBD were measured. **Results:** Using the thyme essential oils improved titer of antibody against ND and IBD disease. The garlic essential oils have shown the good effect on performance. In Rosemary and garlic- treatment groups the leukocyte count, packed cell volume; phagocytic activity and phagocytic index have been increased. Therefore, they increased phagocytic capacity. But the essential have no effect on antibody titer at any age of broilers. **Conclusion:** Based on the result of this trial, thyme and rosemary improved performance of broilers while garlic almost improves the performance of broilers. Three essential oils stimulate the innate immunity in mentioned doses.
Keywords: Essential oils, Humeral immunity, Viral infections, Broilers

Immunology of Chemical Victims

Oral Presentations:

3246O

Serum MMP-9 and its complex with tissue inhibitors of metalloproteinases (TIMPs) in long-term cutaneous complicationsinduced by sulfur mustard: Sardasht-Iran Cohort Study

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Background: The expression of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are important in wound healing and skin repair. Exposure to sulfur mustard (SM) as an alkylation agent creates early and delayed skin injuries. Late skin complications induced by SM are consisting of itching, burning, xerosis, hyper pigmentation, hypo pigmentation, cherry angioma, mustard scar and seborrheic dermatitis. In this study the association of MMP-9 and its complex with TIMPs were evaluated with SM induced late skin problems. **Methods:** This study is a part of Sardasht-Iran Cohort Study (SICS). In this study 372 SM-exposed individuals and 128 unexposed controls were included. All exposed group were visited by clinicians, then SM exposed subjects were divided to participants with and who without skin disorders. Serum levels of MMP-9 and its complex with TIMPs were assessed by R&D Elisa kit. **Results:** Results indicated an association between MMP-9 and itching in SM exposed patient..In addition, MMP-9 of exposed subjects who had hyperpigmentation was significantly lower than exposed who did not have this problem. The results have also shown, MMP-9/ TIMP-2 complex was decreased in exposed group who had xerosisin compared to who did not have it. **Conclusion**: alteration of MMP-9 and its complexes with TIMPs in exposed patient with special complication suggest their interference in presentation of problems. However, more studies on role of MMPs in SM induced late skin complications is needed.

Keywords: sulfur mustard (SM), late skin complications, matrix metalloproteinase (MMP), tissue inhibitors of metalloproteinases (TIMPs).

3184O
Correlation of complement component C5a with pulmonary complications, 20 years after sulfur mustard exposure, Sardasht-Iran Cohort Study

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Background: C5a is produced in the course of complement cascade activation induces chemotactic migration, increases cell adhesion, stimulates the oxidative burst, and releases various inflammatory mediators such as histamine and cytokines. Sulfur mustard (SM) is a chemical warfare agent that induces major injury to the respiratory system. But the mechanism(s) by which SM induces chronic lung pathology is little known. Our previous study has shown no significant difference in the level of C5a in SM-exposed cases in compared to a not exposed group.SinceC5a is involved in the pathogenesis of pulmonary disease, in this study, we have assessed the correlation between salivary $C5_a$ level and SM induced pulmonary complications. **Methods:** In Sardasht-Iran Cohort study 372 SM-exposed cases were selected and classified to normal, mild and moderate - severe based on severity of long term pulmonary complications. They were compared with 128 control individuals. Spirometries were performed and their history was collected by internists. Saliva was obtained by using DRG Sali-Tubes 100 (SLA-4158). C5a levels were measured by ELISA technique (R&D System). **Result:** $C5_a$ shows significant decrease in SM-exposed group with lung problem (0.2 µg/ml) in compared to SM-exposed group without lung problem (0.6 µg/ml) according Gold Classification (P v =0.003). In addition, there is a correlation between salivary $C5_a$ with FVE 1/ FVC in exposed group (0.195%) and PEF (0.126%), in compared to control group (0.063%, 0.031% respectively). (Pv =0.005, Pv =0.033 respectively)**. Conclusion:** Although, the level of C5a is not significantly changed in SM-exposed population long term after exposure but it seems there is an association between salivary C5a level and pulmonary complications.
Keyword: sulfur mustard, Complement component C5a, Lung disease

3185O
Is α1-antitrypsin (AAT) correlate with pulmonary complications 20 years after sulfur mustard exposure? Sardasht-Iran Cohort Study

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Background: Sulfur mustard is used as chemical warfare. The major target of SM is lung however, the mechanisms of SM induced respiratory complications are not exactly clear. α-1-Antitrypsin (AAT) is an acute-phase reactant and a major protective factor against the development of chronic obstructive pulmonary disease. The lung-protective effects of AAT have been attributed to the inhibition of proteases like MMPs. Also, AAT has role in primary lung microvascular endothelial cell activation by relevant cytokines such as TNF-α or IL-1b. Our previous study has shown a significant elevation of salivary α1-antitrypsin (AAT) in SM-exposed civilian population in compared to a not exposed group. Also, it significantly increased in hospitalized cases in compared to non-hospitalized ones. So, in this study, we have evaluated the correlation between salivary AAT levels and pulmonary function. **Methods:** In Sardasht-Iran Cohort study 372 SM-exposed cases were selected and classified to normal, mild and moderate - severe based on severity of long term pulmonary complications;. They were

compared with 128 control individuals. Spirometries were performed and their history was collected by internists. Saliva was obtained by using DRG Sali-Tubes 100 (SLA-4158). AAT levels were measured by ELISA technique (R&D System). **Results:** The results show that SM-exposed patients with chronic caught have higher AAT (174.5µg/ml) than who without chronic caught (39µg/ml) (P_v= 0.001). However, there are no association between salivary AAT and other pulmonary symptoms, signs and pulmonary function tests. **Conclusion:** Although, salivary AAT is significantly increased long term after SM exposure, but it seems it has not strong correlation with pulmonary complications.

Keyword: Sulfur mustard, α-antitrypsin (AAT), Lung disease

3194O

Correlation between serum levels of L, P and E-selectins and sICAM-1 with pulmonary clinical assessment in 20 years after sulfur mustard exposure

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Background: The L, P and E-selectins and intracellular adhesion molecule-1 (ICAM-1) are as adhesion molecules and involved in trafficking of immune cells to inflammatory sites. They have a role in acute and chronic inflammation process and also in inflammatory diseases. Sulfur mustard induced long term pulmonary t complications. This study was design to evaluate the association between these adhesion molecules with clinical pulmonary findings. **Methods:** There were 348 exposed and 120 control participants. The clinical evaluations were done for all subjects and spirometry was performed according to American Thoracic Society Criteria. Respiratory Effects assessed as Severity of Lung Lesions. The serum levels of L,P, E- selectins and sICAM-1 were assessed by ELISA assay. Correlation between ELISA results was evaluated with pulmonary auscultation, pulmonary assessment, and spirometry findings. **Results:** Among the adhesion molecules only sICAM-1 show a correlation with rale and wheezing in SM exposed patient who have pulmonary complication. sE-selectin increased in control and SM exposed groups with mild pulmonary problem, and decrease in control group with moderate – severe status. sP-selectinhas negatively correlated with FEV1/FVC. There are not any significant correlations between other markers and spirometry findings. **Conclusion:** It is concluded that the serum levels of these markers may not represents lung disease in this population, and local evaluation of these adhesion molecules in the lung tissue were suggested.

Keywords: soluble L, P, E-selectins, Soluble ICAM-1, Mustard gas, Pulmonary clinical assessment

3301O

Association of serum level of TNF-α and late SM induced hypopigmentation

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Background: One of the sulfur mustard (SM) late skin complications is hypo pigmentation. Tumor Necrosis Factor (TNF)-α, is a pro-inflammatory cytokine and a paracrine inhibitor of melanocytes with inhibitory effect on pigmentation. TNF-α expression is increased in some skin disorders like hypopigmentation and vitiligo. In other hand, previously we reported lower serum level of TNF-α in a SM-exposed group in Sardasht. Herein, the association of serum level of TNF-α and SM-induced hypo-pigmentation is reported. **Methods:** This study is a part of Sardasht-Iran Cohort Study (SICS). In this study 372 SM-exposed individuals and 128 unexposed controls were included. All exposed group were visited by clinicians, then SM exposed subjects were divided to participants with and who without hypopigmentation. Serum level of TNF-α was assessed by R&D Elisa kit. **Result**s: The serum levels of TNF-α in exposed group who had hypopigmentation (22.431) was significantly higher than those SM-exposed participantswho did not have hypopigmentation (11.113),(Pv = 0.019). **Conclusion**: Although serum level of TNF-α is decreased long term after SM exposure, but it has a relationship with hypopigmentation as the same as vitiligo.

Keywords: sulfur mustard, hypopigmentation, Tumor Necrosis Factor (TNF)-α, skin.

3313O

Evaluation of Transforming growth factor-β1 (TGF-β1) gene polymorphism in –800 (A/G) regoin sulfur mustard exposed people

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Background: TGF-β1 is a growth factor that can be generated by several cell types and it is essential during some organs development. These organs development process often recapitulated in chronic diseases. Some studies have shown an increasing in TGF-β1 in chemical injuriesis associated with long term pulmonary complications. Several polymorphisms of the TGF-β1 gene have been reported. In this study, the polymorphism of the TGF-β1 gene at promoter region position−800 (G/A) is investigated in SM exposed population. **Methods:** The studied population includes 141 of sulfur mustard exposed people and 80 healthy controls. The target fragments of the TGF-β1 gene promoter were amplified and analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** The genotype at position −800 (G/A) in71.6% of chemical- injuriesand 68.8% of controls were homozygote GG, while 24.8% of cases and 30% of normal individuals were heterozygote GA, 3.5% of exposed population and 1.3% of controls were homozygote AA. There was no statistically significant difference of genotype distribution and allele frequency between chemical-injuriesand controls at polymorphic site. **Conclusions:** The promoter region polymorphism of TGF-β1 at position −800 (G/A) has not associated with sulfur mustard exposure.

Keyword: Sulfur mustard, TGF-β_1, Cytokine gene polymorphism

3204O

Sulfur mustard down-regulates SDF-1α (CXCL12)/CXCR4 axis

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Background: Sulfur Mustard(SM) has been used as a chemical warfare agent in Iraq-Iran war. Many years after exposure, people who have been exposed to SM are still suffering from its late complications. Important role of interaction of CXCL12 or Stromal-Derived Factor-1α(SDF-1α) and its receptor CXCR4 (SDF-1α/CXCR4 axis) in recruitment of stem cells from bone marrow to peripheral blood after injury has been indicated in many studies in other disease. The aim of this study was to evaluate the serum levels of SDF-1α and gene expression of its receptor CXCR4 in peripheral blood cells of chemical victims of SM, long term after exposure, compared to an unexposed control group. **Methods:** Serum levels of SDF-1α were studied by sandwich ELISA technique in 174 SM exposed and 39 unexposed participants. Gene expression of CXCR4 was studied by Real time PCR in 123 SM exposed and 37 unexposed participants. **Results:** The serum concentration of SDF-1α in SM exposed group was significantly reduced compared to the control group(p=0.046). CXCR4 gene expression in peripheral blood cells of SM exposed group was reduced about 30% compared to the controls. however this reduction is not statistically significant, but clinically is important. **Conclusion:** According to the significant reduction of the serum concentration of SDF-1α and the 30% fold reduction of CXCR4 gene expression in the SM exposed participants compared to the unexposed control group, it could be concluded that down-regulation of this axis could be one of the critical factors in late clinical complications of sulfur mustard.

Keywords: Sulfur mustard (SM), SDF-1α (CXCL12), CXCR4, Chemical victim

Poster Presentations:

3353P

Correlation between MMP9 and MMP9/ TIMPs complex with pulmonary function in sulfur mustard exposed civilians: Sardasht-Iran Cohort Study

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Background: Long term pulmonary complications in SM exposed injuries dominantly have been associated with protease activation, oxidative injury and inflammatory responses. The proteolytic activity of matrix metalloproteases (MMPs) involved in extracellular matrix degradation must be precisely regulated by their endogenous protein inhibitors, the tissue inhibitors of metalloproteases (TIMPs). In this study the serum level of MMP9 and the MMP-9/ TIMPs complex and their ratio in a SM exposed population have been presented. In addition,

the correlation between these factors and pulmonary finding,IL-1β, TGF-β and TNF-α have been investigated. **Methods**: In context of Sardasht-Iran Cohort Study (SICS) 372 volunteers with a history of SM exposure were recruited and classified to normal, mild and moderate to severe groups based on their pulmonary complications were compared with 128 controls. Spirometries were performed and their history was collected by internists. The serum levels of MMP-9, MMP-9/ TIMPs complex, IL-1β, TNF-α and TGF-β were measured using ELISA kits (R&D System).

Results: It was found that serum level of MMP-9 in moderate-severe subjects was significantly increased compared to normal ones in exposed group. Results demonstrated thatratio of MMP-9 and MMP-9/ TIMP-2 complex were significantly higher in moderate-severe patients than mild and normal individuals in control and exposed groups. Also the ratio between MMP-9 and MMP-9/ TIMP-4 complex was upper in moderate-severe patients than normal group in exposed subjects.MMP-9 level also positively correlated withIL_1β (Rho = 0.7, p-value< 0.01) only in the SM exposed group.The findings showed a significant negative correlation between serum level of MMP-9 and FVC %. **Conclusion:** This study provides evidence of IL-1β effect on MMP-9 concentration and that's impact on severity of disease and pulmonary function.

Keywords: Sulfur mustard, matrix metalloproteases (MMPs), tissue inhibitors of metalloproteases (TIMPs) and pulmonary function

3186O

Secretory Immunoglobulin a (sIgA) in pulmonary complications 20 years after sulfur mustard exposure, Sardasht-Iran Cohort Study

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Background: Sulfur mustard(SM) is used as chemical warfare. The primary targets of inhaled SM are the epithelia of the upper respiratory tract but upper tract complications remain obscure. Secretory Immunoglobulin A (sIgA) in mucous secretion like saliva and mucous surface for example respiratory epithelium is considered as the first line of defense in the respiratory tract whichreduced of expression of the secretory components like sIgA in airway epithelium is associated with airflow obstruction and neutrophil infiltration in lung diseases. In this study, we have evaluated the correlation between salivary sIgA level and pulmonary sign in SM-exposed and not exposed individuals. **Methods:** In Sardasht-Iran Cohort study 500 SM-exposed volunteers were selected and classified to normal, mild and moderate to severe groups based on their pulmonary complications were compared with 128 individuals. Also SM-exposed have been dived to two group hospitalized and non-hospitalized. Spirometery were performed and their history was collected by internists. Then Saliva was obtained by using DRG Sali-Tubes 100 (SLA-4158). Then sIgA levels were measured by ELISA technique (R&D System). **Result:** The SM-exposed group showed a significantly higher amount of salivary secretary IgA compared to the control group (P v =0.018). Also, it was revealed that amount of sIgA in SM-exposed patients with chronic caught and Dyspnea have significant increase (609.20 µg/ml, 604.60 µg/ml respectively) than who without chronic caught (437.7µg/ml, 379.60 µg/ml respectively) (P_v=0.003, P_v= 0.013). However, there are no association between salivary sIgA

and pulmonary function tests. **Conclusion:** Salivary sIgA is significant increase in long term SM-exposed individual but it seems there is not strong correlation with pulmonary complication.
Keyword: sulfur mustard, Secretory Immunoglobulin A (sIgA), Lung disease

3240O

Stromal-Derived Factor-1α(SDF-1α)/CXCR4 axis in sulfur mustard exposed population suffering from late pulmonary complications

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Background: Iranian who have been exposed to Sulfur Mustard (SM) many years ago, are still suffering from its late pulmonary complications. The Stromal-Derived Factor-1α (SDF-1α)/CXCR4axis plays critical roles in trafficking of progenitor cells tothe site of airway injury to contribute to repair it. In this study the serum levels of SDF-1αand gene expression of CXCR4 in peripheral blood cells of three SM exposed group suffering from pulmonary complications compared to an unexposed group. **Methods:** 174SM exposed and 39 unexposed participants as controls were chosen. According to the classification of Medical Committee of Iranians foundation of martyr and veterans affairs, SM exposed population classified to three groups with mild, moderate and severe pulmonary complications. Serum levels of SDF-1α were studied by sandwich ELISA andCXCR4gene expression by Real-time PCR. **Results:** The serum concentration of SDF-1αwas significantly lower in SM exposed group with severe pulmonary complications than in controls (p=0.054). But this was not significant in exposed groups with mild and moderate lung injuries.CXCR4gene expression in peripheral blood cells of SM exposed population with severe pulmonary complications was reduced about 35% compared to the controls. However this reduction was not significant in different exposed groups. **Conclusion:** According to the significant reduction of the serum concentration of SDF-1αand the 35% fold reduction of CXCR4 gene expression in the SM exposed population with severe pulmonary complications compared to the unexposed group, it could be concluded that down-regulation of this axis could be one of the important factors in pathogenesis of late pulmonary complications of SM.

Keywords: SDF-1α, CXCR4, sulfur mustard(SM), pulmonary complications.

2274P

The effect of platelet-rich plasma on CCl_4-induced liver fibrosis in Rat

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Background: Platelet-rich plasma (PRP) growth factors have attracted attentions of scientists and doctors that are involved in wound healing and regenerative medicine extensively, according to their unprecedented potential of promoting and catalyzing healing process. Platelet-rich

growth factors are cost-benefit, available andare more stable than recombinant human growth factors. According to these valuable properties, we decided to assess the effect of PRP on CCl4-induced hepatotoxicity on rat. **Methods:** Rats received CCl_4 (0.5 ml/kg 1:1 in olive oil) twice per week for 8 weeks. Five weeks after CCl_4 injection rats received PRP (100 µl in 100µl PBS subcutaneously) two days a week for three weeks. Twenty four hours after last CCl_4 injection the animals were bled and the livers were dissected for biochemical and histopathological studies. **Results:** Histopathology and biochemical analysis indicatedimproving effectsofPRPtherapy Oxidative stress was attenuated by increase in GSH content of liver tissue.The results showed that PRP itself were not toxic towards liver. **Conclusion:** The results of the present study support our beliefs on hepatoprotective effects of PRP, and could protect the liver from CCl_4-induced damages.

Keywords: Platelet-rich plasma, CCl_4, Hepatoprotective Effect, Rat

Immunology of Gastrointestinal Diseases

Oral Presentations:

2995O

The role of CD14 and CTLA4 gene polymorphisms in risk of celiac disease among patients of Iranian ethnicity

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Background: Celiac disease (CD) is developed via autoimmune reactions against gluten which is mainly found in the grains. Although HLA DQ locus is the most important genetic susceptibility to CD, some other variants have been proposed as CD predisposing genetic factors in many various studies such as A49G and G1359T of *CTLA4* and *CD14* genes respectively. We aimed to assess the possible role of A49G and G1359T polymorphisms in CD in Iranian population. **Methods:** The 100 CD patients and 100 healthy matched controls with average age of 30-33 yrs were selected. They were genotyped for both A49G and G1359T polymorphisms using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** There was no strong association between different genotypes and alleles of A49G variant of *CTLA4* and risk of CD ($p>0.05$). The G1359T polymorphism of *CD14* gene also did not show any significant association with risk of CD in Iranian population. However, patients with *CD14* T/T genotype were more classified in the severe form (marsh III) of CD, showing border line significance ($p<0.05$). **Conclusion:** No association was identified between G1359T or A49G alleles and risk of CD. Furthermore, their combination analysis also did not show any association with CD development. These lacks of association could be due to small sample size and considering further studies in various populations and ethnicity seems to be required.

Keywords: CTLA4, G1359T, A49G, CD14, Celiac disease

2714O

Prevalence of celiac disease in at-risk and not-at-risk groups in the Iranian population

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Background: Celiac disease may develop in only genetically susceptible individuals. The strongest genetic factor that is associated with CD is human leukocyte antigen (HLA)-DQ2 and -DQ8 and is found in virtually all CD patients and the absence of HLA-DQ2 or -DQ8 virtually excludes the diagnosis of CD. The aim of this study is to evaluate the prevalence of celiac disease in at-risk and not-at-risk groups in the Iranian population. **Methods:** Based on the outcome of the HLA-DQ2/DQ8 prediction using tagging SNPs, we divided the Iranian population into three risk groups: low risk (these were DQ2/DQ8-negative), intermediate risk (these were homozygous for DQ2.2 and DQ8, or heterozygous for DQ8, DQ2.5 or DQ2.2), and high risk (these were homozygous DQ2.5 or DQ2.5/DQ2.2). **Results:** According to these results 13.5% of cases and 43.7% of controls are in a low risk group, 57.6% of cases and 39.07% of controls are in an intermediate risk group and finally 25.4% of cases and 4% of controls are in a high risk group. **Conclusions:** The differences were statistically significant for all the risk groups when comparing cases to controls ($P < 0.05$). We also confirmed that CD patients carry more HLA risk alleles than healthy controls and this difference was statistically significant for the DQ2.5 haplotype distribution between cases and controls ($P = 0.0001$).

Keywords: HLA typing, Celiac disease, Iran

2685O

Programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with gastric cancer

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Background: Gastric cancer is the fourth most common cancer in the world. The programmed death 1 (PD-1) is a member of the CD28 super family. PD-1 is a negative regulator of T-cell effector mechanisms which decrease immune responses against cancer. This study aimed to determine the association between PD-1.5C/T (rs2227981, +7785) and the risk of gastric cancer (GC) in an Iranian population. **Methods:** we conducted case- control study to investigate the association of PD-1.5 C/T polymorphism in 122 GC patients and 166 control individuals. DNA was extracted from blood specimens. Genotypes were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. **Results:** The frequency of CC, CT and TT genotypes was 53.6%, 42.2% and 4.2% in control group and 41%, 54.1% and 4.9% in gastric cancer patients respectively. CC genotype was more frequent in control individuals than in patients but we found no statically significant association. The frequencies of PD-1.5CT genotypes were significantly higher in GC patient compared with control individuals (OR= 1.77, 95% CI= 1.077-2.931; *P*=0.026). Allele distribution was similar in patients and healthy individuals (p=0.061).Frequency of C and T alleles was 74.7%, 25.3% in control individuals and 68.03% and 31.97% in gastric cancer patients respectively. **Conclusion:** These results suggest that PD-1.5 C/T polymorphism may affect the GC risk and

prognosis in an Iranian population.
Keywords: Gastric cancer, PD-1, Single nucleotide polymorphism.

2250O

Expression and evaluation of antibody response of dispersin protein of Enteroaggregative *Escherichia coli* as a detection tool

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Background: Enteroaggregative *Escherichia coli* (EAEC) are an important diarrheal pathogen in the world. Dispersin encoded by *aap*gene as an important virulence factor of EAEC decreases bacterial autoaggregation allowing dispersion on the intestinal mucosa. Some assays have been developed for detection of EAEC that could not present the requisite criteria as an ideal diagnostic test. Thus, there is a need to evaluate other tests to achieve an ideal EAEC diagnostic method. The aim of this study was to express and induced antibody response against dispersin to reach a more reliable serological method for diagnosis of EAEC infections. **Methods**: EAEC were isolated from diarrheal patients at the hospital in Tehran, Iran. Bacterial identification was performed by routine methods. The *aap* gene of the isolates was amplified by PCR. After sequencing, the *aap* gene was cloned in pBADgIIIA vector and expressed. The confirmation of the expressed proteins was done by SDS-PAGE and Western blot. Purification of the proteins and endotoxin removal of them was done in Ni-NTA columns and LPS levels were checked by LAL test. Rabbits immunized with purified dispersinprotein and evaluation of antibody responses was performed by ELISA. **Results**: The *aap* gene was amplified in all of the EAEC isolates tested. The *aap* sequence of the isolates showed significant homology with the sequences in Gene bank. SDS-PAGE and Western blot confirmed the expression of the protein that the LPS value of the protein was <0.01 EU/ml. The IgG response was detected after first injection in all rabbits compared to the control that increased significantly after second and third injection. **Conclusion**: Among the serological methods, ELISA has been used widely for diagnosis due to their simple protocols and high sensitivity and specificity. Development of a detection method by ELISA can facilitate the diagnosis of EAEC infections; however, this method largely depends on the selection of target antigens. Dispersin is one the major virulence factors of EAEC containing conserved epitopes which can serve as diagnostic antigen. Our results suggested that dispersin is a conserved and immunogenic antigen in EAEC that could be a promising candidate for detection of EAEC infection. Evaluation of efficacy of the candidate antigen in ELSA is in progress.
Keywords: Dispersin, *aap*, ELISA, Enteroaggregative, *Escherichia coli*

2067O

Evaluation of the expression of CD1d molecule in peptic ulcer and gastric cancer due to Helicobacter pylori infection

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Background: Helicobacter pylori infection is the most important risk factor for dyspeptic disorders and gastric cancer. T-cells may play an important role in the immune response to H. pylori. Natural killer T (NKT) cells are a subset of T cells that share both properties of natural killer cells and conventional T cells and recognize glycolipids by CD1d molecule. CD1d have alternative spliced variants among which, V2, V4, and V5 variants are able to present antigens. To clarify the effect of CD1d in pathophysiology of dyspeptic disorders and gastric cancer, the expression levels of these variants in patients with non-ulcer dyspepsia (NUD), peptic ulcer disease (PUD), and gastric cancer (GC) were compared. **Methods:** Patients with dyspepsia were selected and divided into three groups including: NUD, PUD, and GC according to their endoscopic and histopathological examinations. H. pylori infection was diagnosed by rapid urease test and histopathology. After extraction of RNA and synthesis of cDNA from gastric biopsy specimens, the expression levels of V2, V4, and V5 variants of CD1d molecule were determined by quantitative Reverse Transcriptase PCR and were compared among the three groups of study subjects.**Results:** Fifty patients with NUD, 50 with PUD, and 50 with GC were enrolled in this study. The results indicated that the expression level of V4 variant in GC group was significantly higher than both NUD ($p<0.05$) and PUD ($p<0.05$) groups. Likewise, the expression level of V5 variant in GC was significantly higher than both NUD ($p<0.001$) and PUD ($p<0.001$) groups. V2 variant showed no expression in gastric epithelium. **Conclusion:** It is suggested that V4 and V5 variants of CD1d, but not V2 variant, are involved in pathophysiology of gastric disorders.

Keywords: CD1d molecule, Expression, Peptic ulcer, Gastric cancer, Helicobacter pylori

1778O

The serum levels of CCL28 is elevated in patients with irritable bowel syndrome: another evidence for the role of inflammation in IBS

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Background: Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders in clinical practice. Although its pathophisology is unknown, growing evidence now indicates that immune and inflammatory mechanisms contribute at least to a subset this disorder. Recent studies have demonstrated that CCL28, as mucosa-associated epithelial chemokine (MEC), is highly expressed by columnar epithelial cells in the gut, lung, and

the salivary glands and drives the mucosal homing of T and B lymphocytes that express its receptor. CCL28 is constitutively expressed in the colon, but its levels can be increased by pro-inflammatory cytokines and certain bacterial products. Nevertheless, the level of CCL28 in patients with IBS has not been yet studied. **Methods:** We aimed to examine the level of CCL28 in serum of 41 patients with IBS and 41 matched-normal individuals by Elisa. **Results:** Surprisingly, we observed that the level of CCL28 is significantly higher (p value=0.011) in patients than the control group. When, we determined the level of CCL28 in patients with IBS, we found that the level of this chemokine is higher, although is not significant (P value=0.14), in patients with diarrhea-predominant IBS. **Conclusion:** Overall, we are demonstrating for the first time that CCL28 is elevated in serum of patients with IBS and that CCL28 could be a biomarker for diagnosis of patients with IBS. However, we consider evaluating this biomarker in serum of more patients with IBS as well as inflammatory bowel disease.

2083O
Vascular endothelial growth factor-A and -C geneexpression in patients with Peptic ulcer and Gastric cancer

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Background: To clarify the effect of Vascular Endothelial Growth Factor (VEGF)-A and -C in the pathophysiology of *helicobacter pylori*-induced dyspeptic disorders and gastric cancer, gene expression levels of thesetwo molecules in patients with non-ulcer dyspepsia (NUD), peptic ulcer disease (PUD), and gastric cancer(GC) were evaluated. **Methods:** Patients with dyspepsia who needed diagnostic endoscopy were selected and divided into three groups of NUD, PUD, and GC according to their endoscopic and histopathological examinations. *H. pylori* infection was diagnosed by the rapid urease test and histopathology.Biopsy specimens were taken from each study subject and preserved in RNA later. After RNA extraction and synthesis of cDNA, the expression levels of VEGF-A and VEGF-C were determined by Real-time PCR.**Results:** Fifty patients with NUD, 50 with PUD, and 50 with GC were enrolled in this study. The results indicated that the gene expression level of VEGF-A in PUD group was significantly higher than NUD (p<0.05) group. Moreover, the expression level of VEGF-C in both PUD and GC groups were significantly higher than NUD group (p<0.001 and p<0.001, respectively).**Conclusions:** It is suggested thatVEGF-A and -C are involved in the pathophysiology of peptic ulcer and gastric cancer.

Keywords: VEGF-A, VEGF-C, Gastric cancer, Peptic ulcer

1913O

Normal total duodenal intraepithelial lymphocytes and intraepithelial $CD3^+$ and$CD8^+$ T cells in the celiac patients: the first report on determination of normal values of the intestinal intraepithelial lymphocytes in Isfahan population, Iran

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Background: Although increased numbers of the duodenal intraepithelial lymphocytes (IEL) are one of the key histological findings in celiac disease (CD), this may vary by area and type. There are some borderline histology and seronegative CD cases, which might be differentiated with other causes of the duodenal immunological reactions by measuring the mean percentages of IEL expressing the surface markers of the abTCR, gdTCR, CD3, CD4 and CD8. This study aimed to measure the total duodenal IEL, intraepithelial $CD3^+$ and $CD8^+$ T cells in the celiac patients and healthy controls in Isfahan, Iran.**Methods:** D2 biopsies from definite CD cases (17 cases) and healthy controls (22 cases) were evaluated. The H&E staining method was employed to determine the cut off level of IEL, but the immunoperoxidase staining method was employed to count the $CD3^+$ and $CD8^+$ intraepithelial T cells. **Results:** The immunoperoxidase staining method showed that the mean total IEL was 19 and 40 in the control individuals and CD cases, respectively (P=0.001). The upper normal limit of $CD3^+$ intraepithelial T cells (mean+2SD of IEL cell counts in controls) was 20, while it was 14% for $CD8^+$ T cells. In addition, the H&E staining method showed cut off of 34% for IELs in CD. **Conclusions:** This study suggests that the total IEL of > 34% and CD3 IEL > 20% in the patients with CD by the immunoperoxidase staining technique. This cut off level might be considered as MARSH I among the general population in Isfahan, Iran. This cut off may differ according to the subtype of IEL, genetic backgrounds, alterations of health environment and socioeconomical status. Thus, the local scientists need to determine the normal values in each region. In addition, they need to use the immunoperoxidase staining technique to measure the subtypes of IEL in suspicious cases of CD.

Keywords: Duodenal intraepithelial lymphocytes, Celiac disease, Immunohistochemistry, Duodenal intraepithelial $CD3^+$ T cells, Duodenal intraepithelial $CD8^+$ T cells

Poster Presentations:

1404P

Effects of vitamin A deficiency on mucosal immunity and response to intestinal infection in animal model (Caviaporcelluss)

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Background: Vitamin A deficiency is associated with an increased incidence of infectious respiratory and alimentary tract diseases in children, and vitamin A supplementation can prevent and assist in treating these diseases. To clarify the mechanisms of these associations, we investigated the effects of vitamin A deficiency on mucosal immunity to intestinal infection in animal model (Caviaporcelluss). **Methods**: Specific pathogen-free Caviaporcelluss received a vitamin A-free diet, with (n=40) or without (n=40) vitamin A supplementation. Intestinal infection was induced by oral inoculation of salmonella in 20 Caviaporcelluss in each group. The Caviaporcelluss were killed 4 day after infection was induced, and we measured the number, maturation, and activation of dendritic cells; the expression of Toll-like receptors 2 and 4; mRNA level of myeloid differentiation primary response gene (88) (MyD88, pattern-recognition receptors and their adapter protein); immune cytokine production in the intestinal mucosa; and the amount of secretory immunoglobulin A in the gut.**Results**: In vitamin-A deficient Caviaporcelluss, the number of mucosal dendritic cells and the production of IL-12 markedly increased; the mucosal expressions of Toll-like receptor 2 and MyD88 were up-regulated, and secretions of interferon-γ and secretory immunoglobulin A were decreased. Infection aggravated the damage to the intestinal mucosa and lowered immunity in vitamin-A deficient Caviaporcelluss.**Conclusion:** Vitamin A deficiency damaged both humoral and cellular immunity in the mucosa. Modulation of dendritic cells is likely an important mechanism through which vitamin A deficiency affects mucosal immune responses against infection.

Keywords: vitamin A, Caviaporcelluss, Immune responses, Intestinal infection

1616P

Helicobacter pyloriand IL23R Gene Polymorphism RoleinDegeneration of Gastric Mucosa

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Background: Relationship between *H.pylori* (Hp) colonizes and gastric inflammation is widely accepted. Polymorphisms in inflammation related genes such as cytokines and their receptors were thought to partly determine the outcome of Hp infection. Interleukin 23 receptor (IL23R) may relate to degeneration of gastric mucosa. We evaluate association of IL23R +2199 rs10889677 polymorphism and grade of Hp infection with degeneration of gastric mucosa and grade of Hp infection.**Methods**: Biopsies taken from the corpus patientswere classified

asHp-infected andHp-uninfected. The histological severity of Hp infection and degeneration of gastric mucosa were graded from normal to severe. Polymorphism in IL23R was evaluated by PCR-RFLP.**Results**: AC genotype was related to mild degeneration in Hp-infected subjects (*P*= 0.017).Mild and moderate grades of Hp infection were found related to mild grade of gastricmucosal degeneration (p=0.004 for mild and p=0.037 for moderate grade), sever grade was associated with non-degeneration (p=0.010). We didn't found any association between IL23R +2199 polymorphism and grades of Hp infection (p> 0.05). **Conclusion**: AC genotype of IL23R polymorphism according to presence of Hp andgrades of Hp infection, influences degenerationof gastric mucosa.

Keywords: Degeneration, IL23R, Polymorphism, Helicobacter pylori, Mucosa

2904P

To investigate the linkage disequilibrium between HLA-DRB1 and TLR4 gene polymorphisms in the development of ulcerative colitis

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Background: Ulcerative colitis (UC) is one of the gastrointestinal autoimmune diseases with uncertain etiology. Role of genetic factors and the inheritance of particular gene polymorphisms from HLA, Toll like receptor 4 and several other genes have been proven in UC. The purpose of this study was to investigate the Presence of linkage disequilibrium Presence of HLA-DRB1 and TLR4 gene polymorphisms in the development of ulcerative colitis. **Method:** In this study 85 Ulcerative colitis patients and 95 controls were examined. Polymerase chain reaction-sequence specific primer (PCR-SSP) and PCR-RFLP techniques were employed to determine polymorphisms in HLA-DRB1 and TLR-4 genes, respectively. Statistical analysis was performed to determine linkage disequilibrium between the above mentioned gene polymorphisms. **Results:** The inheritance of 13 alleles of HLA-DRB1 and their linkage disequilibrium with Asp299Gly and Thr399Ile polymorphisms in the TLR4 gene were studied in the development of UC and no significant association was observed (p value>0.05). **Conclusion:** The results of this study indicate Lack of linkage disequilibrium between 13 alleles of HLA-BRB1 and TLR4 polymorphisms includingAsp299Gly and Thr399Ile in the development of ulcerative colitis.

Key word: HLA-DRB1, TLR-4, ulcerative colitis, gene polymorphism, linkage disequilibrium

3021P

Serum levels of anti-cardiolipin antibody in peptic ulcer patients infected with Helicobacter pylori

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Background: The H. pylori were associated with the higher risk of peptic ulcer (PU) diseases. The aim of this study was to evaluate the serum concentrations of anti-cardiolipin (anti-CL) antibodies in H. pylori-infected PU patients, H. pylori-infected asymptomatic (AS) carriers and a healthy non-infected group. **Methods:** Totally, 100 H. pylori-infected PU patients, 65 H. pylori-infected AS carriers and 30 healthy H. pylori-negative subjects (as a control group) enrolled to study. Serum samples of participants tested for the levels of anti-CL antibodies by use of ELISA. **Results:** The mean serum levels of anti-CL antibody was 5.50 ± 0.40 U/mL in PU group, 5.25 ± 0.23 U/mL in AS group and 4.92 ± 0.24 U/mL in uninfected control group. The differences of the mean serum levels of anti-CL antibody were not significant between PU, AS and control groups. The difference of the mean serum levels of anti-CL antibody between total H. pylori-infected subjects (PU patients plus AS subjects) and uninfected control group was not also statistically significant. In PU and AS groups the mean serum levels of anti-CL antibody was not significantly differ between subjects with positive test for anti-CagA antibody and those were negative for anti-CagA antibody (Table 2). In total H. pylori-infected subjects (PU patients plus AS subjects), the difference of the mean serum levels of anti-CL antibody between subjects with positive test for anti-CagA antibody and those with negative test for anti-CagA antibody was not also statistically significant. Moreover, no significant differences were observed between the men and women of PU, AS and healthy control groups with respect to the mean serum levels of anti-CL antibody. **Conclusion:** These results showed no association between anti-CL antibodies and PU disease and also no relation with the CagA+ strains of H. pylori.

Keywords: Helicobacter pylori, Peptic ulcer, Anti-cardiolipin antibody, Anti-CagA antibody

3329P

Effect of lactobacillus acidophilus fermented milk on Escherichia coli O157:H7 infection in mice

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is a highly infectious pathogen which causes gastrointestinal illness with potentially serious consequences in human world wild. Recent reports have focused on novel biotherapeutic agents including

probiotics. The aim of this investigation was to determine effect of consumption of fermented milk by lactobacillus acidophilus **as** a probiotic on enterohemorrhagic *Escherichia coli* O157:H7 infection in mice. **Methods**: In this study 45 mice of 6-8 weeks old were randomly divided into 5 groups, each containing 9 mice. These groups consisted of control group (A), infected group (B), non-infected group (C), pre-infected group (D) and post-infected group (E). Each of the mice in groups (B, D) and E received 1.5×10^8 CFU/ml of *E. coli* O157: H7 through intra gastric tube (gavage). *lactobacillus acidophilus* fermented milk were fed 0.5ml daily for 14 days in Group (C) mice, 0.5ml daily for 7 days after infection in Group (D) mice and 0.5ml daily for 7 days in Group (E) mice prior to infection. Fecal *E. coli* O157:H7 counts were determined from three mice per group on days 1, 3,5,7,9. MacConkey sorbitol agar was utilized for identification of *E. coli* O157:H7. Specific antiserum against *E. coli* O157 was used in confirmation of the diagnosis related to *E. coli* O157. Rate of food and water uptake were measured daily. **Results**: The evidence of *E. coli* O157:H7 in groups (A) and (C) was not observed. Statistical analysis demonstrated the meaningful differences between the group (E), groups (B) and (D) ($p<0.01$). **Conclusion**: Our result suggests that consumption of fermented milk by *lactobacillus acidophilus* can reduce severity and duration of infection. Consequently, this consumption may have a considerable relation with enhancing immunological response against the pathogen.

Keywords: Escherichia coli O157:H7, lactobacillus acidophilus, Fermented milk, Infection, Mice

2354P

Association of HLA-G polymorphisms with Gastric Adenocarcinoma risk and clinical outcome

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Background: Human leukocyte antigen-G (HLA-G) plays an important role in tumor cell escape and HLA-G polymorphisms might service as a potential risk factor for clinical outcomes in GAC (Gastric Adenocarcinoma). We investigated the association between HLA-G polymorphisms as well as soluble HLA-G level and accordance of GAC. **Methods:** This case-control study included 100 GAC and 102 unrelated Iranian individual's samples as a control. The clinical stages ranged from I to IV. PCR-RFLP method was used for amplification of the HLA-G gene. Concentrations of sHLA-G in serum were determined with the sHLA-G-specific enzyme linked immunosorbent assay (ELISA) kit. **Result:** The G*01041 and G*01012 alleles were the predominant alleles in GAC patients and healthy controls. The G*01013 and G*01018 allele distribution is significantly higher among controls comparing to cases and seems to have protective effect (*P* value=0.026and0.007 respectively). There is a substantial differences in G*01012/G*01041 genotype frequencies between cases and controls (OR=2.8, *P* value < 0.001). The G*01013/G*01041 and G*01012/G*01018 genotypes frequency are higher among controls in comparison to patients (*P* value=0.028 and 0.007 respectively).

Conclusion: The polymorphisms in HLA-G could affect GAC induction and its outcome. Also, increased sHLA-G levels in serum might be a useful biomarker for diagnosis.
Keywords: HLA-G, Polymorphism, Gastric adenocarcinoma, Iranian population

2689P

Effect of long term treatment of morphine on enzymes, oxidative stress indices and antioxidant status in male rat liver

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Background: In this study, a biochemical change due to chronic usage of morphine in liver was assessed in rats. **Methods:** Twenty male Wistar rats (180–220 g) were included and divided into two groups. Normal saline (1 ml) was given intraperitoneally as placebo in the control group (n = 10). Morphine group (n = 10) received morphine intraperitoneally at a dose of 4, 8, 10 mg/kg/day in the first, second and the third ten days of the study, respectively. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) levels and liver malondialdehyde (MDA) and reduced glutathione (GSH) levels as well as activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-s-transfrase (GST) were measured in the liver. **Results:** Serum ALT, AST and LDH levels were significantly higher in morphine group compared to the control group. The mean liver MDA level was significantly higher in morphine group compared to control group ($P < 0.05$). The GSH level as well as the activities of CAT, SOD and GST was significantly lower in morphine group compared to control group ($P < 0.01$). **Conclusion:** Our findings pointed out the risk of hepatic damage due to long term use of morphine via disturbance oxidant-antioxidant balance. Although morphine is reported to be effective in pain management, their toxic effects should be kept in mind during chronic usage.
Keywords: Morphine, Oxidative stress indices, liver

2606P

Detection of oipA using PCR method can help to the diagnosis of Helicobacter pylori in the dyspeptic patients

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Background: Helicobacter pylori has been strongly associated with chronic gastritis, gastric

and duodenal ulcers, and it is a important risk factor for gastric cancer. Major virulence factors of H. pylori have been described: the cytotoxin-associated gene product (CagA) and the adhesion proteins BabA2, iceA1, iceA2 and the secretory protein, OipA. H.pylori culture is difficult and CLO test gives various false positive and false negative results. Availability of PCR method for detection of bacterial DNA in biopsy specimens using specific primers implies that detection of one frequent virulence factor can help to the diagnosis H.Pylori. Because of many reports about geographic diversity of the prevalence of H. pylori virulence factors, the second aim of this work was to find their frequency in dyspeptic patients of Shahrekord area of Iran. **Methods:** Gastric biopsy specimens were taken from 438 patients with gastroduodenal problems during endoscopy. Firstly CLO test and PCR using housekeeping genes, glmM and16srRNA was done. After that, samples that were positive at least for two of mentioned testes were selected and PCR analysis of virulence factors was performed. **Results:** In this study 189 patients were infected with strains of H.Pylori that at least in two mentioned tests (CLO test, glmM and 16srRNA) were positive. Further PCR analysis of virulence factors showed that the frequency of oipA, iceA1, iceA2, cagA and babA2 were 93.7%, 83.1%, 69.8%, 57.7 % and 38.6%, respectively. **Conclusion:** In conclusion our results indicated that the oipA virulence factor has most association with CLO test and PCR analysis of H. Pylori housekeeping genes. Therefore oipA can be used as an appropriate auxiliary marker in the molecular diagnosis of H.Pylori infection.

Keywords: Helicobacter pylori, Diagnosis, virulence factor, PCR, oipA

2608P

Comparison of PCR method and RUT test for Detection of Helicobacter pylori Infection in Gastric Tissue

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Background and Aim: Culture has been for long the method of choice to detect infectious agents. However Helicobacter pylori (H.pylori) is a fastidious bacteria and growing slowly that its diagnosis by this method may take several days. This study was done to comparatively evaluate PCR and the rapid urease test in order to determine which of target housekeeping genes (glmM and 16SrRNA gene) was more appropriate in the diagnosis of Helicobacter pylori (H pylori) infection. **Methods:** The specimens used for this study were gastric biopsy samples which were collected from 438 patients undergoing upper gastroduodenal endoscopy for various dyspeptic symptoms. CLO test was compared with the PCR method for detection of selected housekeeping genes of H.Pylori, glmM and 16srRNA. **Results:** Totally 33.78% of the biopsies were positive for H pylori using both CLO test and PCR method for glmM and 16srRNA reference genes. 43.1% of the biopsies were negative for H pylori in all three mentioned methods. 193 of the 438 study subjects (43.6%) were found positive for CLO test.

glmM and 16srRNA were positive in 48.4% and 38.3% of all specimens respectively. In positive CLO test specimens, only 156 and 148 subjects were positive for glmM and 16srRNA gene. Of the 245 negative CLO test subjects, 56 (22%) were positive with glmM gene and 20 (16%) were positive for 16srRNA gene. CLO test gave 7.9% false positive (glmM and 16srRNA Negative) and 4.5% false negative (glmM and 16srRNA Positive) results. **Conclusion:** Our results indicated that the CLO test gave various false positive andfalse negative results and gained poorer performance than PCR for detection of H.pylori infection in biopsy of dyspeptic Patients. This shows that in addition to the CLO test, PCR method using specific primers sets of both glmM and 16srRNA is useful for correctly diagnosis of H.pylori infections in laboratory.

Keywords: Helicobacter pylori, CLO test, 16srRNA gene, glmM gene

2624P

Prevalence of *Helicobacter pylori* infection among patients with β-thalassemia major in Hamedan, Iran

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Background: *Helicobacter pylori* is one of the most common bacterial infections thatcausing abdominal pain chronic gastritis, and probably gastric carcinoma. Patients with β-thalassemia major are in greater risk for infectious diseases due to multiple causative factors and infections constitute the second most common cause of mortality in thalassemia. β -thalassemia major is characterized by a hereditary defect in the synthesis of β-chains of hemoglobin leading to ineffective erythropoiesis. Here we decided to determine the frequency of anti-H.pylori class IgG in patients suffered from thalassemia. **Method**: In this study to determine the prevalence of *H. pylori* infection among patientswith β-thalassemiaof Charity Foundation for Special Diseases, 59thalassemicpatients (27 F, 32 M, mean age: 25.5 ± 15.5years) and for the presence of antibodies in serum by ELISA method for anti-H.pyloriIgG classes detection 50 sex- and age-matched controls were assessed (Pishtazteb-*H. pylori* IgG Antibodies). **Results:** the results showed significant (P<0.001) difference between the anti-H pylori class IgG in case (56.5%) and control (38.7%) groups. **Conclusion:** The rate of anti-H.P IgGwas1.459times higher than control group which is significant. The overall seropositivity did not differ considering gender and age among thalassaemic patients and control group in Charity Foundation for Special Diseases Hamedan.

Keywords: *Helicobacter pylori*, β-thalassaemia major, ELISA method, Hamedan.

2716P

Toxoplasma gondii may have a protective role in the etiopathogenesis of celiac disease

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Background: *Toxoplasma gondii* infection is commonly detected using the immunoglobulin IgG and IgM antibodies in different populations. This parasite has been implicated in the pathogenesis of some autoimmune diseases such as celiac disease (CD). In the present study we examine the association between serological evidence of past infection with *Toxoplasma gondii* in celiac disease patients compared with the controls. **Methods**: In this case-control study, sera originating from 150 healthy subjects and 150 patients diagnosed with CD were analyzed for the presence of antibodies specific for *Toxoplasma gondii* during 2013. After recording the information by questionnaire and preparation of samples, IgG and IgM-*Toxoplasma* were measured by ELISA for detecting the total antibody against *Toxoplasma gondii* and titers above 10 were considered positive. **Results:** High levels of IgG antibodies against *T. gondii* were found in the sera of control group than in the CD group (52.6% vs. 39.4%, P = 0.02) approaching statistical significance. On the other hand, the prevalence of IgM antibodies in the control group compare to CD group was not statistically significant differences (1.4% vs. 0.65%, P = 0.65). **Conclusion:** Our results imply that Toxoplasmosis may generate an immunological environment that disfavors future appearance of certain autoimmune conditions such as celiac disease. About one-third of cases and controls were seropositive and because of the importance of toxoplasmosis, health education is necessary for prevention of this disorder.

Keywords: *T.gondii*, Celiac disease, IgG, IgM

2715P

The value of HLA typing in the diagnosis of Celiac disease in high risk population

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Background: Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. CD predisposing genes are the HLADQ2 and DQ8. The majority of celiac patients have HLA DQ2 or HLA DQ8 genotypes located on chromosome 6, which account for 40% of the total genetic predisposition to CD. Moreover, HLA DQ2 and DQ8 are expressed in 30% of the general population, suggesting the presence of additional factors for CD development. One such factor is *CTLA-4* gene polymorphism, a non HLA gene thought to regulate T-cell immune function. As we have similar genetic background to Western, CD may strongly associated with HLA DQ2 (DQA1*0501 and DQB1*0201) in Iran. Therefore, HLA DQ8 (DQA1*0301 and DQB1*0302) is less strongly associated with CD. Around 40% of the celiac patients are in atypical form and may misdiagnose from other gastrointestinal (GI) disorders. No data are available on the frequency of HLA related CD predisposing genes in Iran. One way to optimize the efficacy of screening would be by using HLA typing as a high-sensitivity rule-out test when there is a high suspicion of CD and to use serologic testing a high-specificity rule-in test when the probability is low. Relying on serology alone might result in overlooking those patients with negative serology even when

the suspicion is low. Perhaps performing HLA typing in seronegatives would give some more degree of reassurance in ruling it out.

Keywords: Celiac disease, HLA typing, Iran

3113P

The relation of ABO blood group phenotypes and gender withseverityof Helicobacter pylori infection

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Background: Helicobacter pylori infection is currently endemic worldwide health problem. The infection causes chronic gastritis, peptic ulcer, and gastric adenocarcinoma. It is clear that blood group (BG) antigens are important to the development of intestinal disorders.Thisstudy determines the relationship between H.pylori and ABO blood group, sex, and density of bacteria. **Methods**: The study included 258patients (18-80 years) with dyspepsia symptoms referred to hajar hospital in shahrekord. Demographic data were recorded. Patients were checked by 16sRNA gene polymerase chain reaction for **H. pylori**. Severity of H.pylori infection graded according to the number of HP bacteria counted in light microscopy by (x1000 magnified). Blood groups was detected by a standard hemagglutination test. **Result**: we observed 60.46% of patients (156 of 258) were positive for **H. pylori**, and 39.54% were negative (102of 258). In the positive patients, 39.74%were male and 60.26% were female; also in the negative patients, 47.05% were male and 52.95% were female. The frequency of the ABO blood groups among positive patients was (A = 32.05%, B = 19.23%, AB = 7.06%, O = 41.66%) and was (A = 34.32%, B = 24.51%, AB = 7.84%, O = 33.33%) in negatives. Also our study showed the frequency of the ABO blood groups among sever infection was (A=18.2%, B=0%, AB=0%, O=81.8%). A significant difference ($P< 0.05$) was observed when we compared severity of infection with ABO blood group phenotypes. **Conclusion**: severity of H.pyloriinfection can be related by ABO blood group phenotypes.

Keywords: H.pylori, Blood groups, Gender, Severity of infection

Immunology of HIV/AIDS

Oral Presentations:

2236O

Constructing novel combinational mosaic-polytopic vaccineagainst Iranian isolates of HIV-1 belongs to 35AD subtypes

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Background: Completion genome project of Iranian drug resistance isolate of HIV-1 belongs to 35AD subtypes, opened new doors to designing novel vaccine based on this subtype. Here, we attempted to design vigorous and effective vaccine based on tow novel technology of mosaic and polytopic rational vaccine design for35AD subtype by wide array of immunoinformatic approaches. **Methods:** At first, all HIV protein sequence of Iranian 35AD subtypes (forgp160, RT, Nef, gag) retrieved from Databases and we made4 different datasets. After alignment and evaluation of mutational/conserve region in each dataset, and then phylogenetic and similarity matrix analysis, by different hybrid approaches sequences tested for T cell epitopes and their potency to attachment to HLA-A* A*0201 (A2) for constructing mosaic-polytopic vaccine. Selected epitopes attached to each other in rational patterns by linker and checked for proteasomal cleavage sites and next presentation on *0201 by docking method.we followed process of designing by evaluation of constructs primary structure, post translational modification, calculation of hydrophobic regions, reverse translation, codon optimization and open reading frame (ORF) checking and finally insertion of start/end codon and Kozaksequence. **Results:** Finally,1polytopicconstruct (gp_2RNp17) and 4 mosaic construct (each construct belong to one35ADprotein) were achieved. Checking of 5 constructs, revealed its reliability and efficacy for in-vitro producing and utilizing. **Conclusion:** AIDS still have no effective vaccine cause highly mutation of this virus, despite many clinical trials. Our designed vaccine can be used as both protective and therapeutic vaccine.

Keywords:HIV-1, 35AD subtypes, Mosaic, Polytopic, Vaccine

3011O

Study the effect of Naloxone-Alum adjuvant mixture on IL-17 cytokine in HIV-1 multi epitopic vaccine model

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Background: Cytokines have important role in the control of bacterial and viral infections as like HIV-1. Interleukin 17 which is secreted by Th17 is one of these cytokines with special role in controlling microbial infection.In the present study, adjuvant activity of Alum and Naloxone mixture on immune responses, especially IL-17 cytokine has been studied. **Materials and Methods:** Naloxone and Alum adjuvant was mixed with 10 µg of recombinant vaccine HIV-1-gag-pol-tat-env. Experimental groups consisting of inbred male Balb/c mice divided into six groups, were injected subcutaneouslyat the days 0, 14 and 28 with total volume of 200 µl. Two weeks after final injection, mouse spleen in sterile condition was removed and cell suspension was prepared. Lymphocyte proliferation response with Brdu test and cytokines IL-2, IL-4, IL-17 and INF-γ with using ELISA kit, total antibody and antibody isotypes IgG1 and IgG2a with ELISA test has been evaluated. **Results:** All results show that the mixture of Alum with Naloxone increased cellular immune parameters and specially rise of interleukin 17 that illustrates significant difference with other groups. **Conclusions:** It is seems that Alum and Naloxone mixture by affecting the Th17 pathway could control viral infections in which IL17 cytokine has critical role.

Keywords: Alum, HIV-1, Interleukin 17, Naloxone

3257O

Evaluation of RT-PCR method to identification of fluconazole resistance gene, ERG11 in native clinical *Candida albicans* isolates from AIDS patients

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Background: *Candida albicans*is pathogenic yeast that causes oral, vaginal and systemic infections. These infections are usually treated with antifungal drugs, such as fluconazole. Azole-resistant strains of *C. albicans*are an increasing problem in human immunodeficiency virus-infected patients. In this study attempt to identify the fluconazole –resistance species of the domestic *candida albicans* isolates from oral candidiasis of ADIS patients using phenotypic and RT-PCR methods with an emphasis on resistance gene ERG11. **Methods:** Evaluation of initial fluconazole susceptibility of *C. albicans* isolates was performed according to CLSI method by broth macrodilution and disk diffusion techniques. RNA was isolated using the glass bead method and synthesized cDNA. RT-PCR was used to analyze evaluation ofERG11 resistance gene. Following the electrophoresis of the PCR products, the patterns of the resulted bands were compared with those of the standard fluconazole resistance strains. **Results:** The results of drug sensitivity test of 66 strains of *C. albicans* isolated from AIDS patients to fluconazole showed, 62.6% were susceptible to fluconazole,8.6% were Susceptible-Dose Dependent(SDD) and 28.7% were resistant. In RT-PCR analysis, positive reaction for ERG11

gene were 9% of patients respectively. The exponential amplification via RT-PCR provides for a highly sensitive technique in which a very low copy number of RNA molecules can be detected. **Conclusion:** Recently Molecular techniques have attracted interest due to their high sensitivity. In this study RT-PCR analysis of the samples showed that 6 isolates had overexpression of ERG11 gene. Though the use of phenotypic methods like disk diffusion which has lower costs together with genotypic methods like RT-PCR which provide the possibility of studying the mechanism of drug resistance and genes involved, recommended.
Keywords: *Candida albicans*, ERG11, RT-PCR, AIDS

Poster Presentations:

1574P

Peptidemimetic designbased on sifuvirtide-a novel HIV-1 fusion inhibitor peptide- using a combinatorial in silico approach

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Background: Human immunodeficiency virus type I (HIV-1), the major cause of AIDS in human, is the most challenging infection to be dealt with. The need for development of new antiviral agent against this virus is stillrising. Peptidic therapeutics is increasingly become popular and has already entered the AIDS treatment process. However, peptides have some important defects such as their short half- life, potential immunogenicity, high molecular weight, having high costs as a therapeutic and most importantly being exposed by enzymes like proteases. Therefore, to resolve these disadvantages, designing peptidomimetics of such effective peptides can be beneficial. **Methods:** Anti HIV-1 peptidomimetics were designed based on the structure of Sifuvirtide (SFT) which is a new-generation fusion inhibitor peptide effective against wild-type and resistant variants of HIV-1. They were found on the basis of the knowledge about critical locations on SFT needed for its biologic interaction with gp41. The six peptidomimetics were then docked with their target molecule gp41 to elucidate the most potential inhibitory peptidomimetics.**Results:** Six potential peptidomimetics were designed for critical locations of SFT in both C and N termini of the peptide. Three of the six peptidomimetics were determined to be the most appropriate ones due to their molecular docking scores.**Conclusion:** The present study is done to reach anti HIV-1 non-peptidic molecules in order to eliminate the disadvantages of the peptide therapeutics. Such knowledge-based *in silico* combinatorial approach seems to be useful in design of other bioactive agents.
Keywords: Anti HIV-1 agent, Sifuvirtide, Drug design, Peptidomimetics

1864P

The prevalence of tuberculosis and the related CD4+ Profile in HIV co-infected subjects referred to Shiraz HIV/AIDS research center

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Background: Tuberculosis is one of the opportunistic infections among patients living with HIV/AIDS and associated with the most common cause of AIDS-related death. The worldwide impact of this co-infection is one of the major public health concerns. The aim of this study was to determine of the presence of co-infecting tuberculosis and probable correlation with CD4+ levels of HIV patients. **Method:** This cross-sectional study conducted from 2010 to 2013 on 824 HIV patients who referred to Shiraz HIV/AIDS research center. Positive HIV patients were confirmed by ELISA and Western Blot tests. Clinical finding, chest X-Ray and culture positive sputum were used to identify tuberculosis. CD4+ lymphocyte counts were also determined. **Results**: A total of 824 HIV positive patients participated. Of them fifty nine (7.2%) were co-infected with tubercle bacillus (TB). From overall tuberculosis infected patients six cases show multidrug-resistant (MDR) with the mean of 163± 166 Cells/mm3 CD4+ lymphocyte count. 22/184 (12.0%) subjects with CD4+ cell counts of ≤200/µl and 37/640 subjects (5.8%) with CD4+ cell counts of >200 /µl were co-infected with TB. The mean CD4+ lymphocyte count of HIV mono-infected and co-infected participants were 374± 237 Cells/mm3 and 325± 307 Cells/mm3 respectively. There were significant relationship between TB infected and CD4+ counts of ≤200/µl (P = 0.006) and prison history (P = 0.03).**Conclusion:** Findings of the current study highlight the importance of CD4+ lymphocyte count monitoring as an early alarm factor for TB co-infection in HIV/AIDS positive patients and helping their treatment to be initiated. quickly.

Keywords: Tuberculosis, HIV, AIDS,CD4+ lymphocyte

1876P

Frequency of hepatitis B and hepatitis C co-infection in HIVpatientsreferred to Shiraz HIV/AIDS Research Center

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Background: Sexually transmitted diseases (STDs) are a major public health concern especially viral related infections. Because of shared routes of transmission co-infection with HBV and HCV are common in HIV patients with variable rate from worldwide. This study carried out to determine the frequency of HBV and HCV co-infection in HIV positive patients who referred to Shiraz HIV/AIDS Research Center.**Method:** 742 HIV infected patients who referred to Shiraz HIV/AIDS Research Center in a cross-sectional study were screened for the presence of co-infection with HBV (HBsAg) and HCV (anti-HCV) by ELISA kits. Positive HIV patients were confirmed by ELISA and Western Blot tests.**Results**: From overall positive HIV infected participated, in 45 (6.1%) patients HBsAg was positive and anti-HCV was positive

in 450 (60.7%) cases. HBV and HCV co-infection was observed in 30 (4.0%) patients. The majority of HBV-HIV and HCV-HIV co-infections were seen among intravenous drug users with 66.7% and 85.7% respectively. Intravenous drug usage is also were mainly infection way 25/30 (83.3%) in HBV-HCV co-infection patients. From a total of HBV-HCV co-infection subjects 10/30 (33.3%) had no treated history with HAART.**Conclusion:** Intravenous drug usage it seems to be a major transmission routes in our area which are the common between HBV, HVC and HIV. Also rate of HBV-HCV co-infected among patients who had no HAART activation recommendation for management and education of HIV patients for risks of co-infection disease and received treatment programs.

Keywords: HIV, AIDS, Hepatitis B, Hepatitis C, HAART

1948P

Molecular analysis of HIV-1 in infants born of HIV-infected mothers

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Background: HIV pandemic is one of the most serious health crises the world faces today. children acquire infection through mother-to-child transmission (MTCT) either during pregnancy, delivery, or by breast-feeding. There are certain drugs decrease the chance of transmission, however, in many developing countries and the poor, such drugs are not available. The purpose of this study was to assess HIV infection in infants who were born from infected mothers. **Methods:** The samples were taken from 88 infants born to infected mothers. Viral infections in newborn children were evaluated by multiplex-nested-RT-PCR protocol and ELISA. **Results:** In the first month of birth, infection status was assessed in 88 children. Initial evaluation of the ELISA technique revealed, 22(25%) of whom are HIV-positive. While evaluating the PCR technique showed, of these 22 cases, only 12 people are infected with HIV and the other 66 cases, 18 cases were positive. **Discussion:** This study showed that 30 (34.09%) of 88 infants are infected with HIV. Mothers who were treated with medication, their children were healthy. It should be noted, however, after one year the number of children who were healthy in the first experiment, in the second experiment were positive.

Keywords: HIV, Mother-to-child transmission, PCR

2741P

The Correlation between HBV, HCV, HIV and injection drug users from Methadone Clinic of Ebn-e- Sina and Hejazi Hospitals

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Backgruond: Narcotic addiction as a serious health problem in communities has economical and social effects as well as health and hygienic complications.Viral infection such as B and

C hepatitis and HIV may be transmitted by addicted persons. Identification and education of these patients is very important and this survey was carried out for determination of the correlation between these infection and injection drug users. **Method:** this case-control study was carried out on 130 self referred IDU who comes to Ebnesina and Hejazi Methadone maintenance therapy (MMT) clinic in Mashhad. After taken 5cc of serum samples, they checked for HIV, B and C hepatitis by Elisa and Western blot test. And a question was filled about the personal information of participents. Data was analysed by spss software and fisher. **Results:** The results of this study suggest that the mean of addiction participents was 40/3 years. That 22 of them was women and 108 of them was men. That 8 of them (6/2%) HBV positive, 14 of them (10/8%) HCV positive and 1 person (0/8%) was HIV positive and from 130 persons who was the control group with 45.8 mean age years that 58 of them was women, 72 of them was men: Only 3 persons (2/3%) HBV positive , 1 person HCV positive (0/8%) and any of them HIV positive. **Conclusion:** the finding of this study revealed that there was a high rate of viral infection in IDU.

Keywords: Injection Drug users, Hepatitis B, C, HIV, Mashhad

Immunology of Infectious Diseases

Oral Presentations:

3319O

***Aspergillus spp*. germ tubes induce stronger cytokine responses in human bronchial epithelial cells in comparison with spores**

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Background: Aspergillus spp are ubiquitous saprophytic fungi that cause a variety of diseases, ranging from hypersensitivity reactions to flu-like pneumonia and life-threatening invasive aspergillosis. As the lung is the primary site of initial infection with airborne conidia, we investigated the innate immune responses of bronchial epithelial cells against different forms of *Aspergillus spp*. **Methods:** Normal human bronchial epithelial (NHBE) cells (Clonetics) were cultured in bronchial epithelial growth medium. Standard species of both *Aspergillus fumigatus* and *Aspergillus flavus* were cultured on S at 25◦C, Both spores and germ tubes were killed by 0.01% thimerosal. RNA was extracted with TRIzol (Invitrogen), and reverse transcription was carried out with Gene Amp RNA PCR (Applied Biosystems). Levels of secreted cytokines (IL6, IL8 and TNFa) into the cell-free supernatant (after 24h treatment) were measured using ELISA kits (R&D system, M N). **Results:** Analysis by real-time PCR showed that inflammatory cytokines such as IL-8 and IL-6 as well as the proinflammatory protease, caspase-5 were strongly upregulated by both treatments in a dose-dependent manner. Consistently, germ tubes induced a stronger response than spores. TNF-alpha and beta-2-defensin were induced by high a concentration of germ tubes, but not by spores. IL1 pretreatment highly induced the expression of both beta-2-defensin, IL8 and IL12. **Conclusion:** Our results show that germ tubes of *Aspergillus fumigatus* and *flavus* are potent inducers of innate immune responses in human airway cells. Considering the presence of Aspergillus spores in the air, differentiation between transient spore contact and invasion, as represented by germ tube formation, is important in order to determine proper immunological response.

Keywords: *Aspergillus spp*, Bronchial epithelial cell, Beta-2-defensin, Cytokine, Hypersensitivity.

3299O

Ectopic expression of micro-RNA-1, 21 and 125a in the peripheral blood immune cells is associated with chronic HBV infection

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Background: Micro-RNAs (miRNAs) play key roles in regulating genes of the immune system. The aim of this study was to examine the expression of miR-1, 21 and 125a in the immune cells taken from the peripheral blood of patients suffering from chronic HBV infection (CHB). **Methods:** This cross-sectional study was performed on 60 CHB patients and 60 healthy controls and expression of miR-1, 21 and 125a were evaluated using Real-Time PCR technique. **Results:** Our results showed that expression of miR-1, 21 and 125a was significantly increased in CHB patients in comparison to healthy controls. **Conclusion:** Based on our results it may be concluded that increased expression of miR-1, 21 and 125a is significantly associated with CHB and may play key roles in induction of impaired immune responses in CHB patients.

Keywords: Chronic hepatitis B infection, miR-1, miR-21, miR-125a, HBV-DNA

3188O

Investigation of the immunomodulatory effects of opioid receptors on munopathogenesis of Respiratory Syncytial Virus in a mice model

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Background: The robust pulmonary inflammatory response observed in severe respiratory syncytial virus (RSV) infection is associated with a misdirected immune response. It has been shown that opioid receptors directly or indirectly leading to substantial changes in immune response during inflammation. Nalmefene,an opioid receptor blockerhas high affinity for all three opioid receptors therefore simultaneous blocking of opioid receptors allowedus to investigate the effect of opioid receptors on RSV immunopathogenesis. **Method:** Female BALB/c mice were infected with RSV and injected daily with 1mg/kg nalmefene.At day 5 after infection mice were sacrificed.The challenge stock was obtained by strain RSV-A2 propagatedon a Hep-2 cell and purification using PEG precipitation. Bronchoalveolar

lavage fluid was collected, and differential cell counts were performed. Viral titers were determined by real-time PCR.Cytokines and chemokineswere determined using ELISA kits. The histology slides were prepared from thesame lungs used for the BAL fluidand analyzed. **Results:**Nalmefenetreatment significantly increased viral titers and weight loss and enhanced cellular influx in the bronchoalveolar space as well as histopoathology scores in all RSV infected mice on day 5 post-infection.Nalmefenealso significantly increased BALlevels of IFN-γ, IL-6, IL10, MIP1-α, MCP1 and MCP3. **Conclusion:** This study expands our knowledge of the antiviral activity of opioid receptors. We have shown that opioid receptors are required to control viral replication in mucosa of the respiratory tract, and thereby decrease immune-mediated disease. This experimental study provides the first evidence that opioid receptor signaling determines the outcome of patients with respiratory viral disease.

Keywords: Immunomodulation, Nalmefene, Opioid receptors, Respiratorysyncytial virus, Viral replication

2286O

Decreased expressions of STING but not IRF3 molecules in chronic HBV infected patients

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Background: The stimulator of interferon genes (STING) induce interferon regulatory factor 3 (IRF3) activation in response to intracellular viral double strand (ds) DNA. The aim of this study was to evaluate mRNA levels of STING and its downstream transcription factor, IRF3, in the isolated peripheral blood mononuclear cell (PBMCs) of patients with chronic HBV (CHB) infection. **Methods:** This study was performed on 60 healthy controls and 60 CHB patients. The mRNA levels of STING and IRF3 were determined using Real-Time polymerase chain reaction (PCR) techniques. **Results:** The results revealed that mRNA levels of STING were significantly decreased in CHB patients in comparison to healthy controls. Our results also revealed that expression levels of IRF3 were not differ between CHB patients and healthy controls. **Conclusion:** In the present study, we found that CHB patients were unable to express appropriate levels of STING. Thus it may be caused to impair HBV-DNA recognition and subsequently disruption of immune responses. These results suggest that a plausible mechanism which may partially define the fact that immune responses are impaired in CHB patients.

Keywords: Chronic HBV infection, STING and IRF3

2012O

Trend of declining of anti-HBs antibody among vaccinated population: 18 years after implementation of Iranian national hepatitis B vaccination program

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Background: The duration of protection following full series vaccination against hepatitis B isn't well known in children and adolescents. It has been shown that the level of anti-hepatitis B surface antigen antibodies (anti_iHBsAbs) declines over years after vaccination. The aim of this study was to evaluate the long-term immunity against hepatitis B virus infection among children and adolescents who had received a complete hepatitis B vaccination series during infancy in national program of hepatitis B vaccination in Iran.**Methods:** In a cross-sectional study, the – anti-HbsAb levels of 840 vaccinated children and adolescents were determined by enzyme-linked immunosorbent assay. **Results:** Hepatitis B seroprotection rates (anti HbsAb ≥ 10 IU/L) among vaccinated children and adolescents aged 1 and 18 years were 90% and 48.9%, respectively. The declining trend of geometric mean titer of anti-HbsAb levels was observed as changed from 272.3 IU/L to 94.1 IU/L in 1 and 18-year-old population, respectively. A significant negative correlation was found between age and anti-HbsAb levels (r = - 0.220, P = 0.0001). **Conclusions:** The results showed a declining trend in anti-HbsAb titers over the time after vaccination against hepatitis B virus in our region. Further studies are warranted to establish the need for a booster dose in cases that are at risk of hepatitis B virus infection.

Keywords: Hepatitis B, Vaccination, Anti-HBs antibody, Sero-conversion, Ahvaz

1937O

Neutralizing single chain antibodies against Influenza virus

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Background: Influenza epidemics have been recognized as a major cause of morbidity and increased mortality. Hemagglutinin (HA) is responsible for attachment of the virus to specific receptors on the host cell surface and has been the main target of influenza virusneutralizing antibodies. Single-chain fragment variable antibodies (scFv) are useful agents for viral immunotherapy due to their small size and high affinity properties. In this study a neutralizing scFv was selected against HA of H1N1 influenza virus.**Method:** A conserved sequence of HA was applied as an epitope and specific scFvs were selected. Panning procedure was carried out to select the phage particles bearing anti-epitope scFvs. The peptide was coated on immunotube. The phage rescue supernatant was added and eluted with Ecoli TG1. After four rounds of panning, specific clones were selected using PCR and DNA fingerprinting. The neutralizing effects of the clones were evaluated by plaque reduction assay.**Results:**Two specific scFvs with frequencies 75% and 20% were selected against the conserved sequence of HA. The neutralising effect more than 90% was obtained for one of the clones.**Conclusion:**

A new prophylaxy and treatment strategy is needed to prevent from influenza epidemic and pandemic spread. Neutalizing scFvs can play a crucial role in this regard.In this study a successful panning process was performed and two specific scFvs with frequencies 75% and 20% were selected.The neutralizing effect of more than 90% of one of the clones offers the usefulness of these recombinant antibodies in the treatment or prevention of influenza in high risk patients.

Keywords: Single chain antibodies, Hemagglutinin, Neutralization, Influenza virus, Selection

1866O

Haemolytic Anaemia in ThromboangiitisObliterans: Is Infection or Autoimmunity to Blame?

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Background: The aetiology and pathophysiology of ThromboangiitisObliterans (TAO) remains puzzling. Plasma samples from many TAO patients show evidence of anaemia and macroscopic haemolysis, which may indicate haemolytic anaemia. This possibility its potential causes were investigated. **Methods:** An Indirect Coombs test, and tests for lactate dehydrogenize (LDH), D-aspartate aminotransferase (AST), D-alanine aminotransferase (ALT), and complements system proteins C3 and C4 were evaluated in 29 plasma samples, which had been banked between 2010 and 2012 from patients diagnosed with TAO. **Results:** The indirect Coombs test was positive in 23 out of 29 samples (79.2%). The mean documented haemoglobin of the patients was 12.28±0.6 g/dl. The LDH level was high in all samples, with a mean of 2552±315 u/l. High levels of AST (mean: 67±7 u/l), normal levels of ALT (26±3 u/l), high levels of C3 (2.08±0.7g/l) and normal levels of C4 (0.38±0.11g/l) were also observed. **Conclusion:** These results indicate that many TAO patients have haemolytic anaemia. However, in light of the clinical manifestation of TAO and the relatively high levels of C3 and C4 in the samples studied, haemolytic anaemia in TAO is likely to be due to the presence of infectious pathogens rather than autoimmune mechanisms.

Keywords: Buerger's disease, Thromboangitis Obliterans, Hemolytic anemia

1753O

The impact of interferon-alpha treatment on clinical and immunovirological aspects of HTLV-1-associated myelopathy in northeast of Iran

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Background: Human T-lymphotropic virus type 1 (HTLV-1)-associatedmyelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic inflammatory myelopathy. The pathophysiology of HAM/TSP is not yet fully understood; therefore, effective therapy remains a challenging issue. This study was designed to evaluate the efficacy of interferonalpha (IFN-α) in HAM/TSP patients in the Northeast of Iran.**Methods:** Forty-nine patients with a definite diagnosis of HAM/TSP were enrolled in this clinical trial. For six months, the patients received three million international units of subcutaneous IFN-α-2b per each injection. The dose regimen was daily injection for the first month, three times administration per week for the months 2 and 3, twice weekly injection for the months 4 and 5 and weekly injection for the sixth month. The clinical and laboratory responses were evaluated based on neurologic examinations and immunovirological markers.**Results:** IFN-α had significant but temporary effect on the motor and urinary functions of the patients. Comparing to the baseline values, proviral load was significantly decreased one month after treatment in responders (495.20±306.87 to 262.69±219.24 p=0.02) and non-responders (624.86±261.90 to 428.28±259.88 p=0.03). Anti-HTLV-1 antibody titers were significantly decreased among responders (1152.1±200.5 to 511.6±98.2 p=0.009) and non-responders (1280.1±368.1 to 537.6±187 p=0.007). Flow cytometry showed no significant changes in CD4, CD8, CD4CD25 and CD16CD56 counts with IFN-α. **Conclusion:**The positive impact of IFN-α was observed during the treatment period with significant effects on some clinical aspects of HAM/TSP.

Keywords: IFN-α, HTLV-1-associated myelopathy,

1830O

Comparison of mononuclear proliferation response to specific *S.aureus* antigen in nasal carriers and noncarries

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Background: Staphylococcus aureus is a potentially pathogenic bacterium that causes a broad spectrum of diseases. Approximately 30% of the healthy populations carry S. aureus in the nose in form permanent and transient carriers. About 80% of invasive nosocomial S. aureus infections are of endogenous origin in nasal carriers. The aim of present study is to investigate the Proliferation of peripheral blood mononuclear cell in S.aureus carrier and noncarrier groups. **Method:**Proliferation was checked by the MTT assay method. A total of 3 × 103 cells in 200µl RPMI 1640 supplemented with 10% FBS were stimulated with 1µg/

mL *S.aureus* specific antigen and PHA in control cells. Next, MTT were added to the cells, followed by incubation, and finally 200µl of DMSO were added. The optical density (OD) values of stimulated and non-stimulated cells were measured at 540 nm.All experiments were performed in triplicates. Proliferation responses for the MTT assay were expressed in terms of the mean stimulation index (SI) and obtained by dividing the OD values of stimulated cells by the respective OD values of the non-stimulated ones. **Results:** Analysis of our result by nonparametric test demonstrated that rate of proliferation in *S.aureus* nasal carriers compare to noncarrier group was significantly reduced (P< 0.001). **Conclusion:** Although previous studies have shown that innate immunity could be suppressed by nasal carriage, our result indicating that cell mediated immunity also might be affected during the nasal carriage of *S.aureus*. This finding can explain the high rate of endogenous infection in nasal carriers.

Keywords: Mononuclear proliferation, S.aureus, Nasal carrier

2009O

High circulating levels of anti-phosphatidylserine antibody in peptic ulcer patients infected with *CagA*-positive strains of H.pylori

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Background: The C*agA*-positive strains of *H. pylori* were associatedwith the higher risk of peptic ulcer (PU) diseases. The aim of this study was to evaluate the serum concentrations of anti-phosphatidylserine (anti-PS) antibodies in *H. pylori*-infected PU patients. **Methods:** Totally, 100 *H. pylori*-infected PU patients, 65 *H. pylori*-infected AS carriers and 30 healthy *H. pylori*-negative subjects (as a control group) enrolled to study. Serum samples of participants tested for the levels of anti-PS by use of ELISA. **Results:** The mean serum levels of anti-PS antibodyin PU group (13.46 ± 2.90 RU/mL) was significantly higher than that observed in *H. pylori*-infected AS group (1.57 ± 0.38 RU/mL, P<0.001) and healthy uninfected control group (0.77 ± 0.32 RU/mL, P<0.001). No significant difference was observed for the mean serum levels of anti-PS antibody between AS group and uninfected control group. In PU group, the mean serum levels of anti-PS antibody was significantly higher in patients with positive test for anti-*CagA* antibody in comparison to patients with negative test for anti-*CagA* antibody. **Conclusions:**These results showed higher serum levels of anti-PS antibody in patients with PU disease. Clinical significance of the anti-PS antibody in *H. pylori*-infected PU patients can be consider in additional follow up studies.

Keywords: Helicobacter pylori, Peptic ulcer, Anti-phosphatidylserine antibody

1494O

Evaluation of NLRC4 and NLRP1, as inflammasomes, in chronic HBV infected patients

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Background: The Nucleotide-binding domain leucine repeats (NLRs) play crucial roles in recognition of different microbial components and induce appropriate immune responses by activation of cytokines. Current study was designed and aimed to examine the expression levels of NLRP1 and NLRC4, which are also considered as inflammasomes, in chronic HBV infected (CHB) patients.**Methods:** This cress sectional study was performed on 60 CHB patients and 60 healthy controls. The mRNA levels of NLRP1 and NLRC4 as well as HBV-DNA were evaluated using Real-Time PCR technique. Serum levels of HbeAg and HbsAg were also examined using ELISA technique.**Results:** The results revealed that expression levels of NLRP1 and NLRC4 were not significantly differ in PBMCs of CHB patients in comparison to healthy controls. The results showed that mRNA levels of NLRP1 were altered in CHB patients with various HBV-DNA copy numbers/mL. **Conclusions:** Based on the results presented here it seems that expression levels of NLRP1 and NLRC4 were not changed in CHB patients and may do not participate in impaired immune responses against HBV in the patients.

Keywords: Chronic HBV infection, NLRP1, NLRC4, PRR, NLRs and Inflammasome.

2290O

Evaluation of Foxp3 gene expression in HTLV-1 infected individuals

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Background: Human T-Lymphotropic Virus Type1 (HTLV-I) is an oncogenic human retrovirus that among the population infected with HTLV-1, only less than 5% develop several inflammatory disorders including the chronic inflammatory disease known as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). Because the immune response plays a major role in the control of HTLV-1 infection, it is important to study the possible role of immune regulation in the control of HTLV-1 infection. One of the major components of immune regulation is the regulatory T cell(Treg). Tregs are specialized subsets of CD4_ T cells that suppress effector T-cell responses in chronic disease, including retroviral infection.It

appears that the best current single marker of Tregs in CD4_ cells, is the forkhead transcription factor FoxP3, therefore, in the present study, we evaluate Foxp3 gene expression to determine the role of Tregs in HTLV-I infected individuals. **Methods:** In This study, 60 cases classified in three groups, including HAM/TSP patients (20 subjects), HTLV-1 carriers (20 subjects) and healthy people (20 subjects) were studied. Real-time PCR TaqMan method was designed and optimized for evaluation of Foxp3 human gene expression in baseline and in activated cells by PMA and Ionomycin. **Result:** The analysed data indicated a significant increasing in Foxp3 gene expression in HAM/TSP patients compared with carrier and healthy controls ($p<0.05$). **Conclusion:** These results might suggest that HTLV-1 infection is associated with abnormal expression of FoxP3 in circulating CD4_ cells. Further studies are needed to determine the role of this transcription factor in imminopathogenesis of HTLV-I-associated diseases.

Keywords: HTLV-1, HAM/TSP, Foxp3

1521O

A large-scale population based survey for HEV Spreading in a ilgrimage-tourism area, in Mashhad city in Iran

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Background: Hepatitis E Virus (HEV) infection is a significant public health concern and responsible for large outbreaks of acute hepatitis in poor sanitary and living conditions. To investigate the impact of population movements on virus spreading, a large-scale population-based survey was performed in a pilgrimage- tourism area, the great Mashhad, capital city of Khorasan province. **Methods:** A cross-sectional study was carried out among 1582 randomly selected individuals from general population of Mashhad, north east of Iran, between May to September 2009. Serum samples were tested for total anti-HEV antibody using a specific enzyme linked immunoassay (ELISA) kit. **Results:** The prevalence of HEV infection was 14.2% (225/1582) with a maximum of 25.5 % (14/55) in densely populated areas. The highest prevalence was observed in visitant areas ($\geq$ 20%) near the holly shrine with crowded hotels and inns. The differences between these areas and other districts were statistically significant ($P < 0.001$). The findings indicated that 13.2% (95/718) of males and 15.0% (130/864) of females were HEV positive; this difference is not significant. Seroprevalence increases with age rising, from 12.8% in subjects less than five years to 28.6% in individuals with more than 65 years old. Although, there were no meaningful differences between HEV seropositivity and socio-economic status, Illiterate individuals were significantly at higher risk for infection than educated persons ($P < 0.001$). **Conclusions:** These findings demonstrated that, high prevalence of HEV is related to populated district, which can reach to the highest rate in hotels and inns close to visitants. Traditional sanitation and water supplying systems are the second important factor for the virus transmission. Therefore, it can be concluded that such areas need efficient surveillance systems to prevent the spreading of infectious diseases.

Keywords: Hepatitis E Virus, Population, Viruses

1626O

Monoclonal antibodies to various epitopes of HBs antigen inhibit HBV infection

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Background: Antibodies against the "a" determinant of hepatitis B surface antigen (HBsAg) are able to neutralize circulating HBV particles and to prevent HBV infection. It has been proposed, that a single amino acid exchange may allow the virus to escape the immune response. We used a set of monoclonal antibodies (MAbs) to investigate whether a single mutation may account for virus escape from humoral immunity.**Methods:** Nine murine HBsAg specific MAbs were raised. Reactivity of all antibodies with 14 recombinant mutants of HBsAg was assessed by ELISA. HBV infection of HepaRG cells was used to evaluate viral neutralization capacity of MAbs *in vitro*. Viral infection markers, cccDNA and HBeAg, were analyzed by real time PCR and ELISA, respectively. **Results:** All MAbs were able to inhibit the establishment of HBV infection in a dose dependent fashion, but recognition of HBsAg variants varied. The MAbs were classified into 3 subgroups based on their pattern of reactivity to the HBsAg variants. Accordingly, three MAbs showed weak reactivity (<40%) to variants with mutations within the first loop of "a" determinant, five MAbs displayed negligible binding to variants with mutations within the second loop and one MAb lost its binding to variants having mutations in both loops of the "a" determinant.**Conclusion:**Our results indicate that antibodies against different epitopes of the "a" determinant of HBsAg are able to neutralize HBV. It seems that mutations within a single or a limited number of amino acids within this determinant can hardly result in viral escape. These results have important implications for the development of antibody-based therapies against HBV.

Keywords: Monoclonal antibodies, HepaRG cell, HbsAg, 'a' Determinant, HBV neutralization

1701O

The HCV NS3 protein longitude Adenoviral vector maintenance in liver by immune modulation through protease activity

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Background: HCV virus has the ability to overcome innate host antiviral responses partly by NS3 protein. Adenovirus induces innate immunity while employed in gene therapy approaches. The purpose of this study was to evaluate the effect of NS3 gene with and without protease activity on maintenance of adenovector in the mice liver. **Methods:** Four groups of mice with similar properties included in study. Plasmids containing NS3, partial NS3(TNS3) or GFP protein was administrated into mice by hydrodynamic injection then 24 hours later

adenovector expresssing luciferase delivered to liver using IV injection. Thereafter, 18 hours post injection total RNA extracted from liver and evaluated for CCl-5, IL-6, IL-1b and TGF-b expression by real time PCR rather than TGF-b ELISA in sera. Liver enzyme and histology performed to examine to extent of inflammatory response as well. Also to detect time of viral maintenance, DNA detection in mice groups evaluated in days 3, 7, 14 and 21 by hexon quantification using real time PCR. **Results:**Evaluation of viral maintenance results showed that adenovector genome was detectable up to 21 days for those groups received pNS3 and dexamethasone while by the day 7 in other groups virus was undetectable. Also in mice group injected by pNS3 the level of TGF-b was significantly higher in compare to partial-NS3 and pGFP plasmid. Furthermore expression level of inflammatory cytokine like IL-6 and CCL-5 were controlled by pNS3 plasmid using Real-Time PCR assessment. As TGF-B expression modulate the immune response it seems that over expression of TGF-b inside the liver rather than modulating the inflammatory response by full NS3 protein improve adenoviral vector persistent and this wasn't the case for partial NS3.**Conclusion:** According to our results it could be concluded that NS3 protein by protease activity can control the induced adenoviral innate response and improve the maintenance of adenovector in liver.

Keywords: HCV, NS3 protein, Adenovector, Inflammation

1506O

Lower circulating levels of chemokine CXCL10 in *Helicobacter pylori*-infected patients with peptic ulcer: Influence of the bacterial virulence factor CagA

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Background*:* Alterations in CXCL10 (a Th1 chemokine) expression have been associated with various diseases. The aim of this study was to evaluate the serum CXCL10 levels in H. pylori-infected patients with peptic ulcer (PU), H. pylori-infected asymptomatic (AS) subjects and healthy H. pylori-negative subjects, and also to determine its association with bacterial virulence factor cytotoxin-associated gene A (CagA).**Methods:** Serum samples from 90 H. pylori infected patients with PU (70 were anti-CagA$^+$, 20 were anti-CagA$^-$), 65 AS carriers (40 were anti-CagA$^+$, 25 were anti-CagA$^-$) and 30 healthy H. pylori-negative subjects (as a control group) were tested for the concentrations of CXCL10 by using ELISA method.**Results:** The mean serum levels of CXCL10 in PU patients (96.64 ± 20.85 pg/mL) was significantly lower than those observed in AS subjects (162.16 ± 53.31 pg/mL, P<0.01) and control group (193.93 ± 42.14 pg/mL, P<0.02).In the PU group, the serum levels of CXCL10 in anti-CagA$^+$ subjects was significantly higher in comparison to anti-CagA$^-$ patients (P<0.04). **Conclusion:** These results showed that the mean concentrations of CXCL10 in H.pylori-infected-PU patients was lower than AS carriers and control group. In the PU group, the serum levels of CXCL10 were associated with bacterial factor CagA.

Keywords: Helicobacter pylori, Peptic Ulcer, CXCL10, Anti-CagA

2194O

Mucosal IL-21 mRNA expression level is high in patients with H. *pylori* and is associated with the severity of gastritis

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Background: Helicobacter pylori (H. *pylori*) infection is associated with gastritis and marked infiltration of the gastric mucosa by several cytokines secreting inflammatory cells. Different clinical forms of the infection may reflect distinctive patterns of cytokine expression. IL-17, IL-21, IL-22 and IL-23 have been reported to be involved in H. *pylori*-induced gastric mucosal inflammation, but the details and relationship to different patterns of inflammation and virulence factors remain unclear. Therefore we examined IL-21 in the gastric mucosa of patients with H. *pylori* infection and evaluated the effects of virulence factors cagA and vacA allelic variants in H. *pylori*-infected on the mucosal IL-21 mRNA level in gastric mucosa. We also determined correlation between mucosal IL-21 mRNA levels and types of disease as well as grade of gastritis**Methods***:*Total RNA was extracted from gastric biopsies of 48 H. *pylori*-infected patients and 38 H. *pylori*-negative patients. Mucosal IL-21 mRNA expression level in H. *pylori*-infected and non-infected gastric biopsies was determined by Real-Time PCR. Presence of *vacA* (vacuolating cytotoxin A) and *cagA* (cytotoxin associated gene A) virulence factors were evaluated using PCR.**Results:**IL-21 mRNA expression was significantly more in biopsies of H. *pylori*-infected patients compared to H. *pylori*-uninfected patients. There was no association between virulence factors and IL-21 mRNA expression. **Conclusion:**Mucosal IL-21 expression level is increased in patients with H. *pylori* and is associated with the severity of gastritis. Therefore, we believe that IL-21 might be involved in the pathogenesis of H. *pylori* and might be an index of the severity of chronic gastritis.

Keywords*:* Helicobacter pylori; Gastritis; IL-21, Virulence factors

2416O

Evaluation of the expression of NOD1 and NOD2 molecules in peptic ulcer and gastric cancer due to Helicobacter pylori infection

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Background: Innate immunity can affect the clinical outcome of *Helicobacter Pylori* infection. Including non-ulcer dyspepsia (NUD), peptic ulcer disease (PUD), and gastric cancer (GC). To clarify this affect we evaluate the gene expression level of 2 molecule of innate immunity, nucleotide-bindingand oligomerizationdomain1 (NOD1) and NOD2 in this patients. **Methods:** One hundredand fifty patients with dyspepsia who needs diagnostic endoscopic were selected and divided into three groups of NUD, PUD and GC according to endoscopic and histopathological finding. *H.pylori*infection diagnosed by Rapid Urease test.

RNA extracted from endoscopic specimen that preserved in RNA later solution by QIAGENE kit. After cDNA synthesis,the expression of NOD1 and NOD2 molecules was determined by RT-PCR. Gene expression level compared in 3 patients groups and also in *H.pylori*+ and *H.pylori*- cases. **Results:** The results indicated thatgene expression levels of NOD-1 was significantly higher in both GC and PUD patients than in NUD subjects (6.11±0.29, 8.12±0.39, and 8.84±0.95, respectively, $p<0.001$ and $p<0.01$). Moreover, it was significantly higher in GC than PUD patients ($p<0.001$). However, gene expression levels of NOD-2 showed no significant difference among three groups. **Conclusion:** Increased NOD-1 expression in gastric mucosa may determine the clinical outcomes of *H. pylori* infection.

Keywords: Helicobacter Pylori, Gastric cancer, NOD

Poster Presentations:

1390P

Virulence factors of helicobacter pylori *vacA* increased markedly gastric mucosal TGF-β1 mRNA expression in gastritis patients

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Background: Helicobacter pylori (HP) infection is the main cause gastric inflammation. Although the induction of H. pylori-specific T and B cells, the immune response is not adequate to clear the infection. Regulatory T cells (Treg cells) suppress the activation and proliferation of antigen-specific T cells and mediate immunologic tolerance. TGF-β1 was shown to be secreted in a subset of Treg cells known as 'Th3 cells'. These cells have not been sufficiently studied in context to H. pylori –induced inflammation in human gastric mucosa.**Methods:** Total RNA was extracted from gastric biopsies of 48 Hp-infected patients and 38 Hp-negative patients with gastritis. Mucosal TGF-β1 mRNA expression in Hp-infected and non-infected gastric biopsies were determined by Real-Time PCR. Presence of *vacA, cagA, iceA, babA2* and *oipA* virulence factors was evaluated using PCR.**Results:** TGF-β1 mRNA expression was significantly increased in biopsies of Hp-infected patients compared to Hp-uninfected patients. There was association between virulence factors and TGF-β1 mRNA expression. TGF-β1 mRNA expression in mucosa was significantly higher in patients with vacA s1 and s1m1. **Conclusions:**TGF-β1 may play an important role in the inflammatory response and promote the chronic and persistent inflammatory changes in the gastric. This may ultimately influence the outcome of Hp-associated diseases that arise within the context of gastritis and vacA may suffice to induce expression of TGF-β1 mRNA.

Keywords*:* Helicobacter pylori, Gastric; TGF-β1, Virulence Factors

1391P

Associations of a TLR4 Single-Nucleotide Polymorphism with Helicobacter Pylori-Associated Gastric Diseasesin Iranian patients

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Background: Helicobacter pylori (Hp) is associated with gastric and gastric adenocarcinoma. Polymorphisms in the host genes coding for Toll-like receptors (TLRs) may influence the innate and adaptive immune response to the infection, affecting the susceptibility to Hp or the disease outcomes. But the details and association to different polymorphisms and different clinical expression in patients infected with Hp (different clinical expression of Hp infection) remain unclear. **Methods:** A case-control study consisting of 195 patients with Hp-infected and 241 Hp-uninfected was conducted. Genomic DNA was extracted and genotypes of TLR4Asp299Gly polymorphism were assessed through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Presence of *cagA* was evaluated using PCR. **Results:**TLR4 (Asp299Gly) G and DG alleles frequency in Hp-infected population was significantly higher in the chronic gastritis group than in the chronic active gastritis group (*p*, 0.021; OR, 2.409; 95% CI, 1.124-5.162). Grade mononuclear (MN) infiltration in Hp-infected patients with DG genotype of TLR-4 Asp299Gly increased significantly. *CagA* positivity was more frequently associated with chronic active gastritis ($p = 0.032$,OR = 0.493, 95% CI = 0.258-0.945) and grade polymorphonuclear (PMN) infiltration.**Conclusion:** TLR-4 Asp299Gly G allele substitution may be modified pattern of immune response in the gastric mucosa of Hp-infected patients and may be Hp-infected patients with gastritis increased risk for the development chronic gastritis. *CagA* positivity may be a risk factor for development gastritis.

Keywords: Helicobacter pylori, Gene polymorphism, Gastritis, TLR-4

2113P

Surveying of Chemiluminescence of neutrophils (respiratory burst) in the patients with chronic brucellosis

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Background: Brucellosis is a worldwide,serious problem.Brucella's pathogen is able to survive within phagocytic cells and these cells then spread to the lymph nodes and replace in reticuloendothelial system.Polymorphonuclears (PMNs) are one of the major defense mechanisms against infection.Because of importance of PMNs in inhibitionof Brucella infection, the study of respiratory burst activity in these cells is necessary.Because the intracellular growth of the pathogen depends on oxidative pathway of neutrophils.The aim of this study was to investigate respiratory burst activity (oxidativepathway)of polymorphonuclear cells in patients with chronic brucellosis, who have been exposed to other organisms, such as opsonized yeast and inactivatedBrucellamelitensis is disabled.**Methods**: Phagocytes of 51

patients with chronic brucellosis were detected and studied with chemiluminescence method. The first group contains 41 patients contains 27 men and 14 women with average age of 35. In this group activity of PMNs cell against opsonizedyeast were surveyed. Butthe second group contains 10 patients (4 women and 6 men) and in this groupactivity of PMNs cell against B.were surveyed. AlsoControl group of 26 were evaluated. **Results:** Rrespiratory burst, or Neutrophilic luminescence, which indicates the production of oxidants or free radicals in the oxidative pathwayin the PMNs of control and patient groups were similar ($p > 0.05$). **Conclusion**: It is concluded that inactivatedB.melitensis, can't inhibit myeloperoxidase activity in the oxidative pathway and can't prevent the formation of phagolisosome. Exactly what ever is seen in cases who have been faced toopsonizedyeast. But live Brucella preventing the phagosome connection to the lysosomes as well aslysosomesdegranulation and preventionof myeloperoxidase activity, and finally from death of own self. So infectious keep on growing and proliferation ,frequent recurrences, and chronicity of the disease.

Keywords: Brucellosis, Chemiluminescence, Myeloperoxidase, Respiratory burst

1539P

Isolation, Identification and Enzyematic Characterization of *Candida spp*isolated oral Candidiasis from the Cancer Patients with Receiving Chemotherapy in Tonekabon

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Background: Candidiaalbicansis endogenous yeast whose disease is emerged as an opportunistic infection. A few disposing elements like age (while natural flora has not been stabled), physiologic changes as pregnancy, taking antibiotics for a long time, general disabilities and deliberating diseases as cancers, provides the conditions for this opportunistic yeast; among which various cancers and using chemotherapy play significant roles in systemic and oral Candidiasis.Production of enzymes out of yeast like phosphatase, protease and lipase, as virulence factors, increase attaching to host and plays an important role in making oralcandidiasis**.** This study aims to ecommen.albicans from cancer patients doubted to oral candidiasis and investigating enzyme production and its relation to yeast virulence. **Methods**: 20 oral swab samples have been gathered from cancer patients doubted to have oral Candidiasis referring to oncology center of Ramsar hospital and clinics in Tonekabon city, during a six month period. Simultaneously 20 oral swab samples have been gathered from non- cancer patients with Oral Candidiasis who have referred to Tonekabon and Ramsar laboratories. The samples have been cultured on SDA containing chloramphenicol. After yeast growing, Candida yeast was determined through microscopic and macroscopic observations and then Germtube and culturing in chrome agar .The determined enzymes were investigated to measure phospholipase, proteinase and lipase enzymatic activities and assessing the corona around each colony**.** **Results**: in 19 samples among 20 provided ones, that is in 95% cancer patients, Candida species have been determined and this has been 30% in non- cancer patients.16 separated candidas, that is 84%, were C.*albicans* and three ones, that is 16% were C.*glabrata*; while all separated Candidia species were*C. Albicans* in non- cancer patients; and

all separated Candida species in non- cancer patients and healthy people contains enzymatic activities and producing Proteinase, lipase and phospholipase were 2.21, 1.46 and 1.36 mm, respectively; and this has been more in cancer patients than non- cancer patients. **Conclusion**: Chemotherapy and radiotherapy are among the important elements predisposing opportunistic fungal infections in which oral candidiasis is the most common. Also, high colonization of: *C.albicans* species including oral enzyme under chemotherapy, indicates increasing the species virulence.

Keywords: Candidaalbicans, Oral Candidiasis, Cancer, Phospholipase, Proteinase, Lipase

1538P

Survey of candidiacidal activity of neutrophils in Cancer patients with oralcandidiasis and receiving chemotherapy in Tonekabon and Ramsar Cities

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Background: Infecting to various microorganisms like fungi,as candida species, has been one of the main problems in cancer patients who receive chemotherapy. The deficiency in various forms in immune system of cancer patients under chemotherapy especially phagocytosis helps emerging the infections particularly systemic candidiasis. Last decades oral candidiasis has been increased in cancer patients. This study aims to investigate neutrophil killing candidia activity after chemotherapy in cancer patients compared to healthy ones through CTT method. **Methods:** In this analytical study, 20 cancer patients and 20 healthy people have been bleed as the samples; Giemsa staining has been applied for counting neutrophils.teh yeast samples of patients with oral candidiosis,especially standard yeast have been cultured on SDA medium containing chloramphenicol, through continuously passage. Separated neutrophils from patients and healthy people have been cultured with healthy people and patients' serums in multiple form and then the fungi was added to the four groups; after one hour incubation, desoxycholic acid lyses the cells and testing cells surviving and measuring killing power has been done by adding TTC to reminded candida. **Results:** This study indicates that neutrophils are decreased in patients normal blood compared to healthy people and there is a significant decrease in anti- candida activity of neutrophils in cancer patients than healthy people. This decrease in anti-candidia activity has been related to deficiency in cells and changing the serum; in a way that, a significant decrease can be seen in killing power of cells in cancer patients to healthy people. Killing candida activity of healthy neutrophils, healthy serums 81.65±6.70, healthy neutrophils, patient serums 73.80±6.16,patient neutrophils, healthy serums 38.70±8.06,patient neutrophils,patients serums33.50±5.90 were observed. Neutrophil killing candida activity has been decreased in cancer patients with oral candidiasis and receiving chemotherapy than normal people.**Conclusion:** According to the findings, if using special drugs like cytokines lead to increasing neutrophils activity, it can be hopeful that it might solve infection problems in cancer patients, especially systemic oral candidiasis.

Keywords: Cancer, Neutrophil, Oral candidiasis, Chemotherapy, Candidiacidal

1498P

Study on frequency of the CCR5 delta 32 mutant allele in thalassemic Patients with hepatitis C viruses

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Background: The chemokine receptor CCR5 has key roles in host responses to viruses in human. Hepatitis C virus is a major health problem in worldwide. TheCCR5 and its ligands might also play a role in HCV infection. In this study, for understanding the role of genetic predisposition to chronic HCV infection, frequency of the CCR5-Δ32 allele was determined in Hepatitis C Virus Infected Patients with thalassemia. **Methods:** In this study, 30 healthy blood donors, 30 responders and 30 non-respondersthalassemic Patients with Hepatitis C Virus infection were randomly selected. Genomic DNA were extracted from blood using the salting out method and CCR5-Δ32 genotype was determined using specific primers by Polymerase Chain Reaction (PCR) method. **Results:** None of both controls and the HCV patients had the CCR5-Delta32 mutant. **Conclusion:** Our results indicated that the CCR5-Delta 32 mutation is not related to lack of the response to HCV infection in thalassemic Patients.

Keywords: CC- chemokine receptor 5, CCR5Δ32, Hepatitis C virus (HCV), Thalassemia

1497P

A study on serologic markers of hepatitis B virus infection among asyptomatic blood donors in Iran

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Background: The most common marker that is used for HBV infection diagnosis in blood donors is HbsAg. But other serologic markers of HBV are needed for detection of disease status and prevention from its spread. The aim of this study was to evaluate hepatitis B infection status in asymptomatic blood donors. **Methods:** In this cross-sectional study, 1000 HbsAg positive samples were collected from all blood centers and tested for anti-HBc, HbeAg, anti-Hbe and anti-HBs. All data were analyzed statistically using SPSS16. **Results:** Out of 1000 blood donors HbsAg positive, 14(1.4%) of them were positive for anti-HBs .Also, about 910(91%) and 9 (0.9 %) of them were anti-Hbe and HbeAg positive respectively. **Conclusion:** The results this study showed that the majority of HBV infected blood donors were in chronic HBV infection phase. Therefore, these patients should be treated and monitored due to inactive disease may be active

Keywords: anti-HBs, HbsAg, blood donor, Chronic HBV, anti-Hbe

1499P

Evaluation of IL-17 and IL-23 genes Expression in Th17 cells in the pathogenesis of tuberculusis

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Background: Tuberculosis is one of the most important infection diseases with high mortality rate in the word especially among developing countries. Interleukin17 (IL-17) is an important acquired immunity cytokine, which is which is mainly produced by TH17 (a TCD4+ subtype). It can recruit neutrophils and macrophages to the infected site in the lungs. Interleukin 23 is the most important inducer of IL-17. In this study, the possible role of IL-23 and 17 in pathogenesis of tuberculosis was investigated. **Methods:** PBMCs were isolated from peripheral blood mononuclear cells (PBMCs) of subjects With Latent tuberculosis Infection(LTB) and newly diagnosed active tuberculos patients (ATB). PBMCs were activated with purified protein derivative (PPD) for 72 hours. Activated cells were harvested and RNA was extracted and cDNA was synthesised. A real-time Taqman method was designed and optimized for evaluation of IL-23 gene expression and SYBR Green method was designed and optimized for evaluation of IL-17 gene expression. We also evaluated the frequency of TH17 cells by flowcytometry in both group.
Results: According to our Real-time PCR findings IL17 level in patients is lower than PPD+ healthy controls at the same time results from Flowcytometrywas exactly like it($P<0.05$), Moreover IL23 expression level in tuberculosis was lower patients than control groups ($P<0.05$). Also Albumin level of serum is lower in patients.In the present study it has been demonstrated **Conclusion:** decreasing of IL23 and 17 play key roles in inflammation of tuberculosis infection and severity of diseases.

Keywords: Mycobacterium tuberculosis, TH17, Flowcytometry, Real time PCR

1405P

Specifity of IL-17 induction by Schistosoma Hematobium infection in Balb/c mouse liver

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Background: Schistosomiasis is a severe tropical disease caused by the parasitic worm Schistosoma Hematobium. Among the most serious pathological effects of S. infe Hematobium infection are hepatic lesions (cirrhosis and fibrosis) and portal hypertension. Interleukin-17 (IL-17) is a pro-inflammatory cytokine involved in the pathogenesis of many inflammatory and infectious conditions, including Schistosomiasis. **Method:** We infected mice Balb/c with S. Hematobium and isolated lymphocytes from the liver to identify cell subsets with high IL-17 expression and release using flowcytometry and ELISA. Expression and release of IL-17 was significantly higher in hepatic lymphocytes from infected mice compared with control mice in response to both non-specific stimulation with anti-CD3 monoclonal antibody plus/anti-CD28 monoclonal antibody and PMA plus ionomycin. We then compared IL-17 expression in two hepatic T-cell subsets, T helper and natural killer T. To determine the major source of IL-17 during infection. We then established a mouse model to further investigate the role of IL-17 in granulomatous and fibrosing inflammation against parasite eggs. Reducing IL-17 activity using

anti-IL-17A antibodies decreased infiltration of inflammatory cells and collagen deposition in the livers of infected Balb/c mice. The serum levels of soluble egg antigen (IL)-specific IgGs were enhanced by anti-IL-17A monoclonal antibody blockade, suggesting that IL-17 normally serves to suppress this humoral response. **Results:**These findings suggest that natural killer cells are the most IL-17-producing cells and that IL-17 contributes to granulomatous inflammatory and fibrosing reactions in S. Hematobium –infected mouse Balb/c liver.

Keywords: Schistosoma Hematobium, IL-17, Mice Balb/c, Flowcytometry, ELISA

1481P

Isolation and identification of *Nocardia* speciesof breast abscess in a woman suffering from pemphigus vulgaris: the first report from Iran

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Background: Pemphigus vulgaris (PV) is autoimmune disease with mucous membrane and skin blistering. Treatment is based on corticosteroids and immunosuppressive drugs. One of the factors causing infection in these patients is Nocardiosis. In this paper have been described the first report case of breast abscess by *Nocardia* species in a patient with pemphigus vulgaris from Iran.**Case report:**A 33-year-old woman was admitted to the department infectious disease (Razi- hospital-Tehran-Iran) in December 2010 for breast pain with a medical history of Pemphigus vulgaris.Physical and clinical examinations were diagnosed breast abscess. The abscess was aspirated and was seen bacteria in direct examination by gram positive and partially acid fast that aresimilar to the genus *Nocardia*.Colonies examined after cultureand the genus *Nocardia* was assessment. *Nocardia* species was identified by variousphenotypic tests such as: Resistance to lysozyme broth, decomposition of Tyrosine, Hypoxanthine, Xanthine, Casein, hydrolysis of Urea, Gelatin and Esculin, production of Nitrate reductase, utilization of Citrate, Acid production of Sorbitol, Ramnose, Glucose, L-Arabinose, D-xylose, Galactose,Manitol, Lactose, Maltose, Sucrose, Raffinose and grown at 45°C , 35°C and was used of polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) (*hsp*65 and16SrRNA genes) for molecular identification. Analysis of biochemicaltests showed similarity to *Nocardia nova* complex and molecular identification recognized *Nocardia cyriacigeorgica*. Antibiogram was done by the Kirby-Bauer method. Our isolate was sensitive to Gentamicin, Imipenem, Amikacin, Cefotaxime and Ampicillin and was intermediate-resistance to minocycline, ciprofloxacin while was resistance to cotrimoxazole.

Keywords: Breast Abscess, *Hsp*65 gene, 16S rRNA gene,Pemphigus Vulgaris, PCR-RFLP

1964P

Association of IL-22 and IL-17A with Peptic ulcer and Gastritis induced by *H.pylori*

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Background: Th17cells, producer of the IL-17A and IL-22, playa key role in the defense against H.pyloriwhich induces gastritis and peptic ulcer. Although IL-22 enhances integrity and regeneration in the stomachepithelial cells, both cytokineaggravate inflammation. Accordingly, we aim to investigate the relationshipof Th17, IL-17Aand IL-22 with peptic ulcer and gastritis in patientsinfectedwith H.pylori.**Methods:** Twenty four*H. Pylori*-infected patients were enrolled in thisstudy, consist of: 12 peptic ulcers, 12 gastritis (Moderate and Mild) and 12individuals negative for H.pylori.Peripheral blood mononuclear cellsisolated from the patients were stimulated with H.pylori antigens and then the levels of IL-17A and IL-22 in the culture supernatantsand the frequency of Th17 were measureduringELISA and Flow- cytometer, respectively.**Results:** Th17 frequency was significantly higher in peptic ulcer and gastritis compared to normal counterparts ($p \leq 0.001$).Although IL-17A was notsignificantlydifferentbetweenpatients groups, the level of IL-22was shown to be associated with gastritis ($p=0.01$).Dividing gastritis to mild and moderate, higher frequency of Th17was observed in moderate and mild gastritis ($p \leq 0.001$) compared to normal groups.Although increased level of IL-22 was significantly observed in moderate gastritis ($p=0.01$),the level of IL-17A was not significantly different between the groups.**Conclusion:**Regarding the Th17 frequency,IL-22may be more effective than IL-17Ain the induction of peptic ulcer and gastritis (especially the moderate form); however,a study with larger sample size is recommended.

Keywords: Th17, IL-21, IL-17A, *H. pylori*, Gastritis

1544P

Total leukocytes count and the neutrophil-lymphocyte count ratio in Helicobacter pylori-infected patients with peptic ulcer: independent of bacterial CagA status

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Background: Elevated leukocyte count has been reported as a marker of inflammation and infection. The aim of this study was to evaluate the total leukocytes count and neutrophil-lymphocyte count ratio (NLCR) in Helicobacter pylori (H. pylori)-infected patients with peptic ulcer (PU) and asymptomatic subjects (AS) and to determine their association with *H. pylori*virulence factor cytotoxin-associatedgene A(CagA).**Methods:** Blood samples were collected from 60 *H. pylori*-infected PU patients, 63 AS carriers and 32 healthy *H. pylori*-negative subjects (as a control). The total and differential counts of circulating white blood cells (WBC)determined by using standard hematological methods. **Results:**The mean count of total WBC and the NLCR in PU were significantly higher than those observed in control

group (P<0.001). Similarly, the mean count of total WBC and the NLCR in AS group were also significantly higher as compared to control group (P<0.005 and P<0.02, respectively). The differences of the mean count of circulating WBC and the NLCR between PU and AS were also significant (P<0.005 and P<0.001, respectively). In the PU and AS groups, the total and differential count of WBC in anti-CagA^{+} subjects was not significantly differ in comparison to anti-CagA^{-} participants.**Conclusion:**These results showed that the total leukocytes count and the NLCR were higher in *H. pylori*-infected subjects in comparison with control group. These parameters were not affected by CagA factor.

Keywords: Helicobacter pylori, Peptic ulcer, Leukocyte count, Neutrophils-lymphocyte count ratio, Anti-CagA

1617P

Is there any association H. pylori and IL-17A and IL-17Fgenes polymorphisms in dyspeptic patients?

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Background: Helicobacter pylori (*H.pylori*) colonize the gastric mucosa of approximately 50 % of the world's population that involved in chronicgastritis. The relationship between Hp colonization and gastric inflammation is widely accepted. Polymorphisms in inflammation related genes such as cytokines were thought to partly determine the outcome of Hp infection and progression of gastritis. Interleukin IL -17A and IL-17Fare inflammatory cytokines expressed by a novel subset of CD4+ Th cells, play important function in inflammation. We evaluate association of IL-17A G197A and IL-17F A7488G polymorphisms with gastritis, Polymorphonuclear (PMN) and Mononuclear (MN) infiltration in related to Hp. **Methods**: According to rapid urease test, PCR 16srRNA, urea and histological examination of biopsies, patients were classified Hp-infected and Hp-uninfected. The histological severity of gastritis was graded from normal to severe based on the degree of MN cell and PMN leukocyte infiltration, chronic gastritis and chronic active gastritis. Polymorphism in IL-17A G197A and IL-17F A7488G were evaluated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results**: AG, GG, AG/AA carriers of IL-17A G197A and AA, GA, GG, GA/Ggcarriers of IL-17F A7488G polymorphisms were not associated with MN infiltration, PMN infiltration, chronic gastritis and Chronic active gastritis in Hp-infected and Hp-uninfected groups (p> 0.05). AA genotype of IL-17A G197A was related to chronic gastritis and PMN infiltration in Hp-uninfected group. **Conclusion**: IL-17A G197A substitution may be a risk factor for development gastritis in Hp-uninfected patients, also affect the pathway MN cell production pathways.

Keywords: IL-17A, IL-17F, Polymorphism, Gastritis

1530P

Helicobacter pylori infection enhances CXCR7 expression on human gastric AGS cells

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Background: The role of *Helicobacter pylori (H.pylori)*infection in gastric damage and inflammation has been extensively investigated. It is well-known that the migration of normal and immune cells to inflamed tissues is governed by chemokines and their receptors. Among these chemokines SDF-1 and its newly identified receptor, CXCR7, has been shown to play a major role in migration of immune cells andmany cancer cells. The purpose of this study was to examine the possible effect of *H.pylori* on CXCR7 expression in AGS cells as human epithelial cells.**Methods:**First, *H.pylori* was isolated from gastric biopsy samples and characterized by microbiological and PCR techniques. The AGS cellswere infected by co-culturing of these cells with *H.pylori* (for one cell 100 bacteria). Then the expression of CXCR7 was examined by RT-PCR and flowcytometry. Finally, CXCR7 expression was evaluated in four*H.pylori* positive and four *H.pylori*negative patients by real time-PCR.**Results:**We have found that the isolated bacterium is an*H.pylori* as it was positive for urease, Cag A genes. When we infected the AGS cells with *H.Pylori*, we found *H.pylori* infection leads to up-regulation of CXCR7 in AGS cells both at gene and protein levels. More importantly, our Real-time data show that the levels of CXCR7 expression are significantly higher in*H.pylori* positive patients than negativeones. **Conclusion:**We demonstrate for the first time that H.pyloriinfection enhances CXCR7 expression on human gastric epithelial cells and that up-regulation of CXCR7 might play a role in pathobiology of *H.pylori*-associated disease.

Keywords:CXCR7, *Helicobacter pylori*, AGS.

1633P

The frequency of CA15-3,CA125,CA19-9in patients with hepatitis B and C in Guilan Province

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Background: hepatitis B is considered as a cause for Serum hepatitiswhich may lead to liver cells cancer. Hepatitis C is major cause of chronic hepatitis in developed countries. Information about tumor markers in patients with HBV and HCV in Iran population is limited.Therefore, this study aimed to determine the role of tumor markers CA15-3, CA125 ,CA19-9 in patients with hepatitis B and Cwho refer to Guilan liver and digestive Disease ResearchCenter. **Methods:**The study on serum samples from 80 patients with hepatitis B and C atGuilan Liver and Digestive Disease Research Center has been conducted from October 2012to October 2013 in terms of listed tumor markers viaELISAmethod.**Results:**The findings showed that no increases in serum levels of tumor markerCA19-9 has been seen in patients with hepatitis (P> 0.05). In patients with hepatitis B, there was an increasing at levels of tumor marker CA15-

3 (P= 0.04). Also in patients with hepatitis C, increasing in the tumor marker CA125 were observed (P = 0.02).**Conclusion:** The study showed that thetumor markerCA 15-3and tumor markerCA125was high respectively in hepatitisBandhepatitisC, but this increasing is not for malignancy, but further studying seemed to be necessary because of low size of samples to find the reasons of the increasing.

Keywords: Frequency,Tumor markers, Hepatitis B and C,Guilan Province

1770P

Evaluation of FoxP3 and CTLA-4 gene expression between Active TB Disease and Latent infection

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Background: Tuberculosis (TB) remains the leading cause of morbidity and mortality due to any one infectious agent worldwide. Regulatory T cells (Treg) constitute key components of peripheral tolerance suppressing potentially autoreactive T cells and preventing autoimmune diseases. Forkhead box P3(FoxP3),isa transcription factor, which characterized the phenotype of regulatory T cells(Treg). There are controversial results regarding the number of FoxP3 expressing cells in the blood of patients with tuberculosis (TB). In the present study, we examined the expression of Foxp3 and CTLA-4 in active tuberculosis and latent infection. **Methods**: PBMCs were isolated from peripheral blood mononuclear cells (PBMCs) of PPD skin test positive healthy donors and active TB patients with TB. PBMCs were activated with purified protein derivative (PPD) for 72 hours. Activated cells were harvested and RNA was extracted and cDNA was synthesised. A real-time Taqman method was designed and optimized for evaluation of Foxp3 and CTLA-4 gene expression.**Results**: Expression of FoxP3 and CTLA-4 in peripheral blood of patients with active TB was significantly lower than control group after and before activation with PPD. **Conclusion***:* In the present study, we demonstrate that the expression of FoxP3 and CTLA-4 in PBMCs of patients with active TB is low which might suggest that Treg cellsmay be sequestered in the lungs.

Keywords: Tuberculosis (TB), T regulatory (Treg) cells, CTLA-4, FoxP3,Real Time PCR

1586P

Comparison of the Tuberculin skin test (TST) and T-SPOT TB test in kidney transplant candidates and follow up the patients after transplantation and using anti-mycobacterial prophylaxis

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Background: Detection of latent tuberculosis infection (LTBI) in transplant candidates is very

important. The tuberculin skin test (TST) and interferon-gamma release assays (IGRA) are standard immunologic tools for LTBI detection. The aim of this study was to compare the accuracy of the T-SPOT.TB test and the TST in kidney transplant candidates for detection of LTBI and follow the patients with positive test for active tuberculosis after transplantation and using anti-tuberculosis prophylaxis. **Methods**: The study was carried out in 44 renal transplant candidates from March 2010 to February 2011 in the teaching hospitals of Isfahan University of Medical sciences, Iran. TST and T-SPOT.TB test were performed and their results evaluated. Patients with a positive skin test and/or T-SPOT.TB test were started on anti-tuberculosis prophylaxis and followed after transplantation for activation of their LTBI. **Results**: Overall, 8(18.2%) patients were positive for TST and 6(13.6%) patients for T-SPOT. TB test. The agreement between TST and T-SPOT.TB test was 86% (κ= 0.49, 95% confidence interval [CI] 0.145-0.839). No relation was found between the clinical risk for LTBI and TST or T-SPOT.TB test positivity. Although isoniazid prophylaxis was used for patients with positive TST or T-SPOT.TB test, one patient had reactivation of TB.**Conclusion**: In kidney transplant candidates both TST and T-SPOT.TB test were comparable for the diagnosis of LTBI with reasonable agreement the tests. However, further studies are needed to determine the ability of T-SPOT.TB test to detect LTBI and to evaluate the need for prophylaxis in these patients.

Keywords: Tuberculin skin test, T-SPOT TB test, Latent tuberculosis, Kidney transplant

1602P

Diagnosis of human hydatidosis using recombinant protein EPC1 by sandwich ELISA

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Background: Cystic echinococcosis (CE) is a major zoonosis of worldwide distribution. A standardized test for the serodiagnosis of human cystic echinococcosis is still needed, because of the low specificity and sensitivity of the currently available commercial tools and the lack of proper evaluation of the existing recombinant antigens. In this regard, we recently proposed the use of a protoscolex recombinant antigen EPC1, for the diagnosis of CE patients by sandwich ELISA.**Method:**A total of 80 human sera comprised of 33 CE cases and 47 healthy donors were used to evaluate diagnostic sensitivity and specificity of the rEPC1 by sandwich ELISA. Hyperimmune serum was raised in two healthy rabbits which were actively immunized with Freund's adjuvant mixed with rEPC1 previously produced. Western blot analysis was carried out by rabbit serum to estimate antibodies production against rEPC1.**Results:** The presence of a single band of predicted size of rEPC1 was confirmed by western blot analysis. A total of 80 human sera yielded an overall sensitivity of 100% and an overall specificity of 92.1% by sandwich ELISA. In the present study, conventional ELISA performed as the gold standard. **Conclusion:** The results of the present study suggest that rEPC1 protein can serve as an alternate source of antigen and sandwich ELISA was found to be highly specific test for detection of hydatid antibodies. Because of the limited facilities to approach to the more human sera, potential cross-reactivity of the rEPC1 has not been examined in this study. Therefore, we are aware that the performance of our antigen should be further investigated in a larger panel of patients with different infections.

Keywords: Cystic echinococcosis, Serodiagnosis, rEPC1, Sandwich ELISA

1653P

Detection of Legionella pneumophilain cooling water systems of hospitals and nursing homes as -compromised cases of Kerman City, Iran by semi-Nested PCR

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Background: *Legionella pneumophila*is involved in more than 95%cases of severe atypical pneumonia. Infection is mainly by inhalation the indoor aerosols through the water-coolant systems. Because some *Legionella* strains may be viable but not culturable, therefore, *Taq* polymerase, DNA amplification and semi-nested-PCR were carried out to detect *Legionella*-specific 16S-rDNA sequence. **Methods:** For this purpose, 1.5 litter of water samples from 77 water-coolantsystem were collected from four different hospitals, two nursing homes and one student hostel in Kerman city of Iran, each in a brand new plastic bottle during summer season of 2006 (from April to August). The samples were filtered in the sterile condition throughthe Millipore Membrane Filter. DNA was extracted from membrane and used for PCR to detect *Legionella* spp. The PCR product was then subjected to semi-nested PCR for detection of *L. pneumophila*. **Results:** Out of 77 water samples that were tested by PCR, 30 (39%) werepositive for most species of *Legionella*. However, *L. pneumophila*Was detected from 14 (18.2%) water samples by semi-nested PCR. **Conclusion:** From the above results it can be concluded that water coolantSystems of different hospitals and nursing homes in Kerman city ofIran are highly contaminated with *L. pneumophila* spp. And pose Seriousconcern, especially in theimmuno-compromised people. So, we recommend avoiding such type of coolant system in the hospitals and nursing homes.

Keywords: *Legionella pneumophila*, Water-coolant system, Immuno-compromised, Semi-nested PCR

1838P

Evaluation of Seroprevalence of Brucellosis in Tooba laboratory during 1391-1392

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Background: Brucellosis is one of the most important zoonotic disease constituting a public health problem worldwide .Brucellosis is usually associated with the consumption of unpasteurized milk and soft cheeses made from infected animals. The purpose of this study is determine the prevalence of brucellosis among people suspicious of brucellosis in Mazandaran province. **Methods:** In a cross-sectional study,A total of 1352 Peripheral blood samples were collected from patientsand their serum were separated. All samples screened for Brucella antibodies by agglutination test. Positive sera were further analyzed by standard tube agglutination tests (Wright, 2ME and coombs wright). **Results:** Theprevalence of brucellosis was 1.62% (22/1352) as detected by agglutination.Among positive cases, there were 17 male (77.3%)and 5 female (22.7%). Tube Wright (1.80 ≤) 22/22 cases (1.62%),

2ME (1.40 ≤) 12/22 cases (0.88%) and coombs wright (1.40 ≤) 13/22 cases (0.96%) were demonstrated to be positive. **Conclusion:** The results of this study show that infectious animals must be control for prevention of disease. It is concluded that screening of brucellosis is necessaryin our population for basic programming.

Keyword: Brucellosis, Seroprevalence, Agglutination

2159P

Fungal infection and increased mortality in patients with chronic granulomatous disease

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Background: Fungal infection presents a serious risk to individuals with compromised immune systems. Chronic granulomatous disease is a primary immunodeficiency with X-linked or auto-somal recessive inheritance. Patients with CGD are predisposed to bacterial and fungal infections. The aim of this study was to determine the incidence of fungal infections, identify the most common fungal pathogens, and determine the risk factors associated with fungal infections and mortality in patients with chronic granulomatous disease (CGD).**Methods:** We reviewed retrospectively 12 patients with CGD, all of them were suspected to fungal infections. The data was gathered from the medical records of all patients as having CGD. Twelve patients had adequate medical records to enter the study. The diagnostic of fungal infections were confirmed by histopathology and direct preparation, culture techniques, histopathology of surgical biopsies, and radiological examination of the affected site. **Results:** We evaluated 12 cases of chronic granulomatosis. Patients that are susceptible to recurrent, severe infections. Patients consisted of seven males and five females. The median age of patients at the time of the study was 11.66 years (3 to18). Neutrophil oxidative burst were absent (NBT = 0) in all patients. Fungal infections were confirmed in five patients (41/7%) by histology and mycological methods. The most common isolated fungi in this study were Aspergillus spp. Out of five cases of fungal infections identified, tree were Aspergillus spp, and two Fusarium spp. The most common manifestations of CGD due to fungal infections (in descending order) were osteomyelitis (42.8%), pulmonary infections (28.6%), lymphadenopathy (14.3%) and skin involvement (14.3%) during their illness.**Conclusion**: High fever for 3-5 days, no response to antimicrobial therapy was the most common sign of fungal infections in patients with CGD. Early diagnosis of CGD and rapid treatment of infections are critical. Lung and bone were the most common site of fungal infections in this study. Present study showed that Fusariosis is also a threat to CGD patients.

Keywords: Fungal infections, Chronic granulomatous disease, *Aspergillus, Fusarium*

2160P

Actinomyces and *Nocardia* infections in chronic granulomatous disease

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Background: Chronic granulomatous disease (CGD) is an inherited disorder of the Nicotinamide adenine dinucleotide phosphatereduced oxidase complex characterized by

recurrent bacterial and fungal infections. We presented in the retrospective review of medical records,the etiology, presentation, clinical characteristics the infections detected, predisposing condition and outcome of Nocardiosis and Actinomycosis involved in a group of pediatric patients diagnosed with CGD. **Methods:** The clinical presentation ofCGD-related infections was reviewed retrospectively from the medical records of all 12 patients with CGD. We studied respectively12 patients and we analyzed two pediatric patients with CGD who acquired *Nocardia* and *Actinomyces* infections, and their clinical and microbiological characteristics were described. The microbiological diagnosis was determined by biochemical tests, histology, microscopy, and culture of clinical samples. **Results:** The medical records of 12 diagnosed CGD patients with suspected Nocardiosis or Actinomycosis were reviewed. One patient was diagnosed with Actinomycosis and one patient with Nocardiosis. Patients consisted of seven males and five females with ranging ages of 3 to 18 years. Nocardiosis and Actinomycosis isolated in the two patients were confirmed by histology and culture methods. Neutrophil oxidative burst were absent (NBT=0) in both patients. The most common manifestations of CGD due to fungal infections, Actinomycosis, and Nocardiosis were osteomyelitis (42.8%), pulmonary infections (28.6%), lymphadenopathy (14.3%), and skin involvement (14.3%) during their illness. **Conclusion:** Nocardiosis and Actinomycosis in children indicate the need for evaluation for an underlying immunological deficiency. Early diagnosis remains critical for decreased morbidity and occasional mortality. Physicians caring for patients with CGD should maintain a high index of suspicion for Nocardiosis and Actinomycosis especially if work up for TB and fungal infections are negative.

Keywords: Chronic granulomatous disease, Nocardiosis, Actinomycosis

1719P

Association of serological and stool antigen responses, against *Helicobacter pylori* with distribution of 16SrRNA gene

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Background: The current study aimed to perform the serology, stool antigen and PCR analysis for amplification of 16SrRNA genes of *Helicobacter pylori* and evaluate their associations together to see the impact of severity of disease on immune properties against the bacteria. **Methods:** About 42 patients with clinical symptoms such as stomachache, gastrointestinal disorders and digestive problem, together with 40 healthy people were randomly selected and enrolled in this study, collecting one blood and one stool sample from each antigens in stools were detected using immunoassay method and cDNA was extracted by Bioneer kit. 16SrRNA was amplified by PCR and sera IgA and IgG were measured by ELISA. Data were analyzed using SPSS package. **Results:** All 42 patients were positive for stool antigen while IgA and IgG were positive for 45.23% and 92.85% of samples respectively. 21% of samples in case group had 16SrRNA gene by PCR. 4 out 19 and 8 out of 39 patients with IgA and IgG had 16SrRNA respectively. **Conclusion**: It seems that stool antigen tests are not highly reliable specifically when detection of *Helicobacter pylori* is the matter of curative medicine so such

results are not either confirmed by PCR or by serology therefore performing another study with higher samples and different genes of bacteria is recommended.
Keywords: Helicobacter pylori,Stool antigen, IgA, IgG, PCR

1718P

TNF-α and IFN-γ variations in patients with *Helicobacter pylori* and their association withstool antigens andCagA and VacA genes

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Background: The aim of the current study is to evaluate the CagA and VacA genes of *Helicobacter pylori* compared to responses to stool antigen and sera levels of cytokine variationsin Ilam to make a comprehensive understanding of the infection processes. **Methods:**82 patients with gastrointestinal disorder were randomly selected and one blood sample together with one stool sample was collected from each individual. DNA was extracted from the stools using Bioneer kit and PCR applied to amplify the VacAm1/m2, VacAs1/s2 and CagA genes. Sera levels of IFN-γ and TNF-α was measured by ELISA. Data were analyzed by ANOVA and Pearson correlation. **Results:** About 42 sample as the case showed positive for stool antigen. The mean level of IFN-γ was 97.63 and 47.72 for the case and control respectively while for the TNF-αit was 177.57 and 85.44 respectively. Only 5 samples (11.9%) of case group had CagA while one sample from the control group were positive. 8 out 42 samples of case group (19.04%) for VacAm1/m2 and 4 out of 42 (9.52%) for VacAs1/s2 were positive. **Conclusion**: Variations of cytokines in case and the control group was significantly different though IFN-γ was directly correlated with responses to stool antigens in case group but about 30% of the case showed positive for the virulence genes and the remaining who are diagnosed as positive for *Helicobacter pylori* are placed among the non virulent strains andhence the necessity for further evaluation regarding the current diagnostic tests.
Keywords: *Helicobacter pylori,* CagA, VacA, IFN-γ, TNF-α

1893P

DNA priming protein boosting regimn using Influenza virus conserved protein induced strong humeral and cellular immune responses

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Background: The M2 protein is translated from a spliced mRNA derived from influenza gene segment 7, which also codes for the matrix protein M1. It is a type III transmembrane protein which, in the form of tetramers, functions as a pH-regulated proton channel, essential in the initial cell infection process as well as in later progeny virus formation. M2 shows remarkable

conservation among all subtypes of influenza A viruses. So, it is considered as a promising candidate target for a broad-spectrum recombinant influenza A vaccine.Here we report the induction of specific immune responses following M2 DNA and/or protein administration in mice model. **Methods:** The confirmed eukaryotic expression vector encoding entire open reading frame of M2 was prepared using Endofree plasmid Mega Kit (Qiagen). Prokaryotic expression M2 protein purified using Ni-NTA Purification System. The recombinantplasmids and protein of interest were injected to Balb/C mice with different formulation and specific anti-M2 immune responses were evaluated. **Results:** The results showed that the administration of DNA vaccine encoding the M2 protein of influenza followed by M2 protein with Alum/CPG adjuvant as booster induced stronghumoral and cellular immune responses. **Conclusion:** DNA vaccines generally consist of plasmids originally derived from bacteria but totally unable to produce infection. The intracellular transcription and translation of the recombinant DNA mimic the replication of a virus during infection. Following pcDNA-M2 immunization intramuscularly, the M2 proteinwill be generated and all arms of the immune responses come into play afterward.

Keywords: Influenza virus, DNA vaccine

1883P

Seroprevalence of rubella, cytomegalovirus and varicella antibodies among pregnant women in urmia district,north west Iran

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Background: Viral infections during pregnancy are major causes of maternal and fetal morbidity and mortality .Aim of present study was to determine the immunity status of pregnant women against Rubella, cytomegalovirus and varicella zoster virus, related factors with seropositivity status and validity of reported varicella history as a marker for immunity.**Methods:** A Cross-Sectional study was carried out on 364 pregnant women were referred to health centers laboratories for blood exam as part of their routine prenatal care. Varicella IgG antibody (VZV IgG) was determined using Eurovir Eliza Leit test. Rubella IgG antibody, IgG and IgM Cytomegalovirus antibodies were measured using Chemilumiescance system .Information related studied factors were collected by questionnaire. Validity indices and predictive values of reportedvaricella history were calculated. **Results:** Rubella and cytomegalovirus specific IgG antibodies were positive among 333(94.3%), 346(98%) pregnant women respectively. CMV-IgM antibody was not found in sera samples and only 8 (2.3%) samples were equivocal. None of investigated factors did have significant effect with seropositivity rate of antibodies in study population (Pvalue>0.05). Reported varicella history was highly predictive of seropositivity in pregnancy (positive predictive value = 97.61%).**Conclusion:** In urmia district, most pregnant women have sufficient immunity against rubella, cytomegalovirus and varicella zoster infections. New infections of these viruses among pregnant women don't appear to be potential factor of congenital infections and related complications in their newborns.Reported varicella history were highly predictive of seropositivity in pregnancy.

Keywords: Cytomegalovirus, Rubella, Varicella, zoster, Pregnant women, Urmia

1949P

Association of HLA Class II Alleles with Clearance and Persistence of Hepatitis C Virus and with Serum Alanine Aminoteransferase Levels in the Iranian Population

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Background: Data on association of HLA class II with clearance or persistence after acute HCV infection in thalassemia patients are rare. **Method:** Class II HLA typing was performed on 117 Iranian thalassemia patients, 54 of whom had viral clearance and 63 had persistent infection. **Result:** A novel protective association for DRB1*0301 (p=0.03) and risk associations for DQA1*0201 and DQB1* 0602 (p=0.007 and p=0.02, respectively) to HCV infection were found. Viral clearance was also associated with DQA1*0501 (p=0.0007), and viral persistence was associated with DRB1*0701 (p=0.004). The haplotype frequencies of DRB1*0301- DQA1*0501- DQB1* 0201 and DRB1*1101- DQA1*0501- DQB1* 0301 (p=0.004 and p=0.04, respectively) were significantly higher in patients with HCV clearance than in those with chronic infection, whereas DRB1*0701- DQA1*0201- DQB1* 0201 (p=0.02) was reversed. Among patients with HCV clearance, DRB1* 0401 and DQA1*0301 alleles (p=0.03 and p=0.04, respectively) occurred more frequently in the normal alanine amino transferase (ALT) group. As compared to chronic patients with abnormal ALT levels, DQA1*0102 and DQB1*0602 (p=0.01 for both) were significantly higher. **Conclusion:** Our findings suggest possible influence of HLA alleles on the serum ALT level in thalassemia patients. Certain class II alleles found in the Iranian thalassemia patients are different those observed elsewhere, suggesting that immunogenetic makeup for HCV clearance or persistence could vary based on the ethnicity.

Keywords: Hepatitis C virus, HLA class II, Thalassemia patients, HCV clearance, HCV persistence

1991P

Characterization of IL-17, IL-22 and HBV escape mutations in low- and non- responders in health care workers to HBV vaccine

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Background: HBV mutations and cytokine polymorphisms are two important factors which determine the quality of immune response to a given HBV vaccine. HBV mutations alter the immune efficacy of HBV vaccine so that HbsAb no longer can recognize the altered immune epitope and cytokines such as IL-17 and IL-22 play central roles in homeostatic, pathologic and protective immune response. In this study, three factors' relationships(HBV mutations, IL-17 and IL-22 polymorphisms and PBMCs proliferation assay) in low- and non-responders in health care workers as a high risk population were investigated. **Methods:** 100 low responded and20non responded health care workers who had been vaccinated against HBV according to the standard schedule were selected and HbcAb, HbeAg, HbeAb were investigated by ELISA thenReal time PCR were performed on sera in order to study escape mutations. The prevalence of IL-17 and IL-22 alleles were determined by PCR-RFLP and the proliferation of PBMC was analyzed in two groups by XTT assay. **Results:** Differences between two groups for IL-17, IL-22 and PBMCs proliferation were not statistically significant but HBV escape mutations had been occurred in 3 low responded and 1 non responded health care workers. **Conclusion:** Although PBMC proliferation and IL-17 AND IL-22 polymorphisms are important factors in immune response to HBV vaccine we did not detect significant differences between low and non-responders of health care workers It is suggested that polymorphisms other cytokines genes such as IL-21 and rate of Th1/Th2 differ in low- and non responders groups to HBV vaccine.

Keywords: HBV vaccine, HBV escape mutations, Cytokine polymorphisms, Health care workers.

2152P

Study counteract between hepatitis C virus infections with Th2 cytokines in liver transplant patients.

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Background: Hepatitis C virus is one of important inducible risk factors of end stage liver diseases and malignancy in orthotropic liver transplant candidates. Th2 immune cells and their products also counteract with hepatitis C virus to design the pathway of viral pathogenesis especially chronic infection in liver transplant patients. Therefore, in this study the level of Th2 cell induced cytokines was evaluated in hepatitis C virus infected liver transplant patients. **Methods:** In a cross sectional study 69 EDTA-treated blood samples were collected from liver transplant patients between years 2007-2010 pre and post-transplantation. Hepatitis C virus molecular prevalence was analyzed by a qualitative in-house nested-RT-PCR protocol. The level of IL-4 and IL-10 cytokines was evaluated by ELISA methods in liver transplant patients. **Results:** The hepatitis C viral RNA was detected in 7 of 69 (10.1%) and 4of 69 (5.8%) plasma samples of patients pre and post-transplantation, respectively. The raising level of IL-4 was detected in 71.4% of hepatitis C virus infected patient pre-transplantation. But the raising level of IL-4 was found in 50% hepatitis C virus infected patient post-transplantation. The level of

IL-10 was raised in 37.5% and 100% of hepatitis C virus infected transplant patients pre and post-transplantation, respectively. **Conclusion:** Diagnosis of higher levels of both IL-4 and IL-10 in transplant candidates in comparing with liver transplant recipients infected with hepatitis C virus and also diagnosis of lower prevalence of this viral infection in post-transplant period emphasize on the determinative role of these cytokines in controlling the risk of hepatitis C virus prevalence and related clinical outcomes in liver transplant patients.

Keywords: Hepatitis C, Th2 immune cells, Liver transplant

2118P

Development of in vitro method for studying of the asthmatic disease due to respiratory syncytial virus (RSV)

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Background: A significant number of Wheezing illnesses due to viral infections and many of clinical asthma exacerbations are frequent in childhood and a reason of morbidity, hospitalization, and application of health resources. In fact Wheezing was found to increase the risk of asthma. **Method:** We have established a new assay for verifying neutralizing antibodies to respiratory syncytial virus in human sera is described. In this experiment, there were increased in the optical densities (Ods) measured after running the MTT assay for different exposures. Following one hour incubation, the serum–virus mixture was transferred to HeLa cells cultured in 96-well plates. After 24 hours, , 25 µL of the)-2,5-diphenyltetrazolium Bromide-Formazan (MTT) solution (5 mg/mL) was added to each well, and the plate was reincubated for 2 hours. **Results:** The amount of formazan crystal was determined by measuring the absorbance using a spectrophotometer. The absorbance values correlate directly to the number of viable cells and also to the neutralizing activity of virus-specific antibodies available in the serum. The test was used to demonstrate the antibody titers of respiratory syncytial virus (RSV). **Conclusion:** This new test format could serve as a valuable laboratory tool for studying of the asthmatic disease due to respiratory syncytial virus (RSV).

Keywords: Respiratory syncytial virus (RSV),Wheezing; asthma,MTT test, Tetrazolium

2151P

Candida Antifungal susceptibility in Primary Immunodeficiency Disease

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Background: Infections due to *Candida* spp. Are common, especially in patients with primary Immunodeficiency diseases (PID) .The aim of this study was to evaluate susceptibility of candida spp. Isolates from PID patients who referred to Immunology,Asthma and Allergy Research Institute to 4 known antifungal antibiotics (Amphotricin B,Fluconazole,Voriconazole and Itraconazole). **Method:** During 2011-2012 from twenty PID patients who had lesion on their body took samples and cultured. Amphotricin B, Fluconazole,Voriconazole and Itraconazole were tested in this assay. Broth microdilution tests were performed according to

the CLSI (Clinical and Laboratory Standards Institute). Each well was inoculated with 1 ml of RPMI-1640 Medium, 1 ml of antifungal agent with 10 serial dilution of each antifungal antibiotics and a fungal suspention contains 10^3Cell/ml. Positive control well contained fungal suspention, RPMI-1640 Medium and negative control well contained RPMI-1640 Medium and distilled water. **Results:** During this study, twenty patients with PID were interred the study who had skin lesion with positive culture.Among these PID patients, 9 male and 11female with meadian age of 7years. Seven positive candida were observed in specific culture that they confirmed with Germ tube test and PCR. An antifungal susceptibility test were done with four antibiotics. Candida spp were susceptible to Amphotricin B; but resistant to the Fluconazole, Voriconazole and Itraconazole respectively. **Conclusion:** Consideration of PID patients showed that patients who referred with recurrent infection resulted in hospitalized, may be resistance to antibiotic because of excessive prescription of antifungal antibiotics ,so prevalence of antibiotic resistance significantly increased in PID Patients.

1689P

Seroepidemiological Study of West Nile Virus Infection in the Cities of Neka and Shiraz in 2013

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Background: West Nile virus is a member of the genus *Flavivirus*, in which to be induced viral infections in human.Unfortunately valuable studies not conducted in our country about that. Therefore this study performed for the detection of IgG antibodies against WNV in patients of two cities of Neka and Shiraz. **Methods:** Specimens from 46 possible WNV case, from Neka (23 persons: 13 women, 10 men) and Shiraz (23 persons: 10 women, 13 men) cities were included in this study. Experiment stages implemented based on the IgG assay against WNV kit (made in Germany institude). Statistical analysis of data was carried out using the SPSS (19 version).**Results and Conclusion:** Results showed that Immune Status Ratio (ISR) in Neka patients were negative for WNV IgG, but 12 sera from Shiraz patients, include 2 women and 10 men, were positive, that varied from 3.12 to 38.6. Statistic analysis of mean of ISR showed that there is significant variation between Neka and Shiraz patients which tested ($p<0.05$). Also, results showed that there is significant variation in WNV infection rate between women and men from Neka and Shiraz cities ($p<0.05$), and in men (39.19%) was more than women (13.04%). Because Shiraz has hot and semi-dry climate, whereas Neka has temperate climate, possibly climate variation influences on this result, because the climate influences the transmission of WNV. Because the job situation of men, they survive more time during a day in out of house than women; thus rather exposure bites of mosquito vectors that transmit WNV.

Based on these results it is recommended that the necessary steps should be done to prevent the disease in Shiraz.

Keyword: West Nile Virus, ELISA, IgG antibody

1562P

Natural immunity to hemophilus influenza type b in children, south of Iran

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Background: Hemophilus influenza type b (Hib) infection has a high morbidity and mortality rate especially in children less than 5 years of age. The incidence of Hib disease in Iran is not known and Hib vaccine is not included in the National Immunization Program. The aim of the present study was to investigate the level of antibody to Hib of children five years or younger living in Jahrom, Iran. **Methods**: Three hundred eighty six children 5 years or younger were selected by random sampling method. A blood samples were taken from those children. Anti-HibIgG antibody (anti-PRP) level was determined in the serum by using anti-Hemophilus influenza IgG EIA kit (IBL, Germany). An anti-PRP antibody levels of 0.15 microgmL(-1) and over were accepted as the natural immunity. **Results**: The mean concentration of Hib antibody was 0.94 +/- 0.480 microgmL(-1). Natural immunity was determined in three hundred and twenty six (84.5%) of the children. The proportion of natural immunity was increased from 64.9% among children = 12 month old to 95.2% in children aged 49-60 month ($p < 0.001$). **Conclusion**: The exposure rate of children with Hib was higher than expected, even in children who were just a few months old. Present data revealed need to be introducing Hib conjugate vaccine in the National Immunization Programs.

Keywords: Natural immunity, Hemophilus influenza type b, children, Iran

1492P

Distribution of CC chemokine receptore5 gene polymorphisms among Persian HBV infected people

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Background: HBV causes the disease hepatitis B, an infectious inflammatory illness of the liver. Patients who get infected with HBV Shown different clinical symptoms and severity. Host immunological and genetic factors could influence the course of the disease.CCR5 is normally involved in immune mechanisms; Down-regulation of CCR5 might have important consequences for the host's immune defense. Distribution of 59029G/A and 59402A/Galleles was determined for the purpose of predicting possible clinical responses to the HBV epidemics in our country, as well as to establish their effects on the expression of surface CCR5. **Methods:** In a case control study 327 patients with HBV infection: 140 inactive carriers, 135 hepatitis chronic and 82 cirrhotic patients were enrolled. All of the subjects with HBV infection were diagnosed according to world health organization standards Genotyping and analysis of SNPs were performed through ARMS PCR.Target sequence, the promoter region of the CCR5 gene, was amplified using sequence specific primers. **Results:** Results in evaluating 59029G/A demonstrated 59029GG allele tended to accumulate in the healthy donors. This accumulation was more than inactive carriers.Evaluating frequency of the59402 A/G genotype revealed us, that distribution of the genotype 59402AG had shown significant difference between the

inactive carriers and other groups (healthy donors or the hepatitis patients) and it is higher ininactive carriers.**Conclusion:** The results of the present study clearly show the significant differences in the frequency of allelic distribution of CCR5 promoter gene.
Keywords: HBV, polymorphisms, CCR5

1624P

Structural studies of KSHV vOX2, a viral orthologue of cellular CD200

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Background: The Kaposi's sarcoma-associated herpesvirus (KSHV) vOX2 protein encoded by open reading frame (ORF) K14 is a lytic cycle protein with roles in modulating inflammatory reactions. To understand the physical properties of this molecule, it was ecommendedd biochemically.**Methods:** Extracellular domains of K14 subcloned into the pDR2DEF1a.Fc expression vector. By Lipofectamine,CHO cells were transfected andwere used to produce the recombinant vOX2:Fcprotein for biochemical and biophysical studies. PNGase F and Xarrest Sepharose kit were used for deglycosylation and cleavage studies, respectively. Jurkat cells transfected with transmembrane KSHV-vOX2 was used to investigate the adhesive properties of the protein *in vitro*. **Results:** vOX2 is a single-pass type I transmembrane protein. It is highly glycosylated;after deglycosylation by PNGase, the diffuse band of vOX2 that originally migrated with a molecular mass of 50 kDa shifted to a sharp band of 25 kDa consistent with that of the predicted molecular mass without glycosylation.Our results revealed that vOX2 is predominantly a beta-folded molecule with RGD motif exposed on the C-terminal domain and exists in a monomer-dimer equilibrium similarly to its IgV-type folded homologs.This extracellular domain of the vOX2 might represent a biologically important region that could ligateone or more cellular receptors. Jurkat cells transfected with transmembrane vOX2 formed multicellular aggregates. **Conclusion:** Taken together, highly glycosylation, adhesive properties, exposed RGD motif, structural similarity to PD-L1 and ability to form homodimer make it a good candidate for potentiation of cellular plasticity in tissue damage and immunomodulation.
Keyword: Viral orthologue, Cellular CD200, KSHV vOX2 protein

2406P

Lack of correlation among Th2 cytokines in latent tuberculosis infection: high IL-5 and IL-13 but low IL-4 levels in response to PPD

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Background: Tuberculosis (TB) is still one of the most important infectious diseases

worldwide. The disease contributes to illness and death. Yet the majority of infected individuals develops a latent TB infection (LTBI) and remains healthy. Defining immune response in these individuals will help to determine what is required for a protective immune response. Several studies have focused on IFN-g due to its strong association with protection against TB. However, given the complexity of the immune response to infection, other cytokines should also be considered. The aim of the present study was to determine the immune response of LTBI and control individuals to PPD. **Methods:** LTBI individuals were selected to be positive to Quantiferon-Gold in Tube (QFT-IT) and control subjects were QFT-IT negative. LTBI and control Individuals were healthy without clinical evidence of active tuberculosis. Peripheral blood mononuclear cells were stimulated with PPD and IFN-g, IL-4, IL-5, and IL-13 were measured in the supernatants of cell cultures by ELISA. **Results:** LTBI showed significantly higher IFN-g levels compared to the controls. Although IL-4 levels were similar in both groups; IL-5 and IL-13 levels were higher in LTBI. There were strong positive correlation between IFN-g and IL-13 (P=0.03, r=0.6), IFN-g and IL-5 (P=0.04, r=0.56), and IL-5 and IL-13 (P=0.001, r=0.8). IL-4 did not show any correlation with IL-5 and IL-13. **Conclusion:** so far there is no specific cytokine profile clearly associated with immune protection against tuberculosis. The findings of this study suggest contribution of IL-5 and IL-13 to the control of disease.

Keywords: Latent tuberculosis, IL-5, IL-13

2750P

Evaluation of Viral risk Factors in HAM/TSP Patients and Asymptomatic Carriers by Assessment of Tax mRNA and Proviral load.

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Background: Human T cell lymphotropic virus type I (HTLV-I) is a human retrovirus that is associated with adult T cell leukemia and with a slowly progressive neurologic disorder, HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP). Virus and host interactions play a significant role in the outcome of the HTLV-I infection. In this study we evaluate the Tax mRNA levels and proviral load as possible risk factors for HAM/TSP disease progression. **Methods:** In this study, two groups of HAM/TSP patients (30 subjects), HTLV-1 carriers (30 subjects) and healthy people (20 subjects) were selected and blood samples (10 ml) were obtained from each subject. To synthesize cDNA, peripheral blood lymphocytes were separated with gradient of Ficoll and RNA was extracted with Tripure isolation kit. Tax mRNA levels was evaluated after and before and after lymphocyte activation. Tax expression levels and proviral load were evaluated by real time PCR. **Results:** An insignificant increase in Tax expression was observed in rest PBMCs of HAM/TSP patients compare to healthy carriers. However, after lymphocyte activation there was a significant increase in Tax expression in HAM/TSP patients (p=0.042). Proviral load in patients was significantly higher than carriers. Moreover, there was a significant correlation between Tax mRNA expression in activated PBMCs and proviral load (R=0.37, P=0.012). **Conclusion:** our results demonstrated that Tax

expression and Proviral load may play an important role as risk factors for prognosis of HAM/TSP disease. Since Tax expression in HAM/TSP patients has higher level than proviral load it may be a better marker for disease progression.

Keywords: HTLV-I, Proviral load, Real time PCR

3302P

Evaluation of Agglutination as Serodiagnosis Test in Brucellosis

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Background: Brucellosis which is a significant public health problem is a zoonotic disease that seen throughout the world as well as in Iran. This study was carried out to show the sensitivity and specificity of the serum agglutination test (SAT). **Methods**: Blood and serum sample were collected from 52 patients were included to the study. Blood cultures and SAT were performed from all the patients. **Results:** 50 patients with similar clinical presentation that the disease ruled out by blood culture and SAT and they were symptoms free without any medication for brucellosis in follow up, were as true negative samples. 54.2% of cases had positive unpasteurized dairy consumption history. Chief complaint was joint pain or fever 56.7% and 41.3% respectively. There was history of perspiration in 61.5% of patients. Brucella spp were isolated in 20 (38.4%) of patients. SAT was found positive in 50 samples (96.1%). When blood culture accepted as the gold standard, sensitivity, specificity, positive predictive value and negative predictive value of the test were calculated as follows: sensitivity 90%, specificity 75.7%, positive predictive value 36% and negative predictive value 98%. C**onclusion**: we found that SAT was still efficient method for serodiagnosis of brucellosis.

Keywords: Agglutination, Serodiagnosis Test, Brucellosis

1606P

Comparsion of pattern of autophagy in mycobacterium bovis (BCG) and Leishmania major in Balb/c mice macrophages

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Background: Autophagy is an evolutionary conserved process that promotes cell survival in periods of stress and starvation by providing nutrients and energy. Autophagy has also been linked with innate and adaptive immune responses against intracellular pathogens, including Mycobacterium tuberculosis, which can survive within macrophages by blocking fusion of the autophagosomes with lysosomes.For protozoan parasites, the autophagic machinery has been shown as essential for Leishmania to differentiate into the infective metacyclic promastigote form and increase in infectivity. The aim of this study was comparsion of autophagy in

Mycobacterium bovis (BCG) and Leishmania major in Balb/c mice macrophages by flourescenc microscopy. **Methods:** 6-8 weeks old Balb/c mice peritoneal macrophages were harvested after injection of 10 ml HBSS medium then separately were cultured in 6 and 24- well of microplates with BCG and Leishmania major. After 5 days, cells were collected and used for acridine orange/ethidium bromide staining and visualized by fluorescence microscopy. **Result:** Differences between pattern of autophagy in Mycobacterium bovis (BCG) and Leishmania major in Balb/c mice macrophages were seen ($P < 0.05$). The percentage of autophagy in the macrophages of BCG group was 100% while in the Leishmania major group was 26.3%. **Conclusion:** Activation of autophagy in macrophage of BCG increases the killing of this bactry and controls its infectivity, while for Leishmania major which is intracellular parasite activation of autophagy is not inhibitory, but also this can be beneficial for more survival.
Keywords: Autophagy, Macrophage, Leishmania major, BCG, Balb/c mice

3134P

Seroepidemiology of Hepatitis E virus (HEV) infection in east IRAN -2012

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Background: Hepatitis E is a viral infection that affects the liver and can range from a simple asymptomatic infection to a fulminant and lethal disease is variable. HEV prevalence in developing countries is between 10 to 35 percent especially in pregnant women. Iran is an endemic area and therefore the importance of studying the prevalence of HEV, and the lack of research in this field in Birjand and history of the epidemic in other provinces, this study was conducted. **Methods**: This study was a cross-sectional study that was in 2012. Random sample of visitors to the city of Birjand for blood transfusion was performed within 3 months And 340 samples were collected. After obtaining informed consent and completed a questionnaire. Infection status was determined using ELISA and data were recorded in SPSS software and analyzed using the chi-square test. **Results**: A total of 340 samples, 50 sample were positive HEV IgG, The prevalence equal was14.7%. 20 to 40 year age group had the highest prevalence (58%). There was no significant association with three variables; sex, history of blood transfusion, job and incidence of hepatitis E (p=0.38). Of total sample, there were 70 people living in rural areas that 21 of them were positive for HEV IgG (20.6%) and was significant according to the chi-square test(p=0.001). Among 60 total numbers of samples that non-purified water used, 20 samples were positive HEV IgG. (40%). In this study a significant correlation was also found between the level of literacy and morbidity (p=0.001). 46% of those with no college education were HEV IgG positive. **Conclusion**: According to the results of this study, Birjand in east IRAN is endemic area for HEV infection. Therefore, it is recommended hepatitis patients routinely checked for the HEV infection that in this review pregnant women should be considered.
Keywords: HEV, Hepatitis, Seroepidemiology, Iran

3242P

Association of interleukin-17 gene variants and susceptibility to *H. pylori*-associated gastric diseases

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Background: *Helicobacter pylori (H. pylori*) is the main cause of chronic active gastritis, which can lead to peptic ulcer, gastric adenocarcinoma, and gastric mucosa associated lymphoma. According to the important role of interleukin-17 (IL-17) in pathogenesis of inflammatory diseases and the known effect of IL-17 gene polymorphisms on the production of this cytokine, we investigated the association between single nucleotide polymorphisms (SNPs) of IL-17A gene and the risk of *H. pylori* related chronic active gastritis and peptic ulcer. **Methods:** The study groups included 100 patients suffering from chronic active gastritis and 50 patients with peptic ulcer, in addition to 226 healthy individuals as the control group. Alleles and genotypes at nine polymorphic sites of IL-17 were compared among the study groups by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** The results showed that the distribution of rs3819024 A allele was significantly more frequent in the gastritis patients than the controls (P=0.0003) and was significantly more frequent in the ulcerative gastritis patients than the controls (P=0.0001). Moreover, homozygous rs3819024GG was significantly more frequent in the controls than the gastritis patients (P=0.0008) and also, it was significantly more frequent in the controls than the ulcerative gastritis patients (P=0.0005). Furthermore, AA (rs4711998A and rs3819024A) haplotype was significantly more frequent in ulcerative gastritis patients than the controls (P=0.0002) and GG haplotype was significantly more frequent in controls than ulcerative gastritis patients (P=0.001). **Conclusion:** It could be suggested that IL-17 genetic variants (rs3819024) can affect resistance or susceptibility to *H. pylori*-associated gastroduodenal diseases among Iranian population.

Keywords: IL-17, Polymorphisms, Gstritis, Helicobacter pylori

2534P

Copro Antigen ELISA assay for the diagnosis of Echinococcosis

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Background: Cystic Echinococcosis (CE) is an important zoonosis. The parasites domestic life cycle is maintained through dogs and a range of domestic livestock as an intermediate host. ELISA for detecting parasite- specific antibodies in serum, have been confirmed in several studies. The main goal of present study was Evaluation of Copro Antigen in ELISA assay for serodiagnosis of canine Echinococcosis. **Methods:** Three puppies were inoculated with 70000 protoscolecis. Fecal Samples were collected before exposure and in day 7, 14, 28 and 35 after inoculation. Fecal samples, 5-10 gram, were applied for preparing Copro Antigens. Protein was quantified by Bradford method and quality of proteins was evaluated by SDS-PAGE. Copro

Antigeng,100µl, was dispensed into each well, serum samples in different dilutions were added and the assay were keep on using Goat anti dog IgG HRP conjugated and TMB as a Substrate. The OD read with ELISA reader at 450nm. **Results**: There was significant difference in OD between positive and negative serums ($p<0.01$). But there was no significant difference in OD between different days. **Conclusion:** In the present study Coproantigen-ELISA test was found to be highly sensitive and specific in the detection of canine echinococcosis. The sensitivity and specificity of test was evaluated 100% and 83%. Nowadays Coproantigen tests have applied successfully in epidemiological studies in many countries. In conclusion, the present result suggests that, Coproantigen is a valid test for detection of *Echinococcus granulosus* infection in living dogs. Thus it is appropriate to apply for epidemiological studies.
Keywords: Echinococcosis, Copro Antigen, ELISA

3074P
Evaluation of the Murine Innate Immune Response to Invasive Aspergillosis

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Background: *Aspergillus ecommend* as an opportunistic fungal pathogen could cause life-threatening invasive fungal disease (invasive aspergillosis (IA)) in ecomm-compromised individuals. **Methods:** In this study, we explored whether the innate resistance to IA in Tumor Bearing Mice (TBM) mice has resulted in altered expression of TLR-2 and Dectin-1 in macrophages (flow cytometry) and production of tumor necrosis factor alpha (TNF-α; ELISA) as well as overall mortality. **Results:** The data demonstrated significant increases in Dectin-1 and TLR-2 on peritoneal macrophages and mortality in aspergillus infected TBM mice. TNF-α levels were not significantly increased in this group. **Conclusion:** Probably, invasive aspergillosis causes some disorganization in inflammatory responses. We hypothesize that concomitance of IA and tumor, may change the micro-environment for local or systemic immune responses. Other complementary studies are required to support our hypothesis.
Keywords: Aspergillosis, Tumor, Cytokines, Dectin 1, Toll-like receptor 2

2299P
The evaluation of rubella virus avidity in early pregnancy women's following vaccination in East Azerbaijan

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Background: Primary Rubella Virus infections during the first 12 weeks of pregnancy have severe consequences for fetus and cause a disease known as congenital rubella syndrome.After new approaches in IgG avidity tests, safe discrimination of primary infections and reinfections

with a single serum sample became possible. During the 1980s modifications of EIA were developed due to efficient use of avidity tests. **Methods:** Sera from 183 pregnant women in the 2^{nd}-4^{th}initial months of their pregnancy period and were at risk of Rubelosis were analyzed for rubella specific IgG avidity during a four year period (Oct-2008 to Oct-2012) using Hedmann method.Avidity test was performed according to the avidity-index (AI) method of Hedman et al. using Euroimmun kit and AI was calculated with this formula: (OD of the sample treated with Urea – OD of the blank)/ (OD of the sample treated without Urea) × 100. Achieved data were analyzed with SPSS14. **Results:** Data were analyzed in three range of avidity. Results showed that 18.57%(34) of cases had AI around 80-90%, 9.28%(17) under 80% and 72.13%(132) above 90%. **Conclusion:** In this study high percentage of women with IgG avidity index above 90 %, showed that the pregnant women had an experience of Rubellosis or vaccination before their pregnancy and results showed that active Rubella infection rarely was observed. Since pregnant women are not usually referred to rubella screening tests before pregnancy, the avidity test used to diagnose the active rubella infection during pregnancy is of a high value.
Keywords: Rubelosis, Pregnancy, Avidity, IgG, Vaccination

2841P

Serotyping of Typhoidal and Non-typhoidal Salmonella species by Serology method and Electrophoresis of proteins soluble in water by SDS-PAGE technique

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Background: The genus of Salmonella is one of the most important of Enterobacteriacea family can cause serious diseases in human and animals. The aim of this study was extraction of whole-cell proteins of typhoidal and non-typhoidal Salmonella from patients with systemic infections by polyacrylamide gel electrophoresis SDS-PAGE method and comparing with serotyping of species. **Methods:** In this study, 100 cases of Salmonella strains were collected from patients who referred to cilinical centers in Hamadan and 4 reference strains of Salmonella species were also included in study. Serotyping of strains were performed by Biomerix and diffco monovalent antisera. Whole-cell proteins of strains were also detected by 10% polyacrylamide gel. Gels were stained by coomassie Brilliant Blue and photogarphed through an orange filter. Rate flow (RF) of each protein band was also determined. The density of protein bands were analysed by densitometery. **Results**: Of 100 cases of Salmonella species isolated from patients,: 43 cases (43%) were *S. typhy*, 20 cases (20%) *S. typhimurium*, 12 cases (12%) *S. paratyphy B*, 10 cases (10%) *S. paratyphy C* and one case was also *S. paratyphy A*. The results of serotyping were compared with the results obtained by SDS-PAGE. Many protein bands from major protein 220 KD_a to minor protein 18.5 KD_a were detected by SDS-PAGE and differentiated the strains well. Protein profiles of clinical strains were compared and showed some variations with results of serotyping and could divided *S. typhy* to 5 subgroups and *S. paratyphy B* and *S. paratyphy C*each to 3 subgroups. **Conclusion :** The our results showed that extraction of whole-cell proteins of typhoidal and non-typhoidal Salmonella speceis by polyacrylamide gel electrophoresis SDS-PAGE technique could be used more reliable than serotyping methods for identification and typing of these microorganisms. However, it is needed more researchs for other organisms to demonostrate this sugestion
Keywords : Serotyping, Salmonella, Typhoid, SDS-PAGE

3063P

Evalation of the relative frequency of IgA in giardiasis patients

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Background: Giardia lambelia is one of the most common intestinal protozoa throughout the world. This parasite inhabits the upper part of the small intestine and has a direct life cycle. In human, the clinical effects of Giardia infection vange frome the asymptomatic carrier state to a severe malabsorption syndrome. Factors possibly contributing to the variation in clinical manifestations include the virulence of te Giardia strain, the number of cysts ingested, the age of the host, and the state of the host immune system at the time of infection also regarding to mechanisms of protection and immunity by IgA , decreas of IgA level could proride a condition for adherence of micro ecommen agents to a mucosal surface and cause infection of intestinal. We conducted a study of the rate of IgA in the serum of patients with Giardiasis. **Methods**: In this ecomm, during 18 months from may2002 to November 2003 from 110 refering patients Giardiasis and 50 healthy controls who were negative for Giardia and other parasites were selected, to the hospital in the city of Mashhad. The patients data including age, sex occupation, residency and educational level were recorded 5cc venous blood were taken and serums were frizzed. Specimens were collected in 2.5% Formaline and in physiological saline solution. For Giardia, specimens by direct smear and Formaline-ether technique we used. And were studied in term of their serum IgA level using single Radial Immuno diffusion(SRID). **Results**: For 110 patient's Giardiasis (58males and 52 females) were examined and control groups (33males and 27 females). The range age of the patient's were 2-59 years 8 case with under normal serum level were observed (<80 mg/dl) and in other 97 case, the serum level rate was similar in both ptiont and control. There was no significant difference regarding in both groups. **Conclusion**: The result we of the study showed that probably decrease IgA could play important in causing develop behavior and longer lasting infections that those who are not.

Keywords: Giardiasis, IgA, Patients

1766P

Prevalence of bla-SHV and bla-GES in burn isolates of Pseudomonas aeruginosa, Zare hospital, Sari, Iran

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Background: Burn patients are at high risk developing nosocomial infection because of their destroyed skin barrier and suppressed immune system,compounded by prolonged hospitalization.*Pseudomonas aeruginosa* remains a leading pathogen causing burn wound infection.The emergence of extended spectrum beta lactamase resistance in this organism is becoming a challenging problem in infection control programmes. The objective of this study was to determine the frequency of bla-SHV and bla-GES among the *P.aeruginosa* isolated from burn infection in Zare hospital,Sari,Iran. **Methods:** A total of 143 strains of *P. aeruginosa* were isolated from burn wound of patients in Zare hospital,Sari,Iran.The antibacterial susceptibility to 9 antibiotics was determined by disk agar diffusion method.ESBL,phenotypic detection was carried out by combined disk method.Polymerase chain reaction(PCR)amplification of

the gene encoding bla(SHV) and bla(GES) was also performed. **Results:** Out of 143 isolates of *P. aeruginosa*,60 (%41.95)were ESBL positive by phenotypic method.The presence of gene encoding bla- SHV enzyme was confirmed in 22(%36.66) isolates.Non of the isolates were producing bla-GES. **Conclusion**: Noticing the increasing rate of the ESBLs producing strains and production of bla- SHV enzyme in *P.aeruginosa* in burn patients,choosing the appropriate protocol for use of antibiotics,and also avoid spreading of the resistance genes among bacteria is highly ecommended.

Keywords: Pseudomonas aeruginosa, ESBL, Burn patients, SHV, GES

1522P

Measurment of serological indexes and liver enzymes level in hepatitis B virus by bio-chemical and molecular method in mashhad

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Background: Hepatitis B virus is the most frequent cause of acute and chronic Hepatitis in the world. HBeAg and other markers should be assessed in the carriers of Hepatitis B virus for the viral replication status. Positive patients with elevated aminotransferase should be treated with anti viral agents. Our aim was to determine the best substitute of viral load in subjects infected by hepatitis B virus. **Methods:** In a case-control study in 2010-2011, 101 infected patients with hepatitis B virus were examined for the presence of serological indexes and the level of liver enzymes by ELISA and biochemical test and PCR. The patients were selected from Jahad Daneshgahi laboratory in Mashhad city. **Results:** The trequencies of male and rivat in 101 hepatitis B infected patients were 58 and 43, respectively.They were between 20 to 75 years old with the mean of 38.71 .serum HbeAg was 0.27 OD and riva also significant differentce between SGOT,SGPT,HbeAG and viral load (respectively ,p-value:0.001,p-vale< 0.001,p-value< 0.01). **Conclusion:** Based on our results the increase of viral load is accompamied with positive HbeAg and increased level of liver enzyme.So the impact for finding the best substitute of viral loud method in patients should be assessed in future studies with more statistic.

Keywords: Serological indexes, Liver enzymes, Hepatitis B virus, Mashhad

2013P

Characterization of the cellular immune function in mice with systemic candidiasis

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Background: Systemic candidiasis is an infection of Candida albicans (*C. albicans*) causing disseminated disease and sepsis, invariably when host defenses are compromised. We

investigated the proliferative responses and cytokine synthesis of splenic cells after stimulation with Concanavalin A (Con A) and Pokeweed mitogen (PWM) in mice with disseminated candidiasis. **Methods:** Lymphoproliferative responses were stimulated *in vitro* with mitogens in RPMI 1640 media, and the production of interferon (IFN)-γ and interleukin-4 (IL-4) in the supernatants was measured by enzyme-linked immunosorbent assay (ELISA). **Results:** The results showed that *C. albicans* organisms multiplied to a greater extent in the kidneys than in the liver and spleen of infected mice. The most predominant forms of *C. albicans* in different parts of the kidneys were yeast mixed with hyphal forms. Infected mice had a significantly increased proliferative response when splenocytes were stimulated with PWM (2.0±0.16) and Con A (1.9±0.19) ($P<0.05$). PWM and Con A-stimulated production of IFN-γ significantly tended to be higher in infected mice (PWM: 68.4±14.0 pg/ml; Con A: 53.7±17.3 pg/ml) when compared to controls ($P<0.05$). Stimulation with PWM and Con A showed no differences in IL-4 production between infected mice and controls. **Conclusion:** These findings demonstrated a significant increase in both cell proliferation and IFN-γ secretion in supernatants of PWM and Con A- stimulated splenocyte cultures obtained from mice with disseminated candidiasis.
Keywords: Systemic candidiasis, *Candida albicans*, Lymphocyte proliferation, Cytokine, Mitogens.

2014P

Evaluation of interleukin (IL)-2, IL-10, IL-17 and Interferon (IFN)-γ in patients with chronic mucocutaneous candidiasis

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Background: Chronic mucocutaneous candidiasis (CMC) is a group of disorders, characterized by persistent mucocutaneous infections with *Candida* species. The underlying defect of CMC has not been elucidated, but a defective cytokine response may be involved. Therefore, we investigated whether an imbalance between Interferon (IFN)-γ and some interleukins may play a role in this disorder. **Methods:** Phytohaemagglutinin (PHA) mitogen and *Candida albicans* (*C. albicans*) antigen proliferation assays were performed by culturing PBMCs in RPMI 1640. The levels of interleukin (IL)-2, IL-10, IL-17 and Interferon (IFN)-γ present in the supernatant of cultures were determined using enzyme-linked immunosorbent assay (ELISA). **Results:** The results showed that most patients (92.3%) had a low proliferative response to *C. albicans* antigens and PHA. PBMCs from CMC patients produced lower levels of Th-1 cytokines IL-2 (78.5±59.8 pg/ml) and IFN-γ (115.1±43.3 pg/ml) in response to *Candida* antigens when compared to controls (Il-2: 177±103.6 pg/ml; IFN-γ: 330.3±21.6) ($P<0.05$). Conversely, we observed a partial enhancement of IL-10 in the patients (213.7±86.1 pg/ml). Production of IL-17 indicated no significant differences between patients and controls when stimulated by *Candida* antigens (21.5±8.6 pg/ml versus 32.4±12.2 pg/ml) and PHA (27.7±11.5 versus 36.2±9.1 pg/ml), respectively. **Conclusion:** These findings suggest that *Candida* antigens trigger a Th2 instead of Th1 cytokine response in patients with CMC. For better understanding, further studies require on a larger number of patients into the future.
Keywords: Chronic mucocutaneous candidiasis, *Candida albicans* antigen, ConA, cytokines secretion.

2288P

Evaluation of IL-17 gene expression in HTLV-1 infected individuals

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Background: Human T-Lymphotropic Virus Type1 (HTLV-I) infection is associated with several inflammatory disorders, including the chronic inflammatory disease known as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). It is unclear why a minority of infected subjects develops HAM/TSP. IL-17 has a pivotal role in many autoimmune and inflammatory conditions such as multiple sclerosis. The aim of this study was to evaluate the effects of the IL–17 in HAM/TSP patients and HTLV-1 asymptomatic carriers and healthy carriers, To arrive at new insights into functions of IL-17 in HTLV-1 infections. **Methods:** In This study, 60 cases classified in three groups, including HAM/TSP patients (20 subjects), HTLV-1 carriers (20 subjects) and healthy people (20 subjects) were studied. The SYBR green real-time PCR assay was designed and optimized for evaluation of IL-17 human gene expression in baseline and in activated cells by PMA and Ionomycin. **Results:** In contrast to previous studies, in the present study, the results showed a significant decreasing in IL-17 gene expression in HAM/TSP patients compared with carrier and healthy controls ($p<0.05$). **Conclusion:** It is unclear whether Th17 cells may contribute to the immune response to HTLV-1 replication as well as to the proinflammatory milieu seen in HAM/TSP patients.Therefore,The biological functions of IL17 deserve further examination to unravel their impact on the course and severity of inflammatory and degenerative HTLV-1-associated diseases.

Keywords: HTLV-1, HAM/TSP, IL-17

2615P

Prevalence of Brucellosis in the Sheep and Cow Herds of Kermanshah Province of Iran

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Background: Brucellosis is a zoonotic disease distributed worldwide and characterized by abortion and reduced fertility in animals. Although brucellosis and its means of transmission were discovered over 100 years ago, the disease remains a worldwide problem; predominantly in developing countries. Since brucellosis eradication programme in Iran uses vaccination, test, slaughter and quarantine as control measures, it is essential to distinguish the prevalence of the diseases among the herds. This study was performed as a cross sectional survey to uncover the prevalence of brucellosis in herds in the Kermanshah province of Iran. **Methods:** As a descriptive cross sectional survey, between year 2008 and year 2013, a total of industrial and semi-industrial husbandry of sheep and cow were screened to determine the seroprevalence of brucellosis. 12000 flock of cow were monitored every year while the sheep flock (average 1000 sheep per year) was surveyed according to the regional focus. The survey was carried out in Kermanshah province of Iran, where the people who lived in near areas of husbandry

had the history of positive result of brucellosis test, reported by the ministry of health. Blood samples were obtained from sheep and cow by venipuncture and transferred to the laboratory under chilled conditions, as soon as possible. Serum was isolated by centrifuging the blood samples at 2000g. All serum antibodies were tested for Brucella genus using slide agglutination by rose rivat test. Tube agglutination test (TAT) by 2-mercaptoethanol just for cow's serum, used for the presence of antibodies against *B. abortus* strain. **Results:** From the total of blood samples, 6 samples of cows were positive for brucellosis in 2008. In this year, the sheep were not sampled. The most seropositive cases were found at year 2009 (2 cases for cows and 2290 cases for sheep). 6, 5, 0 and 2 cases of cows were positive in year 2010, 2011, 2012 and 2013, respectively while in these years, 12, 306, 25 and 0 cases were subjected as seropositive sheep, respectively. **Conclusion**: The results of our investigations indicate that control and eradication programs among animals should be regarded as a priority measures in prevention of brucellosis. The zoonotic aspects of brucellosis from animals must, therefore, be considered because the disease is important from the public health standpoint. When the disease exists in animals, which is a reservoir, it is a concern for human public health. In order to control this zoonotic disease, close cooperation of health and veterinary organizations is necessary.

Keywords: Brucellosis, Kermanshah, Zoonosis

2247P

Role of HLA-B7, B8, B27, and B51 in Protection against Hepatitis B Virus Infection

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Background: It has been argued that unprecedented degree of human leukocyte antigen (HLA) loci polymorphism within a population is required to avoid the devastating effects of infectious diseases. The present study was conducted to determine the associations between some of HLA class I genes and the outcome of hepatitis B virus (HBV) infection. **Methods:** Using sequential sampling method, 64 individuals were selected and categorized into two groups according to their clinical and serological profiles. In the case group were 27 patients with chronic HBV infection and in the controls were 37 individuals considered as HBV natural convalescent. Antibodies against HbsAg (anti-HBs) and hepatitis B core antigen (anti-HBc) were assessed to include primary HBV infection. Individuals with viral hepatitis B infection were positive for HbsAg at two time points. HLA typing was performed by serological method. Collected data were analyzed by SPSS software version 13. **Results:** The most frequent HLA antigens among the studied subjects were B51 (40. 1%), B27 (14.1%), B8 (12.5%), and B7 (10.9%). A significant correlation was found between HBV persistence and HLA-B27 ($P<0.05$). The association between HLA B51, B8 and B7 with HBV clearance was not significant. **Conclusion:** Findings of the present study demonstrated an association between HLA class I and outcome of HBV infection where HLA B27 was linked to an increase in HBV persistence. These findings support the hypothesis that HLA class I-restricted cytotoxic T cells play an important role in HBV chronicity.

Keywords: HLA, Hepatitis B Virus infection

2550P

Identification of *Salmonella Gallinarum* and *Pullorum* by PCR-RFLP

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Background: *Salmonella rivate serotype Gallinarum* (SG) and *Salmonella rivate serotype pullorum*(SP) are non-motile host-specific avian pathogens. *Salmonella pullorum* causes Pullorum disease. *Salmonella Gallinarum* causes fowl typhoid. Some countries are considered free from *Salmonella Gallinarum* and *Sallmonella Pullorum*.However, these are sometimes reported, and are still a matter of concern in the poultry industry. They are very similar, and cannot be distinguished by conventional serological and biochemical methods. The standard methods take approximately 5 to 7 days, and are very time-consuming and expensive. Iochemical methods have been complemented by DNA-based molecular techniques, because of their sensitivity, specificity, and swiftness. Such methods include restriction fragment length polymorphism (RFLP), which is sometimes associated to PCR (PCR-RFLP). Most Salmonella strains have two structural genes (*fliC* and *fliB*) that encode flagellins. Non-motile strains generally exhibit these structural genes, but are unable to build up a functional flagellum. The objective of the present study was to differentiate *Salmnella Gallinarum*and *Salmonella Pullorum* isolated in Iran, by PCR-RFLP using *Hinp 1I* enzyme. **Methods:** The bacterial used in this stady were obtained from Razi Vaccine &Serum ResarchInstitute of Karaj. 1.Culture of bacteria 2.DNA extraction 3. PCR primers: The following two primers were used for the amplification of flagellin gene phase 1(FliC) 4.PCR according *fliC* gene 5.Gel electrophoresis (197 bp) 6. PCR-RFLP with *Hinp 1I* enzyme 7.Gel electrophoresis (197 bp or115 and 82 bp based on bacteria). **Results:** Amplification of the *fliC* gene. The expected 197 bp fragment of the *fliC* gene was successfully amplified from all *Salmonella gallinarum* and *pullorum* strains. PCR-RFLP analysis: Digestion of *Salmnella gallinarum* amplicons with *Hinp 1I* yielded two bands, of 115 and 82 bp, while no change in SP amplicons was observed, since no digestion occurred. **Conclusion:** *Salmonella Gallinarum* and *Salmonella Pullorum* are considered important pathogens, causing, respectively, Pullorum disease and fowl typhoid in poultry . *S. Gallinarum and S.Pullorum* represent the same serovar but different biovars, their identification and differentiation is based mostly on biochemical characteristics. The PCR-RFLP system has been frequently used in differentiation techniques because it is cheap and easy to perform. In our study, the fliC gene in SP and SG were amplified. PCRamplicons(197bp) were digested with the *Hinp1I* enzyme. Two fragments were obtained (82bp and115bp) for all SG strains, whereas no digestion was observed in the SP strains. In the present study, we were able to demonstrate that the use of *fliC* gene restriction patterns is an useful method to allow the differentiation between strains of *S. Pullorum* and *S. Gallinarum* isolated in Iran, including those with atypical biochemical behavior. Therefore, our results reinforce that this method may be adopted to differentiate SP from SG.

Keywords: Salmonella Gallinarum, Salmonella Pullorum, Hinp 1I, PCR-RFLP

2687P

Evaluation of Interleukin-10 Gene Promoter Polymorphism (-819 C/T) in Patients with Chronic Hepatitis B Infection

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Background: Hepatitis B infection is a major epidemiological problem. The clinical outcome of hepatitis B virus (HBV) infection varies between individuals from spontaneous viral clearance to chronic hepatitis. This diversity may be due to alternations in genes encoding cytokines. Interleukin-10(IL-10) as an anti-inflammatory cytokine may have an effect on susceptibility to HBV infection.The aim of this study was to investigate the role of IL-10 gene single nucleotide polymorphism(SNP) -819 C/T as a marker for predicting susceptibility to HBV infection. **Methods:** Blood samples were collected from 143 ELISA positive hepatitis B.Control group included 126 healthy subjects. Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) assay were used for polymorphism genotyping. **Results:** The results suggest a higher frequency of CT genotype among HBV patients in compare with healthy controls, 50.3% and 35.7 % respectively. A statistically significant difference was found in the genotype frequency of IL-10 promoter SNP between HBV patients and healthy controls (*P*=0.013). **Conclusion:** Our findings indicate that IL-10 -819 C/T might be associated with HBV infection and the SNP can serve as a biomarker predictive of risk for the development of HBV chronic infection in Iranian patients.

Keywords: Single nucleotide polymorphism, Interleukin-10, Hepatitis B virus, Genotype

2577P

Prevalence and isolation of *Mycobacterium fortuitum* from environmental water and clinical samples from immunocompromised patients

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Background: Atypical Mycobacterium species are considered as one of the most important factors of nosocomial and occupational infections. Absence of evidence regarding person to person transmission indicates the importance of environment as an infection source. Therefore studying the possible sources, as well as common species in each geographic area is required. Amongst the species *M.fortuitum*is one of the candidates to be studied. Epidemics of hospital-associated infections caused by *M.fortuitum*after surgeries, in haemodialysis unit and in eye, dermal and many other infections show the real importance of this group of

mycobacteria in the health system. It is believed that Non-tuberculous mycobacteria have paramount value to create a variety of infectious and emerging diseases in individuals; both immunodeficient and cases with normal immune systems have been reported. Published case reports from Iran shows the pathogenic potential of this mentioned species. The purpose of this study was to investigate the contamination of surface water, as well as clinical specimens of *M.fortuitum*by means of molecular and phenotypic methods. **Methods:** Water samples were collected from Tehran, Iran as well as some local parts in north and south of Iran. Isolation of atypical mycobacteria from environmental samples was performed by CPC method. Atypical clinical mycobacteria were also isolated from pulmonary and extrapulmonary samples. Followed by isolating and culturing the samples on LJ media differential tests including Nitrate reduction, catalase activity test, Niacin test, and growth rate and pigment production were carried out. RFLP-PCR test was to confirm the result. **Results:** In this study 41 atypical mycobacteria were isolated from environmental samples; based on growth rate and differential test results as well as molecular analysis *M.fortuitum* was identified with the relative frequency of 46.41 %(17 cases). Moreover in clinical samples of study 11 atypical spices were isolated that *M.fortuitum*accounted for 5.54% (6 cases). **Conclusion:** Given the increased prevalence of immune deficiency diseases in the community and presence of Mycobacteria species in environmental samples such as different aquatic sources, awareness and gaining more knowledge species dispersion is essential. Importance of species spreading in different geographical areas is being shown in different treatment strategies and should be clinically considered. Meanwhile, in Iran *M.fortuitum* is the dominant species which has been reported from clinical samples.

Keywords: RFLP-PCR, Mycobacterium fortuitum, Immunocompromised patients

3250P

Interleukin-17A Gene Polymorphisms in Visceral Leishmaniasis

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Background: Immune responses against visceral leishmaniasis (VL) are associated with host genotype, and resistance to infection occurs by Th1 responses leading to macrophage activation and parasite killing. Leishmania parasites can stimulate the production of IL-17A by Th17 cells which strongly associated with protection against VL, independently from Th1 cells. Previous studies had shown that 197A\G SNP can influence IL-17 gene expression in tuberculosis. The aim of this study was evaluating the polymorphisms of 197A\G SNP in VL patients and asymptomatic VL cases. **Methods:** The blood samples were collected from 259 individuals; 88 patients with history of clinical symptoms of VL, and 171 cases with no clinical sign of VL all from the same endemic area of VL in North-West Iran. The causative parasite of VL in this endemic area is *L.infantum*. DNAs extracted by salting out method. ARMS-PCR was performed for detecting IL-17A (197A\G) gene polymorphism. IFA method was used for evaluating anti-leishmanial antibodies. **Results:** In VL patients group 12(13.36%) cases showed A/A genotype and 33(37.75%) G/G genotype. The heterozygote form (A/G) was 43

(48.86%) cases. The proportions of genotypes was12. 3% for A/A, 45.01% for G/G and 42/07% for A/G heterozygote form. In patients group the A/G heterozygote form was most frequent. Our results showed no significant association between IL-17A gene polymorphism in 197A\G region and clinical VL infection. **Conclusion:** The association between IL-17A 197A/G and IL-17F 7488 A/G polymorphisms and susceptibility to gastric cancer has been demonstrated. There is also another results showing association between IL-17A gene polymorphisms and risk of cervical cancer in Chinese population. *L.donovani* stimulates proliferation of Th17 cells and production of IL-17. Our results are the first on association between IL-17A gene polymorphisms and VL due to *L.infantum*. The differences between our studied genotypic alleles were not statistically significant but there is a potential for molecular analysis of other gene polymorphisms of IL-17 in VL patients, preferably in a wider population.

Keyword: Interleukin-17A Gene, Polymorphism, Visceral Leishmaniasis

2770P

Quantification of HTLV- I proviral load in different organs of BALB/c mice by Real–Time PCR

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Background: It has been suggested that Human T-cell leukemia virus type-I (HTLV-I) is able to induce infection in mouse. Therfore, Integration of HTLV-I provirus genome was examined by quantification proviral loads in peripheral blood mononuclear cells (PBMCs), spleen and rivateti lymph nodes. **Methods:** MT-2 HTLV-I cell line were intraperitonealy inoculated (IP) in 4-week-old BALB/c female mice. The mouse were sacrified after 2 month and HTLV-I genome (Tax and LTR) was examined by PCR. Real – time PCR (TaqMan method) was applied to measure the HTLV-I proviral load in different organs including PBMCs, spleen and rivateti lymph nodes. The HTLV-I proviral loads was calculated in 10^4 cells. **Results:** Provirus was detected in the PBMCs, spleen and rivateti lymph nodes. The number of proviral loads was 32±15 copies/10^4 cells, 17±6 copies/10^4 cells and 39±9 copies/10^4 cells in PBMCs, spleen and rivateti lymph nodes, respectively. The rivateti lymph nodes had higher proviral load compared to PBMCs and spleen. **Conclusion:** In conclusion, It seems that animal models such as mice are useful for studing of HTLV-I infection and also rivatetiction of virus behaviours. Furthermore, this model would help to design rivatetic procedures for HTLV-I infection in human. Furthere studies are needed to examine the immune response and genetic factors are involved in pathogenesis of HTLV-I in mice.

Keywords: Human T-cell leukemia virus type-1, Real–Time PCR, animal model

3084P

Analysis & measurement of memory B cell in mice immunized by Botulinum toxin Binding subunit (Hc)

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Background: *Clostridium botulinum* causes botulism disease. BONT/A-Hc toxin subunit could induce two years immunity against disease. It seems the immunogenicity potency of these this subunit maybe influences on the memory longevity. The purpose of this study is analysis & measurement of memory B cells after mice immunization with Hc subunit of botulinum toxin. **Methods**: expression of recombinant protein of Hc subunit was carried out in optimized conditions. After purification of the recombinant protein by nickel affinity chromatography column, it was injected into mice. Spleen cells were extracted after six months. Then the cells were stained with antibodies against CD19, IgD, and IgG and they were analyzed by flow cytometry. **Results:** SDS-PAGE gel was confirmed expression and appropriate purification of recombinant protein of Hc subunit. ELISA data was shown high titer antibody level against BoNT/A-HC in mice. After six-month, there was high percentage of memory B cells in the spleen of test group versus control group. **Conclusion:** After six months, although antibody titration was decline but the memory B cell populations were persistence in the spleen. It seems Hc sub unit could induce a long term memory B cell in mice model.

Keywords: Immunization, Flow cytometry, Memory B cells, Protein BONT/A-HC

2644P

Identification of Immunotopes of Mycobacterium leprae as Immune targets for anti-lepra subunit vaccine by M13-phage display library

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Background: Leprosy is a debilitating condition in physical, mental and emotional terms. Unfortunately patients with leprosy are also found in Iran. One way of preventing the infectious diseases is developing rivat vaccines against disease-causing agents. **Methods:** Blood samples from 34 patients with lepromatous leprosy was obtained and after separation and concentration of serum antibodies, ELISA wells were fixed with the resultant solution, and then added the recombinant M13 phage libraries that expressed short random peptides on their surface. Then the unbounded phages to the antibodies were removed by washing and in the next stage bounded residues isolated and were amplified in an appropriate host (*E.coli* ER2738). **Results:** However there was a significant differences among the 11 clones obtained for 3 clones numbered one, six and nine in first, sixth and final dilutions which are corresponds to 1×10^{12}, $2.2\times 10^{8}$and 2×10^{5} dilutions respectively. Isolated clones have a peptide

sequence at their surface sites which has positive reaction with the sera of leprosy patients. **Conclusion:** In this study we obtain peptides which bind with high affinity to the antibodies against *Mycobacterium Leprae*, we got a large quantities of them and after proliferating these clones in suitable hosts. Monoclonal antibodies can be produced after injection them to the appropriate animals, by which the serological diagnostic kits can be develop for the detection of this bacterium and disease. The immunotope surface antigens of *Mycobacterium Leprae* were obtained based on the assessment of biological activity of monkey topes in animal models, which might be good candidates for vaccine development.

Keywords: Bacteriophage, Vaccine, *Mycobacterium Lepra*e, Random peptide phage display library

2718P

Blood level Anti-HBs due to HBs-Ag vaccine in health care personnel of Torbate heydarieh University of Medical Sciences

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Background: About 3% of the Iranian general population are carriers of hepatitis B virus, and about 15% of infected persons will be prone to chronic hepatitis and get cirrhosis and liver carcinoma. Up to now no therapeutic regimen has been introduced to eradicate completely this infection. **Methods:** This cross-sectional study was performed on 466 health care personnel of Torbate heydarieh Universe of Medical Science. At first, a questionnaire including information about sex, age, vaccination, etc was completed. Anti-HBs were examined by (ELISA method). **Results:** From 466 subjects, 250 persons (53.6%) were female. The mean age of studied cases was 31.5 years. The mean titer of anti-HBs was 135.5 mlU/ml, and with consideration of Anti-HBs =10 mlU/ml as a cut-off value, 92.3% of subjects were immunized against hepatitis B. **Conclusion:** According to the results and decrease of immunity in health care personnel after years, it is suggested that the Anti-HBs titer should be examined one month after vaccination and be controlled every 5-10 years, and then booster vaccine be injected after reducing Anti-HBs titer to below protective level.

Keywords: Hepatitis B, Vaccination , Anti-HBs , Health care personnel

2719P

Seroepidemiological Prevalence of *Helicobacter Pylori* of the Patients Referred to the rivate Laboratory in Torbate heydarieh province, 2012-2013

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Background: *Helicobacterpylorus* is the major cause of inflammation, ulcer and malignancy in stomach,and immunoglobulin IgG is one of the antibodies produced against it,which is important in the course and diagnosis of the previous sufferers. The awareness of the prevalence of this disease can be helpful for the physicians to choose the way of treatment. **Methods:** In these cross-sectional study,1201 patients referred to laboratory was studied. After separating the serum, Antibody *Helicobacter pylori* IgG test was done by ELISA method. **Results:** Of

1201, 407 (33.8 %) of the patients have a positive result, 154 (37.8%) are males and 253 (62.7%) are females. Positive percent of males (36%) is greater than females (32.8%). Over-40-year-old patients (41.6 %) have the highest percentage of disease titers. **Conclusion:** The percent of positive cases in men is more than that the women. Over-40-year-old patients (41.6 %) have the highest percentage of positive case.

Keywords: *Helicobacter pylori* IgG, Torbate heydarieh, ELISA

3137P

A serologic parasitologic study of ocular toxoplasmosis in patients referring to some ophthalmology centers in Tehran

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Background: Toxoplasmosis is one of the most prevalent parasitic infections common between human and animals. *Toxoplasma gondii* is considered as an opportunistic and the most dangerous infection. The parasite reaches to the eye and its retina through circulation. After that, the parasite goes to the choroid and affects its neuronal layers. Toxoplasmosis is known as the most common causes of chorioretinitis worldwide. Attacks to the eye could be chronic and sometimes infection relapses. The damaged tissue which is associated with the eye neural layers such as retina and choroid would not repair. Emergence of clinical symptoms in patients is related to age, the injured site and cyst size. An injury can remain active about four months and often the site of injury is in the posterior pole or proximal to it. The posterior pole scares can persist a serious threat for the vision throughout one's life. Complementary, serologic tests are carrying out for recognition of acquired or relapse infection. The aim of the current study is to represent efficient and effective laboratory methods for precise and quick diagnosis of ocular toxoplasmosis. **Methods:** In this study, sampling had done from 3 major ophthalmology centers in Tehran (Farabi, Labbafinejad and Imam Hossein Hospitals). The patients suspicious to ocular toxoplasmosis examined and their clinical symptoms recognized. Blood sampling taken and the serum was assessed for antibody titers of IgM and IgG by ELISA kit. Thereafter the serum was assessed for antibody titers of IgM and IgG by ELISA kit. **Results:** From the 52 blood samples of the patients suspicious to ocular toxoplasmosis, 20 patients (38%) had positive form of the disease in serologic test (ELISA) as follows: 8 patients (15%) showed positive titer IgG^+ and IgM^+ and 12 patients (23%) demonstrated IgG^+ and IgM^- titer for the disease. **Conclusion:** According to the clinical diagnosis, ophthalmoscope observation and evaluation of active and passive scares as well as clinical symptoms, complementary, ELISA and ophthalmoscope methods are of great importance in the two clinical diagnosis of the disease. While sensitivity and characteristic of ELISA is somewhat higher compared to ophthalmoscope method.

Keywords: Ocular toxoplasmosis, Chorioretinal scar, Toxoplasmosis antibody, IgG Avidity test, Retinochoroiditis, Uveitis, ELISA

2728P

H.pylori infection upregulates embryonic stem cells factors in human mesenchymal stem cells

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Background: Previous studies have shown that *H.pylori* infection plays a crucial role in gastritis and gastric cancer. In addition, previous investigations conducted by us and others comprehensively demonstrated that persistence of *H.pylori* infection leads to migration of bone marrow derived – mesenchymal stem cells (BMD-MSCs) to inflamed stomach. Eventually, migrated BMD-MSC transform to gastric cancers. However, its mechanism has not been yet elucidated. As embryonic stem cells factors have been shown to be involved in induction of cancers and survival of cancer stem cells, we investigated the possible role of *H.pylori* infection on expression of these factors in BMD-MSC. **Methods:** First, *H. pylori* was isolated from gastric biopsy samples and characterized by microbiological and PCR techniques. BMD-MSC were isolated from human bone marrow, cultured and characterized as by flow cytometry. The BMD-MSCs were infected by co-culturing of these cells with *H. pylori* (for one cell 100 bacteria). Then the expression of embryonic factors Sox2, Nanog , Oct4 were examined by RT-PCR. **Results:** BMD-MSC was positive for CD105, CD90 and they were negative for hematopoetic markers such as CD45 and CD34. When we infected the BMD-MSC cells with *H. Pylori*, we found *H.pylori* infection significantly upregulates Sox2 expression in these cells, indicating that Sox2 may play an important role in pathobiology of *H.pylori*-mediated gastric cancers. Conversely, we observed that *H.pylori* infection did not show any effect on expression of other embryonic factors such as Nong and Oct4. **Conclusion:** We demonstrate for the first time that *H.pylori* infection upregulates Sox2 in human BMD-MSC cells which might play a role in pathogenesis of *H. pylori*-mediated gastric cancer.

Keywords: Embryonic stem cells, Helicobacter pylori, Mesenchymal stem cells

2749P

Study of Genetic Susceptibility of Brucellosis

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Background: Th1 cells in *Brucella* immunity produce (IFN-γ) activate the bactericidal function of macrophages and cytolytic activity of cytotoxic T lymphocytes (CTLs) to eradicate the parasite. TNF-**α** production seems to be necessary for full expression for macrophage anti-brucella activity.Variation in the TNF-**α** promoter region has been associated with severe forms of infection diseases with high serum TNF-**α** levels. As a result, it has been proposed that these associations reflect genetic variability in TNF-**α** production which influences the clinical outcome of infectious diseases. We therefore decided to investigate the contribution of the TNF-α gene promoter region polymorphisms and HLA class II genes to susceptibility to or development of clinical forms of *B. melitensis* infection, in east Azrarbaijan population, where the disease is endemic. **Methods:** The present study included 69 patients with brucellosis

from the Infectious Diseases Unit at different hospitals in east Azrarbaijan province. Their mean age was 35·5 years (range 17–74 years), 31 (52%) were women and 28 (40%) men. A control group was composed of 60 healthy volunteers matched for age and sex, living in the same area as the patients. The diagnosis of brucellosis was established by with the presence of high titers of specific antibodies or a four-fold increase or greater of the initial titers in two paired serum samples drawn 2–4 weeks apart. High titers were considered to be 1/160 for Wright's seroagglutination test or 1/320 for Coomb's anti-brucella test. DNA was isolated from anti-coagulated peripheral blood mononuclear cells (PBMC) using standard methods. The distributions of the TNF-α promoter genotypes is distinguished in the patient groups and in the controls .Data were analyzed by χ^2 test with Yates' correction or Fisher's exact test. **Results:** The existence of linkage disequilibrium between alleles at TNF-**α** and HLA loci is well known; therefore we examined the possibility that the TNF-**α** brucellosis observed associations were primary, or secondary to HLA class II antigen associations. TNF-**α** 308.2 allele was in linkage disequilibrium with HLA-DRB1*03 (OR = 7·03; P = 0·0007) and, because of this association, stratification was necessary to rule out an eventual confounder effect of HLA-DR3 to the susceptibility to brucella infection. **Conclusion:** It is generally accepted that host genetic factors are major determinants of susceptibility to or outcome of infectious diseases in humans. Candidate gene studies have implicated several immunogenetic polymorphisms in human infectious diseases, HLA and cytokine genes being the more relevant ones.

Keywords: Genetic susceptibility, Brucellosis, Relationship

3099P

Construction of a chimera hepatitis C virus by replacing with the HVR2 JFH1 genotype 1a E2 gene

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Background: Hepatitis C virus has infected more than 3% of the world population and the infection is asymptomatic or with mild symptoms in most people remains chronically. In many cases, patients themselves are aware of the virus to be disappointed. The large number of patients with chronic infections causes liver disorders such as cirrhosis and hepato cellular carcinoma in severe cases can be very aggressive tumor with poor prognosis. Hepatitis C virus, RNA positive face to the single chain variable region E2 is part of its genome structure. The purpose of this study was to replace the highly variable region HVR2 E2 of genotypes 1a and JFH1 are in this area. **Methods:** We designed specific primers HVR2 area were genotype 1a and Overlap-PCR method was used for the synthesis of the desired piece. **Results:** HVR2 the fragment containing the genotype 1a was synthesized in JFH1 after cloning was confirmed by sequencing. **Conclusion:** The fragment containing the genotype 1a virus JFH1 HVR2 entered and then the resulting virus produced in cell culture will be examined.

Keywords: Hepatitis C, HVR2, Overlap-PCR

2915P

Study of the association between polymorphism of the interferon-gamma receptor 1 gene and susceptibility to chronic HBV infection in an Iranian population

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Background: Hepatitis B virus (HBV) affects millions of people worldwide. The aim of the study was to investigate the association between single nucleotide polymorphism (SNP) in the promoter region of gamma interferon receptor1 gene (IFNGR1) and chronic HBV infection. **Methods:** A total of 225 participants, including 112 chronic hepatitis B patients and 113 healthy control subjects were enrolled in the study. Peripheral blood samples of the both groups were processed for the DNA extraction. Polymerase chain reaction-restriction fragment length polymorphism was used to identify one SNP (–56C/T) in IFNGR1 gene. Logistic regression analysis was performed to calculate the adjusted odds ratio (OR) and 95% confidence interval (95% CI). **Results:** The results indicated that SNP's frequency (IFNGR1 -56C/T) don't have a significant difference in case and control groups (0.426). **Conclusion:** We conclude that –56C/T SNP in IFNGR1 promoter is not related to appropriate susceptibility to chronic HBV infection as a biomarker in Iranian population.

Keywords: Gamma interferon receptor1 gene, Polymerase chain reaction, Restriction fragment length polymorphism, Single nucleotide polymorphism, Hepatitis B Virus

2815P

Anti HBs titer in vaccinated and non-vaccinated students and employees of Shiraz Medical School

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Background: Hepatitis B virus (HBV) infection is a worldwide health concern. Although Vaccination is a superior strategies for HBV prevention especially in high risk groups including health care worker but anti-HBs titer should be follow in this setting. Therefore this study was conducted to evaluate the immune states against HBV infection in students and employees of Shiraz Medical School. **Methods:** A total of 269 individuals including 224vaccinated and 45 non-vaccinated healthy individuals between July and October 2013were included in this study. Blood samples were taken from the participants, subsequently their serum were separated, aliquoted and stored at -20 till assay. Anti-HBs level was measured using an ELISA quantitative kit according to the manufacturer's instructions. **Results:** Out of 224 vaccinated participants, 219 (98%) were found to have anti-HBs titer higher than the protective level (≥10 IU/L), while 5 (2%) individuals had anti-HBs titer below the protective level (<10 IU/L). Out of 45 non-vaccinated subjects, 32 (71%) and 13(%29) had protective and non-protective antibody titer respectively. There were no significant differences in anti-HBs titers according to the age and sex. **Conclusion:** According to the results, three dose vaccinations induce anti HBs protective level in majority of cases. The findings also recommended the necessity of

HBV vaccination in non-vaccinated of high risk groups.
Keywords: Hepatitis B surface antigen, Protective antibody, ELISA, Shiraz

3068P
The CTLA-4 +49A/G polymorphisms are associated with visceral leishmaniasis: a study from north-west of Iran

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Background: Several lines of evidence approved that innate and adaptive immunity play key roles in the defense against visceral leishmaniasis (VL). The polymorphism within the cytotoxic T lymphocyte antigen 4 (CTLA-4) gene alter its expression. Thus, the main aim of this study was to evaluate the polymorphism within +49 position of the CTLA-4 gene of Iranian VL patients in comparison to healthy controls. **Methods:** Inthis cross-sectional study 88 patients with clinical presentation of VL and seropositive for the leishmania (group 1), 86 patients without clinical presentation but seropositive (group 2) and 115 healthy controls (group 3) were assessed with respect to the CTLA-4 +49A/G polymorphism using PCR-RFLP technique. The anti-leishmania antibody titration was evaluated using an immunoflorescence method. **Results:** Our results indicated that the both CTLA-4 +49A/G polymorphism were significantly associated with VL. **Conclusion:** According to the results presented here, it may be concluded that the polymorphism within +49 position of CTLA-4 are associated with VL and may be considered as risk factors for the disease.
Keywords: CTLA-4, Polymorphism, Visceral leishmaniasis.

3066P
Evaluation of FcγRIIA polymorphism in visceral leishmaniasis: a study from north-west of Iran

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Background: Studies demonstrated that either innate or adaptive immunity play key roles in defense against the visceral leishmaniasis (VL). Previous studies revealed that a polymorphism

in the FcgRIIA gene lead to replace of a histidine (H) with arginine ® amino acid at position 166 (H166R) of the receptor which alter affinity of IgG to its receptor. Therefore, the main of this study was to examine the H166R polymorphism in the FcgRIIA gene of VL patients in compare to healthy controls. **Methods:** Inthis cross-sectional study 78patients with clinical presentation of VL and seropositive for the leishmania, 111 subjects without clinical presentation but seropositive and 98 healthy controls was evaluated regarding the H199R polymorphism. The polymorphism was evaluated using PCR-RFLP technique and the titration of anti-leishmania antibody was measured using immunoflorescence method. **Results:** Our results indicated that the FcgRIIA-H166R polymorphism was not associated with VL. **Conclusions:** According to present results, it may be concluded that FcgRIIA-R166H polymorphism is not associated with VL in Iranian patients and more studies on the other Fc gamma receptors may be considered as future studies.
Keywords: FcgRIIA, Polymorphism, Visceral leishmaniasis

3006P

Release of *H. pylori AhpC* to out of bacteria

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Background: Infection with *H. pylori* is associated with a number of diseases in inner and outer gastrointestinal tract, thus, clinical diagnosis of *H. pylori* infection is very important. As one of the abundant antioxidant enzymes in these bacteria, Alkylhydroperoxide reductase (AhpC, the 26 kDa antigen). In this study release of antigen from the cultivated bacteria on solid media was examined using sandwich ELISA and immunoblotting. **Methods:** Stomach biopsies in dyspeptic patients were used as *H. pylori* strains. Modified selective media containing Colombia Agar Base supplemented with lysed horse blood, yeast extract, fetal calf serum and M2 medium was used as culture media. Bacterial cells from culture plates were harvested in phosphate-buffered saline (PBS, 10 mM, pH 7.2). The suspensions were centrifuged, and the supernatants were stored at – 20˚C until use. To investigate the presence of antigen in this solution, the SDS-PAGE and immunoblotting of the collected bacterial washing supernatants were performed, and also two-fold dilution series (1:1 to 1:8) of the mentioned solution were prepared and used as antigen in sandwich ELISA. **Results:** The results of sandwich ELISA indicated a presence of more antigens in out of the bacteria th an the bacterial extract itself. The SDS-PAGE and immunoblotting analysis represented that nearly pure antigen was released to outside of the bacteria. **Conclusion:** Our findings represented that a considerable amounts of approximately pure antigen was released to outside of the bacteria.
Keywords: 26 kDa antigen, AhpC, *H. pylori*, Immunoblotting, Sandwich ELISA

2166P

Relationship between red blood cell distribution width (RDW) and infection in patients undergoing heart surgery

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Background: Surgical site infections (SSIs) are still common and important issues in public health. Red blood cell distribution width (RDW) has recently gained prominence as a prognostic biomarker in inflammatory conditions like rheumatoid arthritis. The goal of this study was to determine the relationship between RDW and sternal wound infection after Coronary Bypass Artery Grafting (CABG).**Methods:**We retrospectively analyzed results of RDW in a cohort of 100 unselected adultpatients who underwent CABG in Tehran Masih Daneshvari hospital. The RDW was determined using an XE-2100 (Sysmex). SSIs were evaluated by use of antibiotics and presence of fever and leukopenia with the use of recorded data.**Results:** Our results showed that patients with higher RDW were more prone to SSIs. A significant reverse correlation was noted between RDW and total count of leukocytes.(r= -0.233, P< 0.01). Patients with higher RDW and leukopenia showed symptoms of infection compared to patients with normal RDW. **Conclusion:** RDW was a predictor of post-surgery infection in patients underwent CABG. RDW is inexpensive and readily measured. In addition to inflammatory conditions it may be used as a prognostic marker in infections.

Keywords:RDW, Infection, Predictor

2176P

Pseudomonas aeruginosa Recombinant Flagellin Induced Poly-Isotypic Humoral Immune Responses in the Balb/C Mice

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Background: Pseudomonas aeruginosa is an opportunistic pathogen that infects people with immunocompromised defenses like neutropenic, burned, hospitalized, and cystic fibrosis (CF) patients.Bacterial flagellin (FliC) is the most effective immunologic effectors of immune system.This study explore the possibility of the recombinant type A flagellin (r-fla-A) in combination of Montanide ISA 70 as a candidate vaccine to promote the humoral and cellular immune responses against r-fla-A.**Materials:** Recombinant flagellin was prepared in Montanide ISA 70 adjuvant; Mice were divided into two groups. The lymphocyte proliferation assay was performed with Brdu/ELISA and IL-4 and IFN-γ cytokine level assay was carried out to determine the pattern of immune response (Th1 *vs.* Th2). Specific antibody responses were measured with an optimized in direct ELISA and finally different isotype-specific antibodies were evaluated with ELISA.**Results:** Immunized mice with adjuvanted flagellin showed a considerably increased lymphocyte proliferation compared with the control group (P = 0.004). High level of IL-4 and IFN-γ secretion was observed in immunized mice compared with the

control group (P = 0.003 and P = 0.006, respectively) with Th1 profile. In addition to the strong antibody-mediated immune response, we found that immunization of mice with r-fla-A induces specific IgG1, IgG2a, IgG2b, IgG3 and IgM antibodies that indicates a statistically significant difference with the control group (P = 0.003, P = 0.004, P = 0.004, P = 0.006 and P = 0.004 respectively).**Conclusions:** Our results demonstrated that r-fla-A could induce cellular and humoral immune response as proper stimulant of poly-isotypic humoral responses.
Keywords: Flagellin, Montanide, Poly-Isotypic Antibodies

3458P
Analysis & measurement of memory B cell in mice immunized by Tetanus toxin Binding subunit (Hc)

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Background: Tetanotoxin- is produced by *ClostridiumTetani*-causestetanusfatal disease. This neurotoxin consist three domains: H_N, Hc and L. The Hc chain is binding domain and immunogenic part of toxin, so it was proposed as vaccine candidate. The purpose of this study is analysis & measurement of memory B cellsafter mice immunizationwithHc subunit oftetanus toxin. **Method:** expression of recombinant protein of Hcsubunit was carried out in optimized conditions. After purification of the recombinant protein by nickel affinity chromatography column, it was injected into mice.Spleen cells were extracted after six months. Then the cells were stained withantibodies againstCD19, IgD, and IgGandtheywere analyzedbyflow cytometry. **Results:** SDS-PAGE gel was confirmed expression and appropriatepurificationof recombinant protein ofHc subunit.ELISA data was shown high titer antibody level against rTHC in mice. After six-month, there was high percentage of memory B cells in the spleen of test group versus control group. **Conclusion:** Aftersix months, although antibody titeraton was decline but the memory B cell populations werepersistence in the spleen. It seems Hc sub unit could induce a long term memory B cell in mice model.
Keyword: Memory B cell, Vaccine, Tetanus toxin, Flow cytometry, Hc subunit

3033P
Evaluation of IL-8 -251 T/A and +2767 A/T polymorphisms in visceral leishmaniasis

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Background: IL-8 plays important roles in recruitment and activation of immune cells during visceral leishmaniasis (VL). The genetic variations in the IL-8 gene can modulate its expression and may be associated with VL. Therefore, this study was designed to evaluate the relation between the polymorphisms at IL-8 -251 and +2767 positions with VL patients in comparison to healthy controls. **Methods:** This cross-sectional study was performed on three groups including; patients with VL clinical presentation and leishmania seropositive, patients seropositive but without clinical presentation and healthy controls were examined regarding the polymorphism at -251 and +2767 positions of IL-8 using PCR-SSP and PCR-RFLP techniques, respectively. Anti-leishmania antibody titration was assessed using the immunoflorescence technique. **Results:** The results demonstrated that either IL-8 -251 or +2767 polymorphisms were significantly associated with VL. **Conclusion:** Based on the results present here, it may be concluded that the IL-8 -251 and +2767 polymorphisms are significantly associated with impaired immune responses in VL and may be considered as risk factors for the disease development.

Keywords: IL-8, Polymorphism, Visceral leishmaniasis.

3193P

Increased Levels of Soluble ST2 in Serum of HTLV1 Patients

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Background: Human T-cell lymphotropic virus-1 (HTLV-1) is a retrovirus with potentially serious outcomes. Many aspects of the pathogenesis of this virus are still unclear. ST2 is a member of IL-1 receptor family which expressed on the surface of Th2 cells. This factor has been shown to be elevated in inflammatory conditions. Considering the inflamatory responces occuring in a chronic viral infection, we compared soluble ST2 levels in serum of HTLV-1 infected patients with control group. **Methods:** This cross-sectional study included 49 HTLV-1 seropositive cases of which 14 were symptomatic and 35 asymptomatic (carrier). All symptomatic patients had associated myelopathy/tropical spastic paraparesis (HAM/TSP). Controls consisted of 30 apparently healthy, HTLV-1, HIV and hepatitis seronegative individuals. ST2 was measured using quantitative ELISA kit fromR&D system. Data were analyzed using SPSS 11.0. A pvalue of <0.05 was considered significant. **Results:** The sST2 levels in HTLV-1^+ individuals showed an increasing trend though was not statistically significant compared to control group ($P= 0.91$). Corroborating the previous reports, ST2 was lower in female group comparing to the male group. ST2 level was not related significantly

to duration of the disease (P=0.78). **Conclusion:** Our results show that increase in ST2 levels occurs in the HTLV-1 seropositive groups in comparison with control in both sexes. Although statisctcally was not significant that could be owing to high normal variations of ST2 in serum. This finding suggest further research with a larger group of patients to explore underlying mechanisms involving interlukin-1 in the pathogenesis of this infection.
Keywords: HTLV-1, Soluble ST2, Interleukin 1

1493P

Evaluation of body mass index and Level of albumin in patients with active tuberculosis and subjects with latent tuberculosis infection

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Background: Limited data are available on the relationship between nutritional status and tuberculosis. **Methods:** In this study, was investigated albumin level in newly detected Active tuberculosis Patients (ATB=17) and to compare them with the level in Subjects With Latent tuberculosis Infection (LTB=17) standard methods were adopted to collect an early morning fasting blood sample for albumin (by the bromocresolgreen method). **Result:** the mean ± SD for albumin in the patients and controls were 3/62±0/56, 4/68±0/25 and BMI in the patients and controls were 19/46±2/79, 25/4±3/46. **Conclusion:** According to our findings demonstrated that all parameters were significantly lower in the patients than control groups.
Keyword: Body mass index, Albumin, Tuberculosis

1887P

Osteomylitis as a late presentation of Disseminated Bacillus Calmette Guerin infection

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Background: Environmental non tuberculous mycobacteria and Bacillus Calmette-Guerin are weakly virulent mycobacteria but most Children with disseminated BCG infection has primary immunodeficiency. SCID, CGD and IL-12/IFN-gamma pathway deficiency accounted for considerable proportion, so special immune function disorder should be detected in these patients. Hematogenouse spread of BCG vaccine may result in osteomyelitis but it is a rare late onset complication under 3 years old .The lesions tend to appear on the same side of the body as the vaccination.We present two patients with late onset osteomylitis of multiple sites in both side of the body result from Disseminated BCG infection. **Methods:** A 5 years old boy who presented with two destructive mass lesion , one in right mandible and the second in mid thoracic vertebral region , history of prolonged intermittent fever many often , right knee joint arthritis with fistule formation and purulent discharge, cervical axillary and medistinal lymphadenopathy , hepatoslenomegaly and a history of an huge BCG lymphadenitis

of right subaxillary under age two . Radiographic studies showed multiple destructive lesions over eight bones. Biopsy of mandibular mass has been shown cassified granuloma and the culture become positive for Bacillus Calmette Guerin . A 13 years old boy presented with osteomylitis and fistula formation in the distal metaphysis of the left ulnar and right tibia . He had a history of right sub-auxiliary BCG lymphadenitis under age of two. Also he had a recurrent mouth ulcers and chronic diarrhea since age 5. He was presented with acute right lower quadrant pain at age six and had gone under laparotomy to rulled out acute abdomen and complicated appendicitis. Because of abdominal pain, chronic diarrhea and query Inflammatory Bowel Disease he has gone under surgery and colostomy six months latter. He started to have pain and bone problems since age eight. Specimens were not processed or were not available for mycobacterial culture in each episode but pathology was positive. Both patients have gone under vast etiologic and immunologic evaluation. **Conclusion:** Antimycobacterial treatment was effective in these patients and resulted in good clinical outcome .The bone lesion has been disappeared and no relapse after four and two year follow up has been occured.

2591P

Evaluating the production of polyclonal antibody against Staphylococcus aureus in animal models

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Background: Staphylococcus aureus is a gram-positive cocci-shaped bacterium that produces enterotoxin causing short incubation period food poisoning. Immunoglobulin Y (IgY) obtained from immunized chicken egg yolk is a rich and inexpensive source of polyclonal antibodies. These antibodies can be considered as reasonable alternative antibodies in instead of producing antibodies by other laboratory animals. In recent years, IgY, is used in medical researches is in order to diagnosis, prevention and treatment of diseases. **Methods:** In this study, seven hens were selected and divided into two groups: treatment group (5 animals) and control group (2 animals). The treatment group was immunized in three stages by antigen prepared from Staphylococcus aureus. **Results and Conclusion:** Finally, eggs were collected and yolk total protein and globulin were measured; so, it was proved that the highest increase in the rate of globulin was at week 10 following the first injection.

Keywords: polyclonal antibody, Staphylococcus aureus, egg yolk

Immunology of Rheumatic Diseases

Oral Presentations:

2675O

Disease activity, organ involvement, and autoimmune parameters in systemic lupus erythematosus

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Background: Disease activity is a requirement for organ damage in lupus patients and autantibodies appear parallel to disease activity might enable better consideration of disease status. Autoantibodies are important pathogenic factors which may possibly cause end organ damage. Understanding the mechanisms linking autoantibodies to organ damage is a challenge for investigators. **Methods:** 98 lupus patients with a mean age of 29.8 ± 9.30 years were evaluated for disease activity, organ damage , and autoantibodies against nRNP/Sm, Sm, SSA, Ro52, SSB, Scl-70, Jo-1, CENP B, dsDNA, nucleosomes, histone, Rib.P-Protein using immunoblotting technique. Correlation of disease activity and organ damage was studied with autoantibodies. **Results:** In present study the mean SLEDAI in lupus patients was 10.27 ± 6.27, and the mean number of autoantibodies was 3.7 ± 1.99. SLEDAI had a significant positive relationship with the increasing number of autoantibodies (p = 0.031).Disease was more active in female, and in anti-dsDNA$^+$ patients (SLEDAI 16.9 for female vs 8.0 for male, and 16.2 in anti ds-DNA$^+$ vs 8.2 for anti ds-DNA$^-$ patients) (p=0.043, and 0.06). Anti-nRNP autoantibody negatively correlated with disease activity (SLEDAI 6.0 in n-RNP$^+$ vs 15.3 for n-RNP$^-$ patients (p=0.001).The number of involved organs significantly related with SLEDAI (p = 0.029), but skin and joint involvement negatively correlated with SLEDAI (p=0.042, and 0.043). **Conclusion:** The more active the disease, the higher the number of autoantibodies, and the higher the number of organs involved. The increased number of autoantibodies is involved in the pathogenesis of disease.

Keywords: Autoantiboies, Human, Systemic lupus erythematosus, Organ involvement

2231O

The effect of Salbutamol on the vascular endothelial growth factor (VEGF) and angiogenesis in the rat air pouch model of inflammation

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Background: The possible role of beta receptors in modulation of inflammation suggests that beta receptor agonists may have beneficial anti-angiogenesis effects. In this study, anti-angiogenic and VEGF inhibitory properties of salbutamol in a rat model for rheumatoid arthritis, namely air pouch model of inflammation was evaluated**. Methods:** Male Wistar rats were anesthetized; 20 ml and 10 ml of sterile air were injected subcutaneously on the back on day 0 and day 3, respectively. On day 6, inflammation was induced by injection of carrageenan into pouches. Saline as control, diclofenac sodium as standard agent, salbutamol (25, 125, 250 and 500 microgram) & salbutamol plus propranolol (500 microgram) were administered intra pouch at the same time as the carrageenan and then for two consecutive days. After 72hr pouches were opened, its fluid was collected in order to determine VEGF level. The granulation tissues formed were dissected and cut into small pieces before being homogenized in Drabkin reagent. The tissue homogenates were centrifuged and the supernatants were filtered through a 0.22µm filter. The hemoglobin concentration in the supernatant was then determined spectrophotometrically using a hemoglobin assay kit. **Results:** Salbutamol with doses of 125, 250 and 500 microgram/rat significantly ($P<0.05$) decreased the production of VEGF in the pouch fluid. In addition, angiogenesis was significantly inhibited by all doses of salbutamol ($p<0.001$). Interestingly, attenuation of angiogenesis by salbutamol was similar to diclofenac sodium. **Conclusion:** The observed VEGF inhibitory property of salbutamol may be responsible for its beneficial anti-angiogenic effect.

Keywords: Salbutamol, Angiogenesis, Air Pouch, Carrageenan, VEGF.

2488O

Blockade of c-Abl by siRNA reduced TGF-β1 response in SSc fibroblasts

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Background: Systemic sclerosis (SSc) is a chronic fibrotic disease with unknown ethiopathogenesis. The overproductions of extracellular matrix (ECM) components is a hallmark of SSc. Fibroblasts, the key cells in the pathogenesis of SSc, are induced by transforming growth factor-β1 (TGF-β1) and produced increased amount of collagen type 1 and fibronectin-1. Cellular abelson (c-Abl), a non-receptor tyrosine kinase, is crucial for the induction of ECM proteins by TGF-β1. The aim of the present study is to determine whether blockade of c-Abl, affects production of extracellular matrix proteins. **Methods:** Fibroblast cells were obtained from skin biopsies of 10 patients with SSc and 10 normal controls. Skin fibroblasts were treated with TGF-β1 after transfected with c-Abl siRNA. The expression of collagen type1 and fibronectin-1 were measured by MGB-TaqMan real-time PCR. **Results:** The results show that the expression of collagen type 1 and fibronectin-1 reduced after

treatment of fibroblast cells with c-Abl siRNA. We also demonstrate that the induction of ECM proteins after stimulation with TGF-β1 was inhibited by c-Abl siRNA. **Conclusion:** Molecular mechanisms underlying the chronic fibrotic response are not fully elucidated. Our findings reveal that tyrosine kinase c-Abl is an important molecule in the pathogenesis of SSc. We also indicate that the inhibition of c-Abl via siRNA might be an interesting candidate for the treatment of SSc patients.

Keywords: Systemic sclerosis, collagen, fibroblast, c-Abl, siRNA

1762O

Serum levels of vascular endothelial growth factor (VEGF) and its soluble receptors sVEGFR-1 and sVEGFR-2 in patients with Behcet's Disease

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Background: Vascular lesions in both arterial and venous systems are often the major cause of complication in Behcet disease (BD). Regarding the importance of vascular endothelial growth factor (VEGF) and its soluble receptors (sVEGFR-1 and sVEGFR-2) in vasculogenesis and angiogenesis, they might be important in the pathogenesis of BD. The aim of this study is to evaluate the levels of VEGF and its soluble receptors in BD patients compared to those of normal controls. **Methods:** VEGF, sVEGFR-1, sVEGFR-2 serum levels were measured in 40 patients and 40 age- and sex-matched controls with enzyme-linked immunosorbent assay (ELISA). **Results:** The serum concentration of VEGF and sVEGFR-2 in patients and controls were not significantly different while the levels of sVEGFR-1 (76.9±21.7 pg/ml vs 62.7±26.1 pg/mL, respectively; $p < 0.014$) as well as the ratio of VEGF/sVEGFR-1 (5.0±1.7 vs 7.5±8.0, respectively; $p < 0.0001$) showed significant difference. **Conclusion:** Increased levels of sVEGFR-1 in patients suggest the possible role of sVEGFR-1 in the pathogenesis of BD.

Keywords: VEGF, Behcet disease,VEGRF1-2

2187O

Expression of Interleukin-21 and IL-21 receptor in peripheral blood of patients with Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the synovial inflammation of the joints. Various cells and cytokines have been identified which may contribute to RA pathology. IL-21 is a pro-inflammatory cytokine mediating pleiotropic functions through the IL-21 receptor (IL-21R). IL-21 is chiefly produced by activated $CD4^+$ T cells but IL-21R has been discovered on different cells. Blockade of IL-21R may represent a hopeful therapeutic approach in RA. The aim of this study was to determine the percentage

of IL-21R expressing $CD4^+$ cells and IL-21 mRNA expression in peripheral blood of RA patients. **Methods:** Surface expression of IL-21R on $CD4^+$ cells in peripheral blood of RA patients (n=32) compared to healthy control subjects (n=20) was evaluated by flow cytometry. Simultaneously, mononuclear cells were taken apart from peripheral blood (PBMC) of individuals on a density gradient (sigma). Finally, the Expression of IL-21 mRNA was assessed by Real-time PCR. **Results:** Expression of IL-21R was evaluated on a flow cytometry analysis. IL-21R expressing $CD4^+$ cells from RA patients showed significantly higher percentage of IL-21R compared with healthy controls (P = 0.000). Moreover, conducting Real-time PCR on synthesized cDNA showed that there was no significant difference between patients and healthy controls (P= 0.089).**Conclusion:** Our results indicated higher expression of IL-21R in RA patients, so we offer that targeting of the IL-21R may be a novel therapeutic idea for the treatment of RA.

Keywords: Rheumatoid arthritis, Autoimmune disease, Cytokine, IL-21, Receptor

3234O

Systemic and Discoid Lupus Erythematous in Four Patient with Chronic Granulomatousis Disease

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Background: Chronic Granulomatousis Disease is a rare inherited disorder that present with recurrent infections.CGD might itself known as a genetic cofactor, which can lowers the threshold of autoimmunity development. There are reports of systemic and discoid lupus erythematosus symptoms in X-linked CGD patients and carriers. Methods and Results: We report three cases of autosomal CGD and one xlinked patient that registered in Iranian Primery Immune Defiency Registry with lupus erythematosus symptoms. First patient is a 24 years old girl with autosomal recessive CGD who evaluated for SLE two years ago because her malar rash and thrombocytopenia and then definite SLE was diagnosed according to American Colleague of Rheumotoly criteria Second patient is 9 year old boy with X linked CGD .He has discoid lupus lesion according skin biopsy without other symptoms of SLE. Other patients are One girls and one boy with autosomal recessive CGD that skin biopsy revealed discoid lupus erythematosusRole of genes located on the X chromosome, CYBB and other CGD-related genes such as NCF1and inability to efficiently clear phagocytized pathogens and continuous foreign antigen stimulation are the main explanation for association between CGD and lupus. Conclusion: In conclusion if clinical symptoms of SLE such as mucocutaneus lesions or arthritis in CGD patients or carriers were significant the patient must be referred for further investigations for autoimmune complication. Appropriate treatment should be initiated because of the potential clinical severity of the disease. Also considering to presence of necrotic and chronic coetaneous lesion in SLE patient CGD screening test could be done.

Keywords: Lupus Erythematous, Granulomatousis

1804O

Effects of vitamin D on regulatory T cells and related cytokines in lupus erythematosus patients

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disease with adverse clinical manifestation with more than 100 autoantibodies that predominantly affects women during child bearing years. Regulatory T cells are CD4+ and constitutively express IL2 receptor alpha chain, CD25. Decreased number of regulatory T cells during active lupus has been reported and several studies indicate that vitamin D deficiency affects many lupus patients. In this research we studied the effects of vitamin D on regulatory T cells and related cytokines in lupus erythematosus patients. **Methods:** We enrolled 30 SLE patients. PBMCs were isolated and cultured in the presence and absence of vitamin D. Then Regulatory T cells were analyzed by flow cytometry. Total RNA was extracted, cDNA synthesized and the expression of FOXP3, TGF-β, IL-6 and IL-10 genes analyzed by Real Time-PCR. **Results:** The percentage of regulatory T cells showed a significant increase in vitamin D-treated PBMCs (6.68 ± 4.47%) compared to non-treated PBMCs (4.63 ± 2.70%; $P<0.007$). Vitamin D increased the expression of FOXP3, TGF-β, IL-10, and IL-6 in 73%, 66.7%, 56.7% and 30% of patients, respectively. **Conclusion:** Addition of vitamin D to the culture medium significantly increased the percentage of regulatory T cells and expression of FOXP3, TGF-β , IL10. The data suggest that one of the mechanisms of immunomedulatory effects of vitamin D in SLE may be associated with its influence on regulatory T cells.

Keywords: Systemic lupus erythematosus, Regulatory T cells, Vitamin D

Poster Presentations:

3203P

Association of a proliferation -inducing ligand (APRIL) polymorphism with juvenile systemic lupus erythematous

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Background: Systemic Lupus Erythematosus (SLE) is a multi-factor autoimmune disorder with diverse clinical manifestations and unclear pathogenesis.Genetic components could play important roles in the incidence and development of SLE. Among these, A Proliferation -Inducing Ligand (APRIL) has roles in the stimulation and antibody production in B cells. This study was performed to assess the involvement of the *APRIL* gene in a group of pediatric patients with SLE. **Methods:** A single nucleotide polymorphism (SNP) for *rs11552708* of the *APRIL* gene were analyzed by Real-time PCR in 60 Iranian children with juvenile SLE and 60 healthy controls. **Results:** There was no significant association for SNP namely, *rs11552708* (allele G: P=0.83) in SLE Iranian children. Likewise, the GG genotype frequency was not significant in SLE patients (86.6%), compared to healthy controls (90.6%) (p=0.68). **Conclusion:** Our results demonstrated that *rs11552708* of the *APRIL* gene is not associated with SLE susceptibility in Iranian children.

Keywords: Systemic lupus erythematosus, Single nucleotide polymorphism, APRIL

3229P

Association between *IRF5* polymorphisms and the risk of systemic lupus Erythematous in children

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Background: Systemic lupus erythematous (SLE) is a chronic and inflammatory autoimmune disease that influenced by several environmental and genetic factors. Generation of autoantibodies against nuclear and glomerular components leads to incidence of disease. As of the role of the inflammatory cytokine in this disease, IRF5 was selected that plays a role in type 1 interferon and pro-inflammatory cytokine induction. The purpose of this study is to analysis of the relationship between polymorphisms of *IRF5* (*rs10954213*) and the risk of SLE in Iranian children. **Methods:** Sixty pediatric patients and 63 health controls were enrolled in this study.DNA extraction was performed by phenol chloroform, analysis of SNPs *IRF5* (*rs10954213*) was performed by Real time-PCR, using a Taqman allele discrimination assay. **Results:** The frequency of G allele was not significantly between children with SLE (37.5%), compared to healthy controls (47.61%)(p=0.14). Also, the frequency of GG genotype was not significantly different between patients (11.66%), compared to healthy controls (23.80%) (p=0.13).**Conclusion:** The current study shows that the polymorphism of *IRF5* (*rs10954213*) are not associated with the risk of SLE in Iranian children.
Keywords: Systemic lupus erythematous, IRF5, Polymorphisms

2785P

Association between the tumor necrosis factor alpha -238 polymorphism with rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease is caused by inflammation of the joints in the structure, and the most common chronic inflammatory joint disease with unknown etiology. Recent studies has been shown tumor necrosis factor-α (TNF-α) plays a key role in the inflammatory response and pathogenesis of RA. The aim of this study was to investigate the effect of TNF-α promoter polymorphism at -238 on the susceptibility of RA. **Methods:** The distribution of -238 TNF-α genotypes was analyzed in 90 RA patients and 90 healthy matched control.by using the RFLP-PCR method. TNF-α serum levels was measured by ELISA. Data analysis and statistical t-test and Kruskal-Wallis Test was conducted by Software SPSS V16.**Results:** The results of research Indicated no significant differences were found in the allele and genetypes frequencies of the polymorphism between patients and controls (p=0.1). However the serum level of TNF-α between patients and controls were significant but no significant among patients with GG, GA AND AA genotypes (P=0.00, p=0.07).**Conclusion:** It seems the TNF-α -238 polymorphism is not associated with rheumatoid arthritis susceptibility. But the polymorphic position is associated with enhanced TNF-α production in patients.
Keywords: Rheumatoid arthritis, TNF-α, Polymorphism

2965P

Measurement of interleukin- 10 and interleukin-23 serum levels in patients with rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a systemic inflammatory disease that mainly affects synovial joints. The cause of RA is unknown but it seems that cytokines produced by different cells such as lymphocyte, monocyte, endothelial and epithelial cells are believed to play major roles in the induction and propagation of the inflammatory conditions. Interleukin (IL)-10 is an important immunoregulatory cytokine produced by many cell populations. Its main biological function seems to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, antigen-presenting cells, mast cells, and granulocytes. Interleukin-23 (IL-23) is a heterodimeric cytokine belonging to the IL-6/IL-12 family that plays a key role in several of autoimmune and inflammatory disorders. The purpose of this study was to measure the levels of IL-10 and IL-23 in the serum of patients with RA, and compare to healthy volunteer controls. **Methods:** IL-10 and IL-23 serum levels of 30 RA patients and 30 healthy controls have been measured using ELISA assay. **Results:** Serum IL-23levels of the RA patients (1360.1±251.06pg/ml) were significantly higher than the IL-23 level the control group (649.8±140.4 pg/ml) P= 0.001.the serum IL-10 levels in rheumatoid arthritis patients (3.79±0.06pg/ml)had no significant changes than the control group (3.58±0.09 pg/ml) P=0.197. **Conclusion:** Our results indicate that IL-23 is highly active in RA and this cytokine might be closely involved to pathogenic mechanisms of rheumatoid arthritis.

Keywords: Rheumatoid arthritis, Interleukin-10, Interleukin-23, Inflammatory response

2657P

Is the C57bl/6 mouse a proper model for the induction of Collagen-induced arthritis?

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Background: Rheumatoid Arthritis (RA) is a chronic autoimmune disease that affects joints and others organs in 1-2% of word population. Due to the involvement of both cellular and humoral immunity, Collagen-induced arthritis (CIA) is a useful model for study the immune-pathogenesis of RA. While DBA/1 strain of mouse is the best model of CIA, however this mice strain is not available in Iran. Hence, the goal of the present study was to evaluate the possibility of CIA induction in C57BL/6 mice which is available in our country. **Methods:** Female C57bl/6 mice (8-10 week-old) were randomly divided into two groups (CIA and Control groups). Mice in CIA groups were immunized by injection of Collagen type II (CII) emulsified in Complete Freund adjuvant (CFA) and boosting Twenty-one days later with the CII emulsified in incomplete Freund adjuvant. Mice in Control group were injected with PBS

instead. During the study, Arthritic indexes and pathological signs were evaluated in both groups. Each experiment was repeated three times with the same conditions and data were analyzed by SPSS software. **Results:** This study showed no significant difference between arthritic indexes in CIA and control mice (P› 0.5). In all CIA mice no swelling and redness of joints was observed. Pathological examinations showed very few lymphocytes, neutrophil and macrophage infiltration in the joints of CIA mice which were not significant compared to control mice. **Conclusion:** We conclude that C57BL/6 mice are genetically resistant strain for CIA induction in Iran. We indicated that these C57BL/6 mice are not suitable for CIA induction. Hence, Using Wistar Rats or pure original C57BL/6 mice is suggested for induction of CIA.

Keywords: C57b1/6 mouse, Collagen-induced arthritis,

2653P

Study of Inflammatory Cytokines in Collagen-Induced Arthritis Compared with Adjuvant-Induced Arthritis in Wistar Rats

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease with inflammation of joints and surrounding tissues. Rodents are useful animal models for study the pathogenesis and therapeutic process of RA. Adjuvant and Collagen are the two commonly used materials for induction of arthritis in rodents. In this study we aimed to compare the immunological and clinical features of Collagen-Induced Arthritis (CIA) and Adjuvant-Induced Arthritis (AIA) in Wistar Rats. **Methods:** Male Wistar rats were randomly divided in CIA, AIA and Control groups (7 in each). Rats in CIA group were immunized with Chicken type-II collagen (CII) emulsified in Complete Freund Adjuvant (CFA), injected intradermally at the base tail. Booster dose was on the seventh day using CII emulsified in Incomplete Freund Adjuvant (IFA). AIA rats were immunized with 200 µl CFA with high concentration of mycobacterium (10 mg/ml). Control mice received PBS. Arthritic Indexes were measured every second day for all groups. Seven weeks after the first immunization, TNF-α and IL-6 were measured in serum and IFN-γ and IL-4 levels were assessed in supernatant of cultured splenocytes in the presence of CII. **Results:** Intensity of inflammatory edema increased in both CIA and AIA groups compared to control group. Statistical analysis indicated that serum level of TNF-α and IL-6 in CIA and AIA groups were significantly elevated compared to control group ($P < 0.05$). Also splenocytes from CIA group produced IFN-γ and IL-4 in response to CII ($P < 0.05$) in comparison with control group. **Conclusion:** This study showed that both CFA and CII are suitable materials for induction of RA in Rats. We showed that the activation of immune system in response to type II collagen leads to increased production of IFN-γ and IL-4 in CIA group but not in AIA rats. We conclude that the activation of T cells against type-II collagen is the main characteristic of the CIA model.

Keywords: Collagen-Induced Arthritis, Inflammatory cytokines, Adjuvant

2395P

Polyamines can changed DNA structure and induce SLE like syndrome in BALB/C mice

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disease with multi factorial etiology and diverse underlying mechanisms. Increased understanding of the pathogenesis mechanisms have been based on analysis of several murine models over the past four decades. The three basic polyamines (putrescine, spermidine and spermine) are essential multifunctional cellular components. Polyamines cause changes in DNA structure turning them to uncommon Z-DNA from routinely available B-DNA.As shown with in vitro or in vivo systems, apoptosis has been considered as a source of nuclear materials in SLE. Better understanding of the lupus network may lead to the specific targeted therapy development based on previous experiments in mice. The disease is T-cell-dependent and antigen-driven, however, more than 100 autoantibodies were detected in the sera of lupus patients with differential correlation to disease activity. **Methods:** Normal BALB/c mice were immunized subcutaneously with 50µgr highly purified DNA. This DNA was extracted from Hep-2 cell line which cultured with or without polyamines (putresine، spermin and spermidin). Serum anti-double-stranded DNA antibody (anti-ds DNA) was determined by enzyme-linked immunosorbent assay. Other SLE-associated autoantibodies were examined by indirect immunofluorescence (ANA) method. Proteinuria was measured with the Bradford assay. T cell subpopulations (Th1 and Th2) percentage evaluated with flowcytometry. **Results:** Proteinuria was significantly increased in the polyamine treated DNA immunized mice**,** high levels of anti-dsDNA antibodies and other autoantibodies were seen more frequently in the sera of immunized mice**.** The level of Th2 was increased in comparison with control group**.** **Conclusion:** DNA structure modification with polyamines can be a valuable tool to induce more vigorous and stable form of SLE animal model in mice. Th1 and Th2 imbalance may be regarded as a probable mechanism of pathogenesis of SLE.

Keywords: SLE, mice model of SLE, T cell subtypes.

2599P

Analysis of Inhibitory Killer Cell Immunoglobulin- Like Receptor Genes with Rheumatoid Arthritis

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Background: Rheumatoid Arthritis (RA) is an inflammatory autoimmune disorder that triggers by an inflammation of the synovial membrane that lead to destruction of cartilage and bone, so it shows extra-articular manifestations. NK cells and CD28null T-cells are presented in synovial membrane of RA and both of them express Killer cell Immunoglobulin- like

Receptors (KIRs). KIRs constitute of activatory and inhibitorygens that may contribute in autoimmune disease. In this study were genotyped inhibitor KIRin RA patient. **Methods:** In a Case and control study 9Inhibitory KIR genes were genotyped in 400 RA patients (according to ACR criteria) and 273 age, sex, and ethnicity matched healthy control, by using sequence-specific primers (SSP-PCR). Differences in KIR genes frequency were determined by x2 test. **Results:** *KIR2DL2* (*P*=0.022; OR=0.688; 95% CI=O.503-0.940), KIR2DL5a(*P*=0.003; OR=0.613; 95% CI=0.449-0.838) and KIR2DL5b(*P*=0.004; OR=0.628; 95% CI=0.460 - 0.859) was significantly decreased in RA patients in comparison with the healthy control group. **Conclusion:** According to our results, decrease of inhibitory KIR genes (*KIR2DL2*, KIR2DL5a, and KIR2DL5b) in patient group may lead to initiation of rheumatoid arthritis in Iranian population.

Keywords: KIR, KIR HLA Ligand, NKCell, RheumatoidAarthritis

2384P

Evaluation of Serum cartilage oligomeric matrix protein (COMP) in rheumatoid arthritis patients who have treated withanti-inflammatory cytokines

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Background: Cartilage oligomeric matrix protein (COMP) is found at elevated concentrations in sera of patients with joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA). We recently showed that COMP activates complement via the alternative pathway and that COMP-C3b complexes are present in sera of RA patients, but not in healthy controls. We now set out to elaborate on the information provided by this marker in a variety of diseases and larger patient cohorts. **Methods:** COMP-C3b levels in sera were measured by using an enzyme-linked immunosorbent assay (ELISA) capturing COMP and detecting C3b. Serum COMP was measured by using ELISA. **Results:** COMP-C3b levels were significantly elevated in patients with RA as well as in systemic lupus erythematosus (SLE), compared with healthy controls. SLE patients with arthritis had significantly higher COMP-C3blevels than did those without. COMP-C3b was furthermore elevated in patients with ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis, systemic sclerosis, and OA. COMP-C3b did not correlate with COMP in any of the patient groups. COMP-C3b correlated with disease activity in RA, but not in other diseases. COMP-C3b levels in RA patients decreased on treatment with tumor necrosis factor (TNF)-a inhibitors, whereas the levels increased in patients with AS or PsA. The changes of COMP-C3b did not parallel the changes of C-reactive protein (CRP). **Conclusion:** COMP-C3b levels are elevated in several rheumatologic diseases and correlate with inflammatory measures in RA. COMP-C3b levels in RA decrease during TNF-a inhibition differently from those of CRP, suggesting that formation of COMP-C3b relates to disease features not reflected by general inflammation measures.

Keywords: Cartilage, COMP, Rheumatoid arthritis, Inflammation

2263P

Inhibitory effects of Verapamil on the angiogenesis and vascular endothelial growth factor (VEGF) in the rat air pouch model of inflammation

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Background: Calcium channel blockers are widely used anti-hypertensive drugs that also have anti-inflammatory activities. In this study, anti- angiogenic and VEGF inhibitory properties of verapamil in a rat model for rheumatoid arthritis, namely air pouch model of inflammation was evaluated. **Methods:** Male Wistar rats were anesthetized; 20 ml and 10 ml of sterile air were injected subcutaneously on the back on day 1 and day 3, respectively. On day 6, inflammation was induced by injection of carrageenan into pouches. Diclofenac sodium as standard agent and verapamil (0.05, 0.1 and 0.2 mg/pouch) were administered intra pouch at the same time as the carrageenan injection and every 24hr as multiple doses. After 72hr of inflammation induction, pouches were opened; its fluid was collected in order to determine VEGF level. The granulation tissues formed were dissected and cut into small pieces before being homogenized in Drabkin reagent. The tissue homogenates were centrifuged and the supernatants were filtered through a 0.22µm filter. The hemoglobin concentration in the supernatant was then determined spectrophotometrically using a hemoglobin assay kit. **Results:** Angiogenesis was markedly decreased by verapamil with doses of 0.05, 0.1 and 0.2 mg/pouch ($P<0.001$, $P<0.001$ and $P<0.05$ respectively). In addition VEGF level of exudates was significantly reduced by verapamil with doses of 0.05 and 0.1 mg/pouch. Interestingly, the reduction of angiogenesis by verapamil was similar to diclofenac sodium. **Conclusion:** Suppression of VEGF production may contribute to the anti-angiogenic effects of verapamil.

Keywords: Verapamil, Air-pouch, Angiogenesis, VEGF, Rat.

2265P

The inhibitory effects of Oxytocin on the angiogenesis and vascular endothelial growth factor (VEGF) in the air-pouch model of inflammation in the rat

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Background: Unregulated angiogenic response is involved in chronic inflammatory conditions such as rheumatoid arthritis. The aim of present study was to investigate the effects of oxytocin on the angiogenesis and VEGF in a rat model for rheumatoid arthritis, namely air pouch model of inflammation. **Methods:** To induce an air pouch model, sterile air (20ml and 10ml) was injected subcutaneously on the back of anesthetized rats on day 0 and day 3 respectively. On day 6, inflammation was induced by carrageenan injection into pouches. Oxytocin (4.25, 8.5 and 17µg/pouch) were administered intra pouch at the same time as the carrageenan and then for 2 consecutive days. Three days after inflammation induction, pouches were opened; exudates were collected in order to determine VEGF level. The granulation tissues formed were dissected, washed in PBS and cut into small pieces before being homogenized in Drabkin reagent. The tissue homogenates were centrifuged and the supernatants were filtered

through a 0.22µm filter. The hemoglobin concentration in the supernatant was then determined spectrophotometrically using a hemoglobin assay kit. **Results:** There was a significant reduction in the angiogenesis in oxytocin-treated rats by all three doses. Interestingly there was no significant difference between oxytocin and diclofenac in the inhibition of angiogenesis. In addition oxytocin showed inhibitory activity against VEGF concentration. **Conclusion:** This study shows that oxytocin can decrease angiogenesis in the granulation tissue. It seems that the anti-angiogenic activity of oxytocin is mediated through modulation of VEGF production.
Keywords: Oxytocin, Angiogenesis, Air Pouch, Carrageenan, VEGF.

3145P
Measurement of serum leptin plasma level in rheumatoid arthritis refer to zanjan hospital during1391-1392

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Background: Rheumatoid arthritis is a chronic autoimmune inflammatory condition characterised by polyarthritis and severe change in body mass. The role of the leptin in modulation of immune response and inflammation has been regarded as important. so we decide to investigate relation between rheumatoid factor and leptin level in serum. **Methods:** To investigate plasma levels of leptinin patients with rheumatoid arthritis and to compare them with levels in healthy controls leptin concentrations were measured in 50 patients with rheumatoid arthritis and 50 healthy controls by using specific enzyme-linked immunosorbent assays. **Results:** Patients with rheumatoid arthritis showed considerably higher plasma levels of leptin, adiponectin than healthy controls. **Conclusion:** A marked increase in plasma levels of leptin was noted in patients with rheumatoid arthritis. There was also positive correlation between ESR and leptin level in patient with positive in rheumatoid factor. Coordinated roles for leptin are suggested in the modulation of the inflammatory environment in patients with rheumatoid arthritis.
Keywords: Rheumatoid arthritis, Leptin level, Zanjan

3252P
Correlation between rheumatoid factor levels and tumor markers elevation in patient refer to Zanjan hospital in 1391-1392

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Background: Rheumatoid factors (RFs) are autoantibodies directed against the Fc portion of IgG. Tumour markers (eg, CA-125, CA-19-9) are tumour-expressed proteins that are usually measured in the serum by means of ELISA sandwich assays.We systematically sought to determine whether patients with active RF-positive rheumatoid arthritis (RA) and no evidence of a neoplastic disorder could have “falsely” increased levels of some commonly tested serum tumor markers. **Methods:** A total of 50 patients (31 women and 19 men; median (range) age 54years (22–61)) that refer in zanjan hospital. Patients with other autoimmune or inflammatory diseases associated with increased RF titres were excluded. **Results:** Data for the following

variables were collected: RF (measured by latex agglutination test; normal value ⩽15 IU/ml), CA-125 (measured by ELISA; normal value ⩽33 U/ml), carcinoembryonic antigen (CEA; measured by ELISA; normal value ⩽5 ng/ml) and CA-19-9 (measured by ELISA; normal value ⩽37 U/ml). Patients were monitored for the development of cancer over a 3-year period. **Conclusion:** Interestingly, 7 (13.2%) patients had high values of CA-125, 2 (3.8%) patients had increased CEA and 1 (1.9%) patient had a high value of CA-19-9. No significant correlation was detected between RF titres and levels of CEA, CA-125 and CA-19-9.

Keywords: Rheumatoid factor, Tumor marker, Zanjan hospital, 1391-1392

2489P

Fibrosis of the skin in systemic sclerosis: role of microRNA-21

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Background: Systemic sclerosis (SSc) is a multisystem autoimmune disease which induces fibrosis of the skin and internal organs. In pathological condition dermal fibroblasts differentiate into myofibroblasts, which are resistant to apoptosis and continuously secret extra-cellular matrix components especially collagen type I. At present, there are no effective approaches for curing skin fibrosis. MicroRNAs (miRNAs) are a new part of gene regulatory network. It has been shown that many physiological and pathological processes depend on miRNAs. In this study the possible role of miR-21 in fibrosis of the skin in systemic sclerosis was investigated. **Methods:** Human dermal fibroblasts were obtained by skin biopsy from forearms of 8 SSc patients and 8 controls. Proteins were extracted with RIPA buffer and DOC-TCA method. Total RNA including microRNAs was extracted from fibroblasts using miRNeasy Mini Kit (Qiagen). **Expression of fibrosis** related **genes** at RNA and protein levels were measured by using Real-Time PCR and western blotting technics respectively. **Results:** MicroRNA-21 (miR-21) was significantly up regulated in SSc patients. Transfection of normal fibroblast by miR-21 mimic induced myofibroblast differentiation and collagen production. In addition, inhibition of miR-21 in SSc myofibroblasts by using miR-21 inhibitors reduced α-SMA (the phenotypic marker for myofibroblast differentiation) and collagen production. **Conclusion:** The majority of SSc patients suffer from skin fibrosis. However, how miRNAs regulate skin fibrosis remains unclear. Our data suggest that miR-21 inhibitor can be considered as a therapeutic option to control myofibroblast differentiation and collagen production.

Keywords: Systemic sclerosis, mir-21, Dermal fibroblast

2524P

Assosiation of killer immunoglobulin-like receptor (KIR) genes polymorphism with Behcet's disease

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Background: Behcet's disease (BD) is a systemic vasculitis with unknown pathogenesis. However, it has been supposed that defects in the natural killer (NK) cell repertoire may be involved. Killer cell immunoglobulin-like receptors (KIR) of NK cells constitute the key receptors to mount early immune response against infections. The number and type of KIR genes may vary in different individuals, populations and disease conditions. The aim of this study was to compare the KIR genes polymorphism in Iranian BD patients and healthy control group. **Methods:** In a case control study,19 KIR genes were typed by PCR-SSPon genomic DNAof 400 BD patients (according to ICBD criteria) and compared the results with 300age, sex and ethnicity matched healthy controls. Differences in KIR genes frequency were compared by x^2 test. **Results:** KIR-2DL3 gene was less frequency in patient group as compared to healthy control (OR=0.606; CI=0.381- 0.964, *P*=0.041).There were no statistically significant differences between BD patients and healthy control group in other genes. **Conclusion:** Distorted inhibitory KIR receptor repertoires, due to the absence of KIR-2DL3, may lower the threshold for NK cell activation, and this may be involved in autologous tissue injury of BD. However, we can't ignore the fact that the activation of this inhibitory receptor depends on the simultaneous presence of its corresponding HLA ligands. This needs to be elucidated in further studies.

Keywords: Behcet's disease, KIR, NK cells, Pathogenesis, Polymorphism.

2517P

Anti-nuclear auto-antibodies in 200 Iranian patients with systemic sclerosis: correlation with characteristic clinical features

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Background: Scleroderma is a chronic systemic autoimmunity characterized by fibrosis, vascular alteration and existence of auto antibodies. In this study we aimed to investigate the correlation of different antinuclear antibodies (ANA) in patients with systemic sclerosis and its clinical features. **Methods:** Sera of 200 patients with systemic sclerosis (SSc) were analyzed by an indirect immunofluorescence (IIF) technique with HEp-20-10 cells as a substrate. Anti-centromere antibodies (ACA), anti-topoisomeraseI (TOPO), anti-RNA Polymerase III (Pol 3), anti-Pm/Scl (Pm/Scl) and anti-Histone were determined by ELISA. Presence and frequency of clinical features associated with a specific antibody were reported cumulatively over the follow-up period. **Results:** We detected ANA in sera of 91.5% of the patients (ACA:11.5% ;anti-TOPO:78%; anti-pol3:11% ;anti-Pm/Scl:3.5% ;anti-Histone: 4.5%).ACA was related to a high prevalence of Raynaud's phenomenon as first symptom, esophageal reflux, lung fibrosis and low prevalence of diarrhea. Anti-TOPO were associated with higher prevalence of diffuse subtype of SSC, digital ulcer/gangrene, pulmonary fibrosis, calcinosis and reduction of pulmonary diffusion (DLCO<60%).Patients with anti-pol3 were older at time of first symptom & had more diffuse subtype and less pulmonary fibrosis. Anti-Pm/Scl antibodies correlated with younger age at disease onset but not with specific clinical features. Anti-Histone antibody

was associated with pulmonary fibrosis. **Conclusion:** Anti -TOPO antibody had higher prevalence and correlation with diffuse disease subtype in Iranian SSc patients. There was a direct association between anti Pol3 antibody and higher age at disease onset.
Keywords: Systemic sclerosis, Auto-antibodies

2533P

Evaluation of *ANTXR2* (rs4333130) gene polymorphism in Iranian patients with Ankylosing spondylitis

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Background: Ankylosing spondylitis (AS) is a chronic inflammatory arthritis of unknown origin that affects sacroiliac joints and axial skeleton. A recent genome-wide association study (GWAS) found anthrax toxin receptor 2 (*ANTXR2*) to be associated with AS in Caucasians. This gene encodes a receptor for anthrax toxin. We aimed to investigate whether the *ANTXR2*polymorphism is also associated with AS in Iranian population. **Methods:** We studied 363 AS patients fulfilling the modified New York Criteria and 502 age, sex, and ethnicity controls. DNA extraction was performed by Phenol-Chloroform method. We selected one SNP of *ANTXR2* (rs4333130) and genotyped it by ARMS PCR (amplification refractory mutation system polymerase chain reaction) procedure. **Results:** The frequencies of the C allele rs4333130in the patients with AS was significantly decreased in comparison with the control group (18.5% vs. 31.4%, *P*=0.01); whereas the frequencies of T allele at the same position were significantly increased in the patient group (81.5% vs. 68.86%, *P*=0.01). Comparison of genotype frequencies at this position showed that the frequency of CC genotype in comparison with other genotypes was decreased in the patient group (2.5% vs. 7.2%, *P*=0.004), while the TT genotype in comparison with other genotypes was increase (51% vs. 44.2%, *P*=0.012). **Conclusion:** This study shows that C allele of rs4333130 on *ANTXR2* is protective for AS in Iranian population. The results of our study confirm the results of other studies on Caucasians population.
Keywords: Ankylosing spondylitis (AS), *ANTXR2*, SNP, ARMS PCR

2972P

Investigate role of stress with disease activity in rheumatoid arthritis

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Background: Rheumatic diseases are common among humans and a significant percentage of people are suffering from chronic diseases and many of them are long and can cause numerous problems for the person with dementia. Many rheumatic diseases are caused by genetic factors and environmental factors such as infection, smoking , stress, poor nutrition , and various

other factors that are involved in the incidence of rheumatoid arthritis is different . This study examines the impact of stress on disease activity in rheumatoid arthritis. **Methods:** A cross sectional study on 200 women with rheumatoid arthritis rheumatology clinic of Shariati Hospital in 1391-1390 was conducted. Sampling was easy. Cohen perceived stress scale was to collect data. Had higher scores indicate less stress. Score of disease activity was measured using the DAS-28. Data were analyzed using SPSS v.15 software and descriptive analysis of the mean, standard deviations and frequency of testing and statistical analysis of T-test, Chi square was used and a significance level of less than 05 / 0 was considered. **Results:** The results showed that the stress in women with mild and moderate disease activity 4/7 ± 4/36 and in women with severe disease 4/5 ± 4/32, respectively. The results showed that the relationship between stress and disease activity score scores were significantly higher in women with more severe disease activity. **Conclusion:** There was significant correlation between disease activity and disease stress and more attention is given to the emotional state of immunological diseases.

Keywords: Rheumatoid arthritis, Perceived stress, Disease activity

2952P

Survey of sexual disorders in women with rheumatoid arthritis attending rheumatology clinic of Shariati Hospital, Tehran

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Background: Rheumatoid arthritis is an autoimmune systemic disease that can affect various systems of the body. Disease in the female to male ratio is 3 to 1. These patients due to disability and reduced quality of life and reducing the level of sexual dysfunction are sexual problems. Patients with rheumatoid arthritis may have problems such as reduced feelings of sexual attraction, decreased libido and sexual satisfaction, sexual arousal dysfunction and decreased sexual sensation during sex have a. **Methods:** A cross sectional study on 200 women referred to the rheumatology clinic of Shariati Hospital in 1391-1390 was conducted. Convenience sampling method on 100 women with rheumatoid arthritis and other diseases of 100 women matched for age were studied. The subjects for the study of sexual dysfunction in women, sexual function questionnaire (FSFI) for sexual dysfunction in men study groups were compared. Data were analyzed with SPSS v.15 software and descriptive analysis of the mean , standard deviations and frequencies and statistical analysis tests, Mann-Whitney, T-test, Chi square was used and significance level is less than 0 / 05 were considered**. Results:** Mean age was 29/6 ± 6/32 years. Comparison of sexual desire in patients with rheumatoid arthritis had a mean gain score, 5/2 ± 84/22 in the control group and 94/2 ± 63/24 libido was higher in the control group (p = 0/033). Women with rheumatoid arthritis had a mean arousal score 47/2 ± 89/13 and controls 41/3 ± 27/15 that there was a higher score in the control group, the difference was not significant. In comparison Lubrykasyvn, orgasm, sexual satisfaction, and pain between the two groups did not differ. **Conclusion:** The results showed that compared to other acts of sexual desire sexual response in women with rheumatoid arthritis were

most affected. Therefore, patients proceeding mental and sexual counseling of women with rheumatoid arthritis seem to require.

Keywords: Rheumatoid arthritis, Sexual dysfunction, Sexual performance standard questionnaire FSFI

2996P

Study of Tumor Necrosis Factor Receptor Type II (Polymorphism 196R) And Association with Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is a long-term autoimmune disease that identified by inflammatory responses with mainly affecting joints and surrounding tissues. Tumor necrosis factor (TNF) plays a key role in the pathogenesis of rheumatoid arthritis (RA). It binds to two receptors namely TNF receptor (TNFR) I and TNFRII. Several studies have suggested an association between *TNFRII* 196R/R genotype with RA and increases in production of inflammatory cytokine. The objective of the present study was to evaluate the predictive value of the *TNFRII* 196R allele for RA diagnosis of patient's arthritis rheumatoid. **Methods:** A total of 100patient's and 100controls were subjected to a case–control study performed to investigate the association of the functional 196R polymorphism of TNF-RII with RA. The functional M196R polymorphism of TNF-RII was genotyped by using analysis for screening, followed by nucleotide sequencing with Real-Time PCR**. Results and conclusion:** There were revealed significant difference between cases and controls for distribution of TNFRSF1B genotypes (TT, TG, GG,) and frequencies of mutant allele G with P >0.05%in patient's and association between the functional 196R polymorphism of TNF-RII with RA.

Keywords: *TNFRII*, Polymorphism, Rheumatoid arthritis

1588P

Reduced IL-17 but not IL-6 in peripheral blood mononuclear cells: immunomodulatory effects of mesenchymal stem cells on rheumatoid arthritis

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Background: rheumatoidarthritis (RA) is an inflammatory chronic disease that destroys cartilage and bone. The role of Effector T cells and failure in immunoregulation by Treg cells is proved in RA pathogenesis. IL-17 is produced by TH17 by IL-6 and TGF-β and has vigorous effects on cells of the immune system playing important roles in pathogenesis of autoimmune disorders. On the other hand, mesenchymal stem cells (MSCs), because of their

therapeutic roles in many diseases can be used as cell therapy in RA. In current study the effect of MSCs on peripheral blood mononuclear cells (PBMCs) by IL-17 and IL-6 detecting in RA patients was assessed in order to effect of MSCs on reduced inflammation. **Methods:** MSCs of adipose tissue was isolated from 10 patients undergoing caesarean section under cell culture. On the other hand, PBMCs that isolated from 15 RA patients and 11 healthy controls were added to MSCs in culture. Then, IL-17 and IL-6 expression was determined by ELISA on co-culture supernatants of PBMCs from both groups. **Results:** As results,analysis of cytokine production profile revealed that IL-17 level in MSCs-PBMCs co-culture was less than PBMCs culture lonely in patients ($P<0.005$) but in healthy individuals was not seen any significant differences. However, no statistical difference was found in the IL-6 level in MSCs-PBMCs co-culture compared to PBMCs culture lonely.**Conclusion:** In conclusion, because of MSCs role in reducing IL-17 as an inflammatory cytokine in PBMCs, can be as suitable candidate for treatment RA diseasein immunomodulation.
Keywords: RA, MSCs, PBMCs, IL-6, IL-17

2120P

Autoantibodies to small RNAs and clinical manifestations in systemic lupus erythematosus patients

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Background: Antibodies against small RNAs and common RNA proteins such as Sm, nRNP, Ro, and La are frequently found in patients with systemic lupus erythematosus. These autoantibodies are more prevalent but less specific for SLE and are thought to be the first developed antibodies in SLE patients and arise sequentially over time. Once first autoantibody initiated, epitope spreading provides development of other pathogenic antibodies. Anti-Ro and anti-La in some studies are associated with dermatological manifestations of cutaneous lupus. **Methods:** Serum samples from 98 patients with systemic lupus erythematosus were studied for autoantibodies against anti-nRNP, anti-Sm, anti-SSA, anti-Ro52, and anti-SSB autoantibodies by using immunoblotong technique. All patients fulfilled at least 4 ACR criteria .**Results:** Anti-RNA autoantibodies were frequent in our SLE patients. Anti-SSA in 42.8%, anti-Ro52 in 37.7%, anti-nRNP in 30.6%, anti-Sm in 14.2%, and anti-SSB in 9.2% of our SLE patients was detected. Anti-SSA, anti-SSB, and anti-Ro52 were more frequent in female in comparison to male SLE patients (p=0.028, 0.005, 0.006 respectively). In male SLE patients kidney involvement is more prevalent than females, and none of male patients has anti-SSB. Anti-SSB was seen in any of patients with kidney involvement (p=0.005). Anti-nRNP negatively associated with disease activity (SLEDAI 6.0 in anti-nRNPvs 15.3 in anti-nRNP positive patients) (p=0.11). **Conclusion:** Kidney involvement is more prevalent in male SLE patients, while in none of male patients anti-SSB autoantibody was detected, and it seems that anti-SSB was associated with decreased chance of renal involvement. Anti-SSA, anti-SSB

and anti-Ro52 autoantibodies are more frequent in female SLE patients, and perhaps because of lower pathogenicity of these autoantibodies, their presence could protect female patients against more serious manifestations.

Keywords: Autoantibody, Small RNA, Clinical manifestation, Systemic lupus erythematosus, Orga

2119P

Anti-dsDNA, Anti-chromatin and Anti-histone antibodies in systemic lupus erythematosus

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Background: Antibodies directed against chromatin components (ds-DNA, chromatin, histones) are of principal importance in systemic lupus erythematosus. Anti-dsDNA serum levels have been correlated with disease activity, and are often used as representative of SLE activity.Several studies report conflicting data relating disease activity and ds-DNA level. **Methods:** 98 patients with a mean age of 29.8 ± 9.30 years were evaluated for anti-dsDNA, anti-nucleosomes, and anti-histone using immunobloting technique. **Results:** In SLE patients, the presence of anti-nucleosome in joint involvement (p=0.04), and anti-dsDNA in skin involvement, was significant compared to patients without above symptoms. The presence of anti-histone and anti-dsDNA correlated with disease activity. In anti-dsDNA positive patients, anti-nucleosome, and anti-histone were significantly more than anti-dsDNA negative patients (p value of 0.001, 0.001 respectively).Anti-nucleosome positive patients have significantly more anti-dsDNA and anti-histone autoantibodies than the patients lacking anti-nucleosome (p value of 0.001 and 0.001, respectively).In patients with anti-histone autoantibody, anti-dsDNA and anti-nucleosome was more frequent than in patients without anti-histone (p value of 0.002, 0.001 and 0.001, respectively). **Conclusion:** Increased presence of anti-nucleosome in joint involvement (p=0.04), and anti-dsDNA in skin involvement, was significant, and presence of anti-dsDNA and anti-histone autoantibodies was associated with disease activity. Anti-dsDNA, anti-nucleosome, and anti-histone autoantibodies have concurrent presence with together.

Keywords: Anti-dsDNA, Anti-nucleosome, Anti-histone, Systemic lupus erythematosus, Organ

1735P

The effects of Nitric Oxide (NO) and Adiponectin in autoimmune disease systemic lupus erythematosus patients

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Background: Systemic lupus erythematosus (SLE) is one of the most autoimmune diseases with multiple organ complications. Nitric Oxide (NO) is a free radical with numerous physiological functions and involved in pathophysiologic state of many diseases. Adiponectin as a cytokine has anti-inflammatory and anti-atherogenic effects. With regard to complications and unknown ethiopathogenesis of SLE, the current study was designed to investigate the possible role of NO and adiponectin on immune system in SLE patients. **Methods:** blood samples were collected from 40 SLE and 40 control groups. Plasma nitric oxide levels were measured by Griess method and adiponectin levels were assayed by ELISA. Student t-test was used for statistical analysis and $P \leq 0.05$ assigned as significant differences between groups. **Results:** Plasma NO concentration was significantly increased in SLE patients (32.31µmol/ml) as compared to control group (14.15µmol/ml, $P \leq 0.001$). In addition, Adiponectin concentration was significantly increased in SLE patients (17.6µg/ml) as compare to control group (13.00µg/ml, $P \leq 0.02$). **Conclusion:** current study showed a significant increase in both NO and adiponectin concentrations in SLE patients as compared to control group. The increased level of NO might be due to increased level of adiponectin and subsequent induction of iNOS enzyme in SLE patients with damaging property. Although adiponectin has modulatory effect on immune system its real role in SLE patients needs further investigation.
Keywords: Nitric Oxide (NO), Adiponectin, Systemic Lupus erythematosus (SLE)

2311P

Evaluation gene expression of cytokines in Rheumatoid Arthritis patients

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Background: Rheumatoid Arthritis (RA) is a systemic inflammatory disorder influencing synovium of the patients' joints.Pro-inflammatory cytokine and TGF-β have a crucial role in maintaining the balance of regulatory T cells and TH17 cells. Therefore determination of these cytokines may be helpful to predict the treatment and progress of the disease.**Methods:** Peripheral blood mononuclear cells (PBMCs) were gathered from 35 active RA patients who treated for at least 3 months with disease-modifying anti rheumatic drugs (DMARDs: Methotrexate) and steroids (Prednisone) and the same number of the healthy individuals. Total mRNA was extracted from PBMCs and cytokine mRNA expression of IL-6, TGF-β, IL-21 and IL-23 was determined by RT-PCR. **Results:** the mRNA expression of TGF-β, IL-21 and IL-23 was lower in patients in comparison to healthy controls whereas in some patients (n=6) the level of mRNA IL-6 was more than healthy control(the other the same as control, p= 0.001). **Conclusion:** In some of our studied patients, the therapy couldn't regulate IL-6 levels the same as healthy individuals and seems level of IL-6 can be used to predict treatment process in RA patients.
Keywords: Rheumatoid Arthritis (RA), Cytokines, Real time PCR (RT-PCR)

2333P

The effect of mesenchymal stem cell supernatant for prevention of collagen-induced arthritis in rats

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Background: The present study aimed to investigate the effect of molecules secreted by mesenchymal stem cells (MSCs) for the prevention of collagen-induced arthritis (CIA) in rats. **Methods:** Supernatant of MSCs prepared from rat bone marrow derived MSCs culture .In prevention group ,rats treated with collagen-adjuvant and MSC supernatant on days 0 and .7 In control group ,we used from clutter medium instead of MSC supernatant for rats treatment and other cases were similar with prevention group .From day 7 through day35 after immunization ,we investigated onset of CIA ,paw swelling and clinical score in rats .We also determined histopathological features of joints and bones .**Results:** We have found that supernatant of MSCs significantly delayed the onset of CIA and decreased paw swelling, bone destruction, inflammationof synovial and clinical score in prevention group compared with rats treated with culture medium in control group. **Conclusion:** Taken together, we conclude that application of soluble factors produced by MSCs if the time of CIA induction can decrease severity of arthritis and local and systemic tissue lesions, but cannot prevent the onset of CIA.

Keywords: Rheumatoid arthritis, Collagen-induced arthritis, Mesenchymal stem cell

3082P

Neurological effects in patients with Rheuma toidrathritis.

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Background: Rheumatoid arthritis (RA): Is a chronic sickness in which system organs conflicted by 1-3 percent. Outer joint involvement including: Lungs, Eye, Vascular, Neurological manifestations. Neurological effects of the disease are divided by tow, 1_involving central nerve system, mainly Cervical spinal cord. 2_ Involving peripheral nervous system includes involvement of peripheral nerves, Poly neuropathy, Mononorit multiplex (sensory & motor). Assessment and a better understanding of neurological effects in RA patients provides the possibility of early diagnosis and appropriate treatment. To achieve this goal, from 88_87 years ,for 18 months a search has been done in Torbal-heydariyeh. **Methods:** This search is of the cross-sectional that due to it, the patient were 60 and base on ACR criteria the diagnostic actions was under staging of disease improving. All patients were performed under Nasvltral neck radiography & upper extremity Electrodiagnostic studies. The result of research in this perusal 72/2% female and 72/2% male were between 50/21±14/9. The most common clinical symptom was morning stiffness (84/2%) and the most common involving joint was the wrist (75/6%).ERS was increased in 53/7% of patients and CPR was positive in 61/7% and also RF in 51/2%. 0/4 of patients were in Stage I, 48/9% in Stage II, 25/2% in Stage III and 21/9% in Stage IV. In 57/3% of patients observed nervous system involvement, in 23/4%

involvement of central nervous and in 33/8% peripheral nervous system involvement. The most common effects of central nervous system: involving the lower cervical spine in11/3%, 8/2% involving cervical spine, 3/8% involving the Atlantoaxial. The most common effects of peripheral nervous system was obstruction of nerve 19/3 percent and sensory Polyneuropathy 12/2 percent.The most common type of nerve involvement was Carpal tunnel syndrome that reported In 9 patients. **Conclusion**: One of the serious and threatening symptoms of Rheumatoid arthritis was nervous system involvement,rate of neurological effects increases with disease progression .performing periodic neck radiography and studies in patients with disease duration of more than 2years, Deformity or Erosions is one of necessary doing in order to control the neurological effects.

Immunoparasitology

Oral Presentations:

3304O

The FcgRIIIB-NA1/NA2 polymorphism is significantly associated with visceral leishmaniasis: a study from north-west of Iran

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Background: Several lines of evidence demonstrate that innate and adaptive immunity play important roles in the defense against visceral leishmaniasis (VL). A polymorphism within the FcgRIIIB gene can lead to the expression of three variants including NA1, NA2 or in combination (NA1/NA2) the isoforms demonstrate alter affinity of IgG to its receptor. Thus, the main aim of this study was to evaluate the FcgRIIIB-NA1/NA2 polymorphism in the FcgRIIIB gene of Iranian VL patients in comparison to healthy controls. **Methods:** In this cross-sectional study 54 patients with clinical presentation of VL and seropositive for the leishmania (group 1), 104 patients without clinical presentation but seropositive (group 2) and 104 healthy controls (group 3) were evaluated with respect to the FcgRIIIB-NA1/NA2 polymorphism using a PCR-SSP method. The titration of anti-leishmania antibodies was analysed using an immunoflorescence technique. **Results:** Our results indicated that polymorphisms within the FcgRIIIB gene that lead to the expression of the NA1/NA2 isoforms are significantly associated with VL. The results demonstrated that the genotype heterozygotic for FcgRIIIB-NA1/NA2 expression was significantly increased in group VL patients when compared to groups 2 and 3. Conversely, there is a decrease in homozygous NA1 and NA2 genotypes in VL patients, however, the overall allele frequencies for NA1 and NA2 appear similar across the three cohorts examined. **Conclusion:** According to the results presented here, it may be concluded that the increased frequency of the FcgRIIIB-NA1/NA2 genotype could be associated with impaired immune responses against VL and its subsequent clearance from the patient.

Keywords: FcgRIIIB, Polymorphism, Visceral leishmaniasis

2928O

Genetically-different strains of *Leishmania major* show distinct virulence patterns and divers cytokine mRNA expression levels in C57BL/6 mice

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Background: *Leishmania major*, the causative agent of zoonotic cutaneous leishmaniasis (CL), exhibits several diverse clinical manifestations in different areas and experimental models. This diversity may cause difficulties in selection of reliable strains for the vaccine studies. The present study was aimed at choosing an ideal strain of *L. major*, capable of inducing reliable virulence and higher mRNA expression of the prototypic Th1 cytokines. **Methods:** Four genotypically-distinct *L. major* strains from the lesion of patients residing in four endemic areas of CL in Iran, including Damghan (north), Kashan (center), Dehloran (west) and Shiraz (south) were used for evaluation in C57BL/6 mice. The virulence of the parasites was evaluated by measurement of parasite load in the lymph nodes (LN) using limiting dilution assay. The immunogenicity of the strains was estimated by analysis of the cytokine mRNA expression levels in LN of C57BL/6 mice at weeks 1, 3, 5 and 8, post-infection. The mRNA expressions of major cytokines, including IFN-γ, IL-4, IL-10 and IL-12 were analyzed using real-time PCR.**Results:** Our data revealed the lowest parasite load in LN of mice infected with Damghan strain. The Damghan strain (DA39) showed higher expression of *Ifng* and *Il12* at week 8 post-infection. However, the highest *Ifng/Il4* ratio was expressed by the Kashan strain at week 8 post-infection.**Conclusion:** Taken together, our results indicated that the Damghan strain which induced lowest parasite load and higher expressions of *Ifng* and *Il12* mRNA in the LN might be an ideal candidate strain for vaccine studies in leishmaniasis.

Keywords: Leishmania, C57BL/6 mice, Cytokine

3279O

Comparison the effect of TGF-β1 suppression by ShRNA mediated lentiviral vector on BALB/c and C57BL/6 macrophage response to Leishmania major infection

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Background: Immediately after leishmania parasites enter the skin, leishmania trapped by macrophages and dendritic cells and inhibit the induction of type I immune responses. One of inhibitory mechanisms isincrease in production and secretion of TGF-β1 by infected macrophages. In this research we study the effect of ShRNA mediated TGF-β1 gene expression on macrophage response to L.major infection.Because of functional differences between sensitive (BALB/c) and resistance (C57BL/6) strains to L.major, we compared the effect of TGF-β1 suppression in both groups. **Methods:** Inbred BALB/c and C57BL/6 mice were purchased from pasture institute of Iran. Peritoneal macrophages prepared after 4days intraperitonealthioglycolate injection. Macrophages cultured in 10^6cells/well and divided to four experimental groups: macrophages(I), macrophages+L.major(II), TGF-β1 ShRNA treated macrophages+L.major(III) and TGF-β1 ShRNA treated macrophages. Five days after lentiviral treatment of macrophages for TGF-β1 suppression, L.major added at 3:1 ratio to macrophage culture. Supernatant collected at day 0-2, day 2-4, day 4-7 and day 7-10 intervals.

IL-10, IL-17 and TGF-β1 production measured at supernatants. **Results:** TGF-β1 measurement showed that L.major infection can induce TGF-β1 production in infected macrophages. BALB/c macrophages produced significantly($p<0.05$)more TGF-β1 compare to C57BL/6 mice. ShRNA mediated TGF-β1 suppression, reduced TGF-β1 production during 4-7 days after transduction. Results showed that IL-10 production increased after L.major infection in both BALB/c and C57BL/6 macrophages. IL-10 productionhad significant decrease in ShRNA mediated TGF-β1 suppressed groups compared to only L.major treated group. Results showed that IL-17 production increased in TGF-β1 suppressed group in response to L.major infection. This increase is significantly ($p<0.05$) higher in BALB/c mice compare to C57BL/6 mice. **Conclusion:** The results showed that TGF-β1 suppression had significant effect on IL-10 and IL-17 production in both mice groups. IL-10 production decreased and IL-17 production increased in macrophages in response to L.major infection.This result showed that TGF-β1 suppression can induce inflammatory responses through reduction of IL-10 effect and enhancement of IL-17 effect in both macrophage groups.

Keywords: TGF-β1, macrophage, Leishmania major

2500O

Vaccination against Leishmaniasis using low pathogenic strain of *Leishmania major* in BALB/c mice

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Background: Although the host immune system has an important role in the outcome of leishmaniasis, the species and strains of *Leishmania* can also play a pivotal role in the development of clinical symptoms and host immunity. In this study, a low virulent *Leishmania major* strain was used for immunization of BALB/c mice against a virulent *L. major* challenge. **Methods:** A new strain of *L. major* with low virulence, isolated from an endemic area of cutaneous leishmaniasis in North-East of Iran (Damghan) was used for immunization of BALB/c mice. The mice were immunized with three doses (two-week intervals) of killed promastigotes ($1x10^5$) in the left footpad with or without Imiquimod adjuvant and challenged using a live virulent *L. major* strain in the right footpad, two weeks after the last immunization. DTH and IgG isotypes were measured before the challenge. Parasite burden, footpad swelling and mRNA expressions of major cytokines of Th1 and Th2 responses were measured by qRT-PCR in lymph node cells after the challenge. **Results:** Increase of DTH and IgG2a were shown in the pre-challenged periods. The parasite burden and footpad swelling were significantly lower in the immunized mice compared to the control ($p<0.05$). A significant difference was observed in the ratio of IFN-γ/IL4 mRNA expressions at the first week post-challenge in the immunized mice compared to the control ($p=0.001$). **Conclusion**: The results indicated that the application of killed low virulent *L. major* strain could lead the immune response toward protection against leishmaniasis, especially when combined with an appropriate adjuvant such as Imiquimod.

Keywords: low pathogenic *Leishmania major*, Imiquimod, Vaccine

2114O

Cytokine profiles following immunization with alum precipitated autoclaved *Leishmania major* mixed with BCG plus Imiquimod in dogs in VL endemic area

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Background: The objective of this study was to investigate cytokine profiles of peripheral blood mononuclear cells (PBMCs) collected from dogs immunized with alum precipitated autoclaved *Leishmania major* (Alum-ALM) plus BCG along with Imiquimod as an adjuvant in an endemic area of Kala-azar, north-west of Iran (MeshkinShahr). **Methods:** A randomized double blind control trial was designed to evaluate cytokine profile of dogs immunized with alum-ALM plus Imiquimod. Production of cytokines and expression of cytokines mRNA were checked on ~ 10 ownership dogs in Meshkin Shahr district. Periodical peripheral blood sampling was carried out on vaccinated dogs at days 30, 80 and 300 post vaccination. PBMCs were isolated by Ficoll Hypaque gradient. Supernatants were collected and used for measurement of canine IFN-γ, IL10, IL-12 and TGF-β cytokines. A part of isolated PBMCs was used for expression of cytokines (IFN-g, IL-2, IL-4, IL-10, IL-12 and TGF-²) mRNA by real-time PCR. **Results:** The results showed an increase of IFN-γ at 30 and 80 days post immunization and a considerable increase of IFN-γ/IL-10 ratio at days 30 post immunization. However, increase of IL-10 at days 80 or 300 and TGF-β at day 30, 80 and 300 post immunization were also detected. Moreover, the higher expression of IFN-γ mRNA at days 30 and 300 and also increase of IFN-β/IL-4 mRNA ratio were observed at day 30 post immunization. **Conclusion:** The data showed initiation and establishment of a Th1 immune response in day 30 post immunization which then was converted to a mixed Th1 and Th2 cytokine profiles.

Keywords: Canine visceral leishmaniasis, Vaccine, Cytokines, Iran

1651O

Evaluation of protective effect of IL-22 on cutaneous leishmaniasis in BALB/c mice

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Background: Since decades ago, many attempts have been carried out to develop appropriate drugs and vaccines against different types of human leishmaniasis. The role of IL-22 in development of protection and innate immunity mechanism in control of infectious disease, maintenance of hemostasis, and tissue regeneration has been confirmed recently. Protective effect of IL-22 on cutaneous leishmaniasis resulted from *Leishmaniasis major (L. major)* in BALB/c mice were investigated. **Methods:** In this study we evaluated the effect of IL-

22 on infected BALB/c mice with *L. major*. The experiment groups were evaluated for the cellular and humoral responses by measurement of IL-4, INF-γ, after challenge by *L. major* of the standard Iranian strain MRHO/IR/75/ER. Clinical evaluations were performed by measurement of lesion diameter, and survival rate of the mice. **Results:** In week 27, the mortality rates for control groups were 100%. While the survival rates for the IL-22-5ng/g groups were 100%. The size of lesions decreased in the presence IL-22-5ng/g of mice weight, which was statistically significant in comparison with other groups ($p<0.05$). Measurement of INF-γ, IL-4 demonstrated that IL-22 increased INF-γ production and decreased IL-4 production after the challenge with *L. major*. The difference with other groups was statistically significant ($p<0.05$). **Conclusion:** The results obtained indicate the effectiveness of IL-22 in protection of cutaneous leishmaniasis.

Keywords: *Leishmaniasis major,* IL-22, protection, BALB/c

1533O

Immunoregulatory effects of chitin microparticles on *Leishmania major*-infected BALB/c mouse

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Background: While chitin microparticles (CMPs) are shown to have immunopotentiating activities, their exact contribution to the in vivo regulation of immune response in Leishmania-infected BALB/c mouse is not studied yet. To address this issue, we utilized *L. major* infected BALB/c mice and assessed the immunoregulatory effects of CMPs injection as well as its outcome on the course of Leishmania disease.**Methods:**BALB/c mice were infected with *L. major* promastigotes at their base of the tail. CMPs (100µg/100µl) were injected 3 days before and every 2 days after the parasite inoculation. Cytokine concentrations (TNF-α, IFN-γ, IL-5 and IL-10) were quantified using ELISA assays. The onset of lesion formation and their size were also determined.**Results:**According to the obtained results, CMPs treatment modulated immune response toward Th1 responses with significantly increased IFN-γ/IL-5 ratio as well as TNF-α level. Interestingly, IL-10 level, as an anti-inflammatory cytokine, also elevated in CMPs treated group, however, IFN-γ/IL-10 ratio was significantly higher in this group than untreated infected control animals. Accordingly, the onset of lesion formation was significantly postponed and the lesion size was smaller in CMPs treated mice than that of control group. **Conclusion:** These findings clearly show that CMPs mediate in vivo immunoregulatory effects via the production of IFN-γ and IL-10 and provide enough motivation to design further studies on potential application of this naturally nontoxic substance in prophylactic or therapeutic interventions against Leishmaniasis.

Keywords: Chitin microparticle, Leishmania major, immunomodulation, cytokine

Poster Presentations:

1955P

Evaluation of immune response induced by DNA vaccine cocktail expressing complete LACK and TSA genes against Leishmania major

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Background: Leishmaniasis is an important disease in humans. Leishmania homologue of receptor for Activated CKinase (LACK) and thiol specific antioxidant (TSA) as immuno-dominant antigens of Leishmania major are considered the most promising molecules for a DNA vaccine. **Methods:** A DNA cocktail, containing plasmids encoding LACK and TSA genes of Leishmania major constructed and evaluated the immune response and survival rate in BALB/c mice. **Results:** IgG and Interferon gamma values were noticeably increased in the immunized group with DNA cocktail vaccine, which were significantly higher than those in the single-gene vaccinated and control groups ($p < 0.05$) following the immunization and after challenging with Leishmania major. Interleukin 4 values were decreased in all immunized groups, but only in DNA vaccine cocktail and single-gene vaccination with pc-LACK there were statistical differences with control groups ($p > 0.05$). The immunized mice with the cocktail DNA vaccine presented a considerable reduction in diameter of lesion compared to other groups and a significant difference was observed ($p < 0.05$) in this regard. The survival time of the immunized mice with the cocktail DNA vaccine was significantly higher than that in the other groups ($p < 0.05$) after their being challenged with Leishmania major.**Conclusion:** The findings of this study indicated that the cocktail DNA vaccine increased the cellular response and survival rate and induced protection against infection with Leishmania in the mice.

Keywords: DNA vaccine, Leishmania major, cocktail pcTSA+pcLACK, immune responses

2030P

Immunosuppressive effects survey of Lavandula spica extract on cutaneous leishmaniasis in BALB/c mice

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Background: Different kinds of Lavandula speca have been used traditionally in throughout history. Considering the aspects of mint family plants, in this survey, the effects to suppress the immunosystem of the BABL/c mice, which were infected by cutaneous Leishmaniasis, were studied. **Methods:** sufficient twigs of lavender sterilized and prepared as topical with concentration of 40, 60 and 80%. 40 BABL/c mice were infected with the parasite Leishmania (L) major [MRHO/IR/75/ER]. Extract- lavandula was applied every two days. As well, the foot and the size of the lesion were measured; the weight was also taken in all mice in the four groups by using ale and *coils* every week until the death of the last mouse in the control group. **Results:** The mean of the weight of the mice that were received 40 and 60% of the extract –lavandula showed significant difference comparing with the mean of the weight of the mice in control group($P = 0.000$), but the mean of weight in the mice received 80% did not show significant difference($P > 0.05$).The mean of lesion size of the mice that received 40 & 60 of extract –lavandula showed significant difference with the mean of the lesion size in controls groups ($P = 0.000$), but, the mean of 80% of the extract –lavandula did not show significant difference($P > 0.05$). **Conclusion:** The process ofweight lossandincreased in sizewith increasing concentrationup to 80% of the animal'swoundcontinuedand theother80%had no effectconcentration.

Keywords: Lvandula Extract, Cutaneous Leishmaniasis, Immunosupressive, BALB/c Mice

1468P

Increased levels of interleukin-4 (IL-4), interleukin-10 (IL-10) and IFN-γ in Iranian Visceral Leishmaniasis (VL) Children

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Background: Leishmaniasis is a disease caused by obligate intracellular parasites. Visceral leishmaniasis (VL) as a prevalent type of it is a public health threatening in developing world. Functional immunity to VL depends on cytokine profile. IFN-γ, major cytokine of TH_1, plays a crucial role in immune responses against leishmaniasis in the contrast with IL-4 and IL-10 activity. **Methods:** All active cases in the endemic area were children under 12 years old. Sera from VL patients of north-west area of Iran were analyzed for the concentration of IFN-γ (patients=23, controls=9), IL-4 (patients=26, control=9) and IL-10(patients=26, control=9) by ELISA method and were studied TH_1/TH_2 paradigm via their associated signature cytokines. **Results:** We detected circulating levels of IFN-γ in 23 patients (mean=40.57pg/ml) higher than control group (mean=7.18pg/ml) and also the results show increased concentrations of IL-4 in

the sera of 26 patients (mean=72.92pg/ml) in comparison with control group (mean=33.28pg/ml).Results achieved from measurement of IL-10 serum levels showed 4 cases with very high IL-10 levels (case1=1512pg/ml, case2=2255pg/ml, case3=1713pg/ml, case4=2031pg/ml) and mean of IL-10 level in 22 patient (34.78pg/ml)was higher than control group(mean=9.37pg/ml, n=9). **Conclusion:** Accumulating evidence points toward an antagonism between TH_1 and TH_2 and also their associated cytokines in leishmaniasis. In acute phase of VL, TH_2 responses are dominant and TH_1 cytokines are protective. Our findings suggest that the cytokine pattern in VL patients is not polarized and profile of these cytokines is not characterized by TH_2 phenotype as in mice. Although both TH_1 and TH_2 cells appear to be proliferated and involved in host immune responses.

Keywords: Leishmaniasis, interleukin-4, interleukin-10, IFN-γ, TH_1/TH_2

1655P

Study of serum levels TNF-α in leishmaniasis patients

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Background: It is generally accepted that the host cell-mediated immunity plays a pivotal role against the protozoa parasite leishmania spp. It is reported that tumor necrosis factor (TNF-α)appears to play an important role in host against leishmaniasis. TNF-α is a cytokine produced mainly by macrophages, with a wide range of biological activities and may be important in inflammatory processes. However, so far there is little information on the possible role of TNF-α in leishmaniasis. The aim of this study is to evaluate the role of TNF-α in the reactive phase of leishmaniasis. **Methods:** We evaluated plasma levels of TNF-α in 100 patients with active leishmaniasis and 50 normal individuals in an endemic area of Iran (Isfahan) to investigate the correlations between the clinical outcome of infection and plasma cytokine levels. Blood sample obtained from patient and control groups and the separated plasma was stored in the -70°c until tested. Plasma level of TNF-α was measured by Sandwich ELISA (USCN,China) according to the manufacture´s protocol. Data were analyzed by the Kolmogorov-smirnov and Mann-Whitney test using SPSS software. **Results and Discussion:** The mean of cytokines in the patient and control groups were 44±5pg/ml and 55±6.4pg/ml respectively. The plasma levels of TNF-α in patients were significantly lower than controls ($P<0.05$). It is being suggested the predictive value of for disease reactivity.

Keywords: Leishmaniasis, Cytokine, TNF-α

2093P

Comparison of immune modulating effects of chitin microparticles with chitosan microparticles on leishmania infected lymph nodes cell suspensions of the BALB/c mice

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Background: There are contradictions about the immune modulating properties of chitin in

comparison with chitosan. To respond to these contradictions, we investigated stimulatory effects of chitin micro-particles in comparison with chitosan micro-particles on L.major infected cell suspensions. **Methods :**BALB/c mice were intradermally infected with 2 × 10^5 stationary phase of L.majorpromastigotes into their base of the tail and two weeks later infectivity were confirmed by PCR then leishmania infected lymph nodes cell suspensions of the mice were isolated . Chitin micro-particles were prepared by sonication and passing the filter and after size determination by Master sizer, used to cell stimulation. Finally concentrations of TNF-α and IL-10 were measured by ELISA in cell culture supernatants. **Results:** We observed Chitin micro-particles significantly increase T NF-α and also IL-10 production ($P \leq 0.001$) in compare with chitosan. **Conclusion:** Chitin micro- particles can stimulate production of TNF-α and IL-10 in leishmania infected cell suspensions. Chitin seems to be utilized as an immunomodulation in Leishmania vaccine or treatment.

Keywords: Chitin, Chitosan, Leishmania major, Cytokine

2486P

Infectivity rate comparison of *Leishmania* expressing EGFP or EGFP-LUC proteins with wild-type *in vitro* evaluation for drug potency

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Background: Leishmaniasis remains an uncontrolled disease in the world due to lack of an effective drug or vaccine. Still, there is no powerful method for assessment of rapid and precious drug effectiveness against intracellular form of parasite. Reporter genes technology has proved to be an excellent tool for drug screening. In this project, for the first time, we compared the infectivity rate of recombinant *Leishmania major* stably expressing enhanced green fluorescent protein (EGFP) alone or luciferase fused (EGFP-LUC) with the wild-type strain using MTT and NO assay. Also, both mouse (B10) and human macrophage cell lines (THP-1) were used in combination with several classical and specific methods. In order to measure the activity of each reporter gene within 24, 48 and 72 h post infection we used flow cytometry and fluorescent microscope for EGFP and luminometer for luciferase. **Results:** Expression of EGFP and EGFP-LUC was stable in promastigote and amastigote stages of parasite. We observed a linear correlation between the number of parasites and reporter gene activity. *In vitro* infectivity rate of three strains were similar. EGFP expression was directly visualized with fluorescent microscopy and measured by FACS analysis and showed significant differences between untreated and treated cells with different concentrations of amphotricin B. Also, luciferase activity in infected cells with *Leishmania* expressing EGFP-LUC decreased when treated with drug in comparison to control cells. **Conclusion:** Our results indicated that using more than one reporter gene allows evaluating of drug efficiency faster and more sensitive on infected cells harboring amastigotes.

Keyword: *Leishmania*, Reporter gene, Amphotricin B, Infectivity, in vitro

1670P

Seroepidemiology of Toxoplasma gondii in blood donors in Jahrom, Iran

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Background: Toxoplasmosis is a cosmopolitan zoonotic disease which is caused by an intercellular obligate protozoan, *Toxoplasma gondii*. Most of these infections are asymptomatic or benign, but may cause severe or fatal consequences in immunodeficient patients, transplant recipients and in the fetus. Transmission may occur by eating unwell-cooked meat, contaminated vegetables, blood transfusion, organ transplantation and transplacental to fetus. IgG antibodies to *T. gondii* may persist in the serum at high titers for years. The aim of this study was to determine prevalence of anti- *T. gondii* IgG and IgM antibodies in Jahromblood donors in 2010. **Methods:** This cross sectional-descriptive study was carried out on 420 blood donors in Jahrom in 2010. Sera were collected and kept at -70°C. IgG and IgM anti-*Toxoplasma gondii* antibodies were detected using ELISA. **Results:** Due to data, 13.4% and 1.7% were seropositive for IgG and IgM, respectively, Toxoplasma antibodies. Seropositivity was 13.6% in men and 9.1% in women. The highest seropositivity incidence for IgG was seen in age group 40-50 years. There was no difference in frequency of seropositivity between group of people with exposure to cat or raw meat or soil and who had no exposure. **Conclusion:** IranianBlood Transfusion Organization does not examine blood samples for *Toxoplasma gondii*. This research suggests the importance of serological tests for *Toxoplasma gondii* in blood donors.

Keywords: Toxoplasmosis, *Toxoplasma gondii*, Blood Donors, Jahrom,

1534P

A real time polymerase chain reaction assay for quantifying *Leishmania major* in lymph nodes of infected mouse

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Background: To investigate efficiency of vaccines and drugs against Leishmaniasis in animal models, parasite loads in lesions and other organs are evaluated. In the present study, we developed a real time PCR assay to estimate parasite numbers in lymph nodes of *Leishmania major* infected BALB/C mice and then utilized a limiting dilution assay to estimate and compare parasite burdens with those of real time PCR assay. **Methods:** The SYBRGreen based Real Time PCR assay was performed to amplify a 75bp fragment of superoxide dismutase B1 gene in the inguinal lymph nodes of *L.major* infected BALB/C mice. A standard curve was established using 10-fold serial dilutions of *L. major* DNA corresponding to 5×10^6 parasites to 0.5 parasites per reaction. A limiting dilution assay was performed for estimating and comparing parasite burdens with those of real time PCR. **Results:** The standard curve was linear over an at least 6 serial dilutions of parasitic DNA with a correlation coefficient (R^2) value of 0.993 and we were able to detect as little as 50 parasites. The melting curve of PCR product demonstrated single peak with the melting temperature 82.5˚C. Pearson's correlation coefficient of parasite burdens between two techniques was statistically significant

(p value = 0.016).**Conclusion**: These results demonstrated that our real time PCR based on the amplification of a fragment of superoxide dismutase B1 gene of *L. major* may replace limiting dilution assay for estimating parasite burdens in *L. major* infected BALB/c mice.
Keywords: Real time PCR, limiting dilution assay, *Leishmania major*, Quantification

1855P

The usage of 21KDa antigen Leishmaniainfantum in diagnostic immunized dogs from unvaccinated dogs

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Background: *Leishmania infantum* (*L. infantum*) is an obligatory intracellular protozoan and causative agent of visceral leishmaniasis in human and dogs. An attenuated line of *L. infantum* H-line, developed in the pressure on gentamicin, has been shown to protect dogs against canine visceral leishmaniasis. The aim of this study is to confirm the immunogenicity of 21 kDa antigen of promastigotes of *L. infantum* H-line as a diagnostic marker to distinguishing between the dogs vaccinated with the attenuated parasite with dogs naturally infected with *L. infatum* wild-type (WT) using Western blotting (WB). **Methods:** Sera from 2 groups of dogs, vaccinated with the attenuated line and dogs naturally infected with *L. infantum* WT collected. WB analysis was applied using lysates of stationary phase promastigotes of *L. infantum* H-line and WT. **Results:** In the present study we found that sera from vaccinated dogs recognized a 21 kDa antigen of *L. infantum* H-line but not of *L. infantum* WT. Whereas, the sera from naturally infected dogs with L. infantum WT, recognized a 21 kDa antigen of promastigotes of *L. infantum* WT but not of *L. infantum* H-line. **Conclusion:** These data are suggesting that 21 kDa antigens of L. infatum H-line and WT could be used for distinguishing between vaccinated dogs in field vaccine trial in dogs.
Keywords: 21KDa antigen, *Leishmania infantum*, Western blotting (WB)

2125P

Effects of rosmarinic acid and linoleic acid on leishmaniasis

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Background: Leishmaniasis is caused by parasitic protozoa transmitted by the bite of female sand fly and is currently endemic in 88 countries. BALB/c mice are highly susceptible to the infection with *Leishmania major* and this susceptibility has been attributed in part to the expansion of Th2 cells and down-regulation of Th1 cells. We have previously showed that both aqueous and alcoholic extracts of Iranian Borago (*Echium amaenum* Fisch & C.A. Mey) had immunomodulatory properties with increasing the level of IFN-γ and lowering the parasite burden in the lymph nodes and preventing the necroses of the footpad in BALB/c mice. In this report, we show the effects of two purified constituents of the Borago on leishmania infection. **Methods:** *Leishmania major* promastigotes were injected into the footpad and at

the same time rosmarinic acid and linoleic acid were injected into the peritoneal cavity. The treatment continued weekly for five weeks. At weeks 5, 9 and 12 after infection, proliferation assay, footpad swelling and parasite burden were determined. **Results:** Both rosmarinic acid and linoleic acid, at different concentrations, showed strong immuno-stimulatory activities and displayed a significant reduction of the footpad swelling for more than 12 weeks after infection with *L. major*. Lymph node parasite burden at 5, 9 and 12 weeks after infection were lowered than control group without treatment. **Conclusion:** These data indicate that anti-leishmania effects of Borago may be due to the rosmarinic acid and linoleic acid. Since there is no effective vaccine against leishmaniasis, the medicinal plants could be valuable means for development of novel therapeutic agents against leishmaniasis.

Keywords: *Leishmania major*, Rosmarinic acid, linoleic acid, Borago, *Echium amaenum*

2305P

Comparison of infectivity rate in three lines of *Leishmania* major wild type and transfected with reporter genes (EGFP and EGFP-LUC) in susceptible BALB/c mice

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Background: Leishmaniasis is a major infectious zoonotic disease which is produced by different *leishmania* spp. There is a necessary need to develop new diagnosis method for better investigating of infection expansion both *in vivo* and *in vitro*. This disease hits developing countries such as Iran. It has been shown that reporter genes could be as an excellent tool for studying leishmaniasis progression. Hence for the first time, in this project the infectivity rate of transgenic *L. major* strains expressing enhanced green fluorescent protein (EGFP) and EGFP-Luciferase (LUC) compared with *L. major* wild type. **Methods:** Transgenic *L. major* with EGFP, EGFP-LUC and wild type line, injected into the foot pad of BALB/c mice. The *in vivo* infectivity progression was monitored by fluorescence emission in Imaging System and foot pad swelling measurement using caliper. In addition, parasite burden by EGFP expression in the lymph nodes was evaluated and compared using *in vitro* assays like flow cytometry, Epi fluorescence microscopy and micro titration assay in 1 and 2 months post infection. **Results:** Parasite burden and footpad swelling in infected mice by EGFP transgenic line was higher than two others groups. *In vivo* imaging was shown progressive infectivity in mice within 1 and 2 months after infection. **Conclusion:** *In vivo* and *in vitro* studies have showed more infectivity potential in transgenic *L. major* lines in comparison with wild type of *L. major*. Our findings have indicated the immunogenicity of EGFP in infected BALB/c mice.

Keywords: *Leishmania*, Reporter gene, EGFP, EGFP-LUC, Infectivity.

1898P

TTG and IgE serum levels in patients with celiac infected with *Toxoplasma gondii*

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Background: *Toxoplasma gondii*, is an obligate intracellular parasite in coccidian group. Invasion of parasites impairs the function of Th cells in regulating antibody production resulting in increased IgE levels. Celiac disease is an autoimmune disorder of the small intestine. Purpose of this study was to determine the level of IgE antibodies in the sera of patients with celiac disease infected with *Toxoplasma gondii*. **Methods:** This case-control study was conducted during 1390-1391 on 90 patients referred to the gastroenterology clinic. 45 patients with celiac disease as case group and 45 persons without celiac diseaseas control group were assigned to the study. The groups were matched for gender and age. ELISA was used to determine the IgE and TTG levels in the sera of both groups. Data collection and analysis were performed bySPSS.16 software. **Results:** The results of this study showed that the mean concentrations of IgE antibody were 1/96 (IU/ml) and 1/41 (IU/ml), in patients with celiac and patients without celiac, respectively. The difference was statistically significant (P=0/03) implying significant association between celiac disease and Toxoplasmosis. There was also a significant association between TTG (anti-tissue transglutaminase)levels and IgE antibody titer (P=0/03).**Conclusion:** Celiac disease is an inflammatory disease and an autoimmune disorder of the small intestine. Presence of IgE antibodies in patients with celiac disease along with increased concentrations of IgE in patients infected with *Toxoplasma gondii* might be useful in the detection of chronic and particularly acute *toxoplasma gondii* infections. It would be also of practical importance in the treatment of patients with celiac disease.

Keywords: TTG, IgE, celiac disease, *Toxoplasma gondii*

2358P

Effect of N-((5-nitro -1*H*-imidazol-yl-2)methylene)benzohydrazid derivatives on Reactive Oxygen Species production

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Background: *Leishmaniasis*is caused by protozoan parasites of the *Leishmania* genus. Susceptibility of many parasites to oxidative stress is a well-known phenomenon. The redox system plays an important role in the survival of the parasite in the host. All aerobic organisms are exposed to reactive oxygen species (ROS) such as superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$) generated by their metabolism. Leishmania can also cope with the oxidative (or respiratory) burst of the host immune system. Redox imbalance occurs in the parasite when the endogenous antioxidants fail to cope with the excessive ROS, and this leads to the development of oxidative stress. In general, antiparasitic drugs, which have the ability to inhibit vital redox reactions or promote oxidative stress in parasites, are considered redox-active antiparasitic drugs. In the present study, we have evaluated the effectiveness of N-((5-nitro-1H-imidazol-yl-2)methylene) benzohydrazid derivaties on Redox-

active antileishmania. **Methods:** We used IC50 of N-((5-nitro-1H-imidazol-yl-2) methylene) benzohydrazid derivatives on reactive oxygen species production by spleenocytes of BALB/c mice. ROS production was evaluated by reduction of DCFH-DA to DCF. **Results:** AK1> AK5> AK4>AK13>AK3 derivatives can produce ROS in spleenocytes. **Conclusion:** Our results demonstrate that nitroimidazole derivatives not only can be cytotoxic for leishmania parasite but also can stimulate lymphocytes to protect the host.

Keywords: N-((5-nitro -1H-imidazol-yl-2) methylene) benzohydrazid derivatives, Reactive Oxygen Species

1698P

Production and Purification of polyclonal antibody against attenuated and wild type leishmania infantum in dog

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Background: Polyclonal antibodies are mixtures of monoclonal antibodies that are produced against different epitops. The aim of this research is preparing, purifying (attenuated and wild type) and characterizing the anti leishmania infantum with the nature of IgG class in dog. **Methods:** purification of anti-leishmania was done through precipitation with ammonium sulfate solution ,ion exchange and affinity chromatography. purity of the fraction was done and confirmed by polyacrylamide gel electrophoresis in denaturating condition(SDS-PAGE). The product was conjugated with FITC for detection of leishmania infantum in fluorescent microscope. **Results:** The SDS-PAGE results indicated that the antibody product was the IgG class with 95% purity. The FITC conjugated anti-leishmania infantum suitably detect the leishmania infantum in slide as standard conjugate. **Conclusion:** our conjugated product could detect the leishmania infantum as a standard one.

Keywords: Production, Purification, Dog immunoglobulins

2940P

Stable transfection and expression of GFP gene in Leishmania mexicana H.line and its infectivity rate in BALB/c mice macrophages

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Background: Genetic manipulations of protozoan parasite such as *Leishmania* parasite feasible analysis of new genetic changes and biological function. They can be used for improving of immune responses against infectious agents. In the present study *Leishmania meicana* wild-type (*L. mexicana* WT) and gentamycine-attenuated *L. mexicana* H-line were used for expressing green florescence protein (EGFP) as a reporter gene. The aim of this study was to investigate the expression of GFP gene in *L. mexicana* H-line. **Methods:** To render *Leishmania*

to express EGFP we used a recombinant plasmid, pLEXSY-EGFP-Neo, after removing a piece of 2.9kb by swaI restriction enzyme, was linearized and then electoroporated into the parasite to lighted in 18srRNA of chromosome 27 through homologous recombination. Transfected parasite after screening and selection on solid medium containing G418 was used for further steps. EGFP gene presentation and expression was analysed by PCR and flow cytometery. Transfected parasites were also observed using fluorescence microscopy and analysed by Western blotting. The ability of transfected parasites to enter BALB/c derived bone marrow macrophages and surviving within infected macrophages were examined. **Results:** The results confirmed EGFP expression and protein production in high level in *L. mexicana* WT and *L. mexicana* H-line. The percentage of macrophages infected with *L. mexicana* WT was significantly higher than that macrophages infected with the attenuated line parasites after 96 hours. **Conclusion:**The transfected *L. mexicana* H-line infected macrophages but unable to survive within infected macrophages. Whereas, transfected wild-type parasites multiplied within infected macrophages.

Keywords: Leishmania, EGFP, Gentamycine

2528P

Leishmania lymphadenitis diagnosis by fine needle aspiration biopsy in Fatemi hospital of Ardabil City, Ardabil Province, Iran

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Background: Visceral Leishmaniasis (kala-azar) is one of the important infectious-parasitic diseases that make human injuries and economical issues. It includes clinical observation from skin damaging to lethal visceral symptoms. Nodals in neck region have different reasons that one of the common reasons is dermoid cystic. Leishmaniasis is appeared in nodals neck rarely. **Methods:** Patient was a 11-yaers-old girl with nodal in neck region (left shift carotid), that it makes previous several years. After hospitalizing the patient in Fatemi Hospital, fine needle aspiration (FNA) was performed from nodal. Cytology and pathology assessment showed kala-azar. **Results:** In study of lymphatic nodes samples by optical microscopic, between small lymphocyte and plasma cell that surrounded macrophage cells were founded intercellular leishman bodies. **Conclusion**: Leishmaniasis is chronic cutaneous, mucocutaneous and visceral disease that is due to granulomatous response or systemic mononuclear in skin, respiratory mucosa and reticoloendothelial system against specific genus of Leishmania. According to prevalence of kala-azar in Iran especially in North West region (Ardabil Province), control of vector and source of this disease is impossible in this country. It was concluded that the best way is protection healthy people, early treatment patients and introduces them to health centers.

Keywords: Leishmaniasis, Lymphadenitis, Leishman bodies

2866P

Antiparasitical effect of *Olea europaea* on amastigotes of *Leishmania major* in vivo

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Background: Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis that is still treated with drugs, which present side effects. Conventional healers usually are cheaper and sometimes more effective than chemical drugs. *Olea europaea* is one of them. This plant has therapeutic value. This study was conducted to assess the in vivo efficacy of olive leaf extract (OLE) in animal models for cutaneous leishmaniasis. **Methods:** Mice were inoculated with *L.major* poromastigotes. Then they were divided randomly to test and control groups. The treatment was continued for eight weeks with different concentrations of OLE. **Results:** The results show that the size of lesions in the test groups with OLE was significantly decreased. Also the mortality ratio in test groups significantly was less than control. **Conclusion:** These results indicate that OLE effect on preventing death of infected mice. Also the survival rate in the treated groups compared with control groups was more. According to the results of this research, it seems that not only OLE has antifungal, antiviral, antibacterial effects but also antileishmanial activity.

Keywords: *Olea europaea*, *Leishmania major,* Cutaneous leishmaniasis**,** Mice

2617P

Construction of Whole recombinant yeast vaccines against Leishmaniasis

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Background: Leishmaniasis, an infectious disease caused by Leishmania **(L.)** parasites ranges in severity from self-healing skin lesions to fatal systemic infection. With an estimated prevalence of 15 million, leishmaniasis is second in mortality among the tropical infections but no vaccine for human use is approved to date. A protective anti-leishmania vaccine should be capable of activating DCs for induction of strong cellular and MHC-I immune responses. Herein, we describe construction of whole yeast vaccines (WYV) expressing LmSTI1 (L. major stress-inducible protein1) antigen of L. major and immunization studies in murine model. **Methods:** The LmSTI1 gene was PCR-isolated from L. major (strain: MRHO/IR/75/ER) and cloned into pET15b, pPICZA and pYES2 vectors for intracellular expression of the target protein in E.coli, P.pastoris and S.cerevisiae yeasts. Three groups of BALB/c mice were immunized at days 0, 14, 28 with Ni-NTA-purified protein plus montanide-50, recombinant P.pastoris and S.cerevisiae yeasts and analyzed for various immunological responses. **Results:** Analyses by PCR, sequencing reactions and Western Blotting confirmed the accuracy of the LmSTI1 harboring Yeast and E.coli hosts and their expression efficiencies, respectively. Assessment of Lymphocyte proliferation, cytokines (IL4, IF-γ, TNF-α) by ELISA, antibodies

and CD4/CD8 ratio by flow cytometery indicated comparable immune responses for whole yeast vaccine candidates and montanide-50 formulated LmSTI1(purified from E.coli) immunogen. **Conclusion:** Stimulation of cell-mediated immunity through engulfment of yeast cell by DCs and their unique ability to process antigens via MHC-I pathway makes WYV an ideal vaccination approach against leishmaniasis

Keywords: Leishmaniasis, Whole recombinant yeast vaccine, Immunization

2766P

Genetic diagnosis of bovine *Fasciola gigantica*

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Background: Fascioliasis is an important zoonose disease caused by *Fasciola hepatica* and *Fasciola gigantica*. In Iran, the distribution of these two species overlaps in most area, including northern Iran. Morphological examination and biologic characters cannot cause a certainty in the accurate and precise differentiation and intra/inter- specific difference of Fasciola spp. In this study, *Fasciola gigantica* identified using 18srDNA in cattle isolates from Guilan province. **Methods:** Adult worms were collected from cattle livers at an abattoir in Guilan. Total DNA of them was extracted by MBST kit, PCR was performed to amplify 18srDNA by universal specific primers (annealing temperature: 58 ̊c). Morphological characters were studied too. **Results:** The primers were used in this study amplified a region of approximately 506 bp in *F. gigantica*. The morphometric analysis of the samples showed all data were *F. gigantica* and they were distinguishable from *F. hepatica*. **Conclusion:** 18srDNA is an internal control because it showed less variance in expression than other genes. In this study, we used 18srDNA as a target to distinguish these two parasites. It is so abundant, it amplifies rapidly during PCR, PCR amplification strongly supported the view that the isolates belong to *F. gigantica*. According to Fascioliasis as one of the most important zoonose in Iran, differential diagnosis of Fasciola species is important. The pattern of prevalence and incidence of infection in domestic animal and humans should always be explored. *F.gigantica* is dominant species in many of the studied queries in Iran and the results of this experiment is the demonstration of this fact.

Keywords: *Fasciola gigantica*, 18srRNA, Iran

2427P

Evaluation of ELISA method for detection of Cryptosporidium parvum antigens in stool samples of Immunosuppressive patients

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Background: Cryptosporidium parvum is a protozoan pathogen with worldwide distribution. It localizes on the intestinal cells and causes prolonged diarrhea in immunocompromised patients. The aim of this study was to estimate the prevalence of cryptosporidiosis in pediatric

patients with lymphohematopoietic malignancies. **Methods:** In this cross-sectional study stool samples were collected from 100 children (67 boys, 33girls) with lymphohematopoietic malignancies who underwent chemotherapy between the ages of 6 months and 17 years (mean age 7.5 years). All of the specimens were examined for the oocysts of C. parvum by modified Ziehl Neelsen (MZN) staining technique and coproantigens of C. parvum by ELISA. **Results:** Cryptosporidium infection was detected in 22 patients. 16 (72.7%) of the infected patients were male and 6 (27.3) female. 7 (31.8%) patients were <5 years, 8 (36.4%) 5-10 years and 7(31.8) >10 years old. Parasites were detected in 19/85 (86.4%) patients with ALL, 2 of 5 (9.1%) with AML, and 1 of 10 (4.5%) with NHL. Clinical symptoms were found in 11 (50%) of the patients. We found longer duration of chemotherapy in patients who were positive for cryptosporidium infection (Mean=2067 days) in comparison to negative group (Mean=258.5 days). **Conclusion**: The incidence of cryptosporidium infection was 22% among pediatric patients with lymphohematopoietic malignancies. We recommend evaluation of these patients with at least two different diagnostic methods in order to prevent possible life threatening outcomes.

Keywords: Cryptosporidium sp, Cryptosporidiosis, Immunosuppressive patient

2473P

Evaluation of ELISA for detection of Giardia lamblia antigens in stool samples of Immunosuppressive patients

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Background: Giardia lamblia is an intestinal protozoa with widespread prevelance, especially in the tropical and subtropical regions.In this study,routine parasitolgy methods are compared with ELISA test. **Methods:** In this cross-sectional study stool samples were collected from 89 children (53 boys, 36 girls) with lymphohematopoitic malignancies under chemotherapy,the age between 1 and 18 years (mean age 7.5 years). Three fresh stool samples taken for three consecutive days were examined by direct smear, formalin-ether method, trichrome staining and ELISA test for Giardia lamblia coproantigens. **Results:** In this study 35.9% of our patients had parasitic infections and the following parasites were identified; Giardia lamblia (the most prevalent parasite in children) 16 (18%), Entamoeba coli 6 (6.7%), Blastocystis hominis 5 (5.6%),Iodamoeba butschlii 2 (2.2%), Chilomastix mesnili 1 (1.1%), Hymenolepis nana 1 (1.1%) and Enterobius vermicularis 1 (1.1%). **Conclusion:** With regards to the high incidence of gasterointestinal parasitic diseases and also because of asymptomatic cases of giardiasis, we recommend evaluation of pediatric patients with malignant lymphohematopoitic disease by at least two different diagnostic methods and three rounds of stool examination in order to prevent possible life threatening outcomes. Coproparasitoscopic study for oncologic patients should be performed and anti-parasitic treatment provided before starting chemotherapy to prevent disseminated parasitic infections. The coproantigen-ELISA is especially advantageous in situations where only a single stool sample can be examined.

Keywords: Giardialamblia, ELISA, Immunosuppressive patients

2462P

Study the effect of parasite dose on Interfron gama response of *Leishmania tropica* infection of BALB/c mice

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Background: Aim of this study is to move towards a suitable experimental model for *Leishmania tropica*. A proper experimental model for this disease can help us in finding new approaches for prevention and treatment of this disease. **Methods:** *Leishmania* species were cultured. Appropriate concentrations of the parasites were injected into the ear of mice. Mice were killed at 3 intervals of one week, one month, and 4 months after infection. Lymph node draining ear were obtained and single cell suspension were prepared from them. The cells were cultured and stimulated by soluble leishmania antigen and Interferon-γ was assayed in supernatant of cultured cells. **Results:** our results showed:Low dose of Leishmnia tropica resulted in no Interferon-γ production.High dose of *Leishmania tropica* resulted in no Interferon-γ production at one week and one month after infection, but Interferon-γ was produced at four months after infection.Low dose of *Leishmania major* resulted in production of Interferon-γ only at four months after infection and no Interferon-γ was produced at one week and one month after infection.High dose of *Leishmania major* resulted in production of Interferon-γ at one week after infection and its levels were reduced at one month and four months after infection. **Conclusion:** our study showed that the dose of *Leishmania tropica* affects Interferon-γ response. The results also showed the immune response up to 4 months after infection, while the low dose may need longer time to affect immune response, which need further studies.

Keywords: *Leishmania tropica*, cytokine, Interferon-γ, BALB/c mouse

2598P

Study the effect of parasite dose on interleukin-10 response of Leishmania tropica infection of BALB/c mice

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Background: *Leishmania tropica* is among the causative agents of cutaneous leishmaniasis. It is necessary to know the variables in *Leishmania tropica* in order to find treatment or prevention for this disease. No study has yet been performed on the effect of parasite dose on infection pattern of this parasite. In our study the effect of low and high dose of the parasite on the pathogenesis of this parasite is studied. *Leishmania major* has been used in this study as a control species. This species results in destructive lesions, visceralization of the parasite and progressive disease in BALB/c mice. **Methods**: *Leishmania tropica* was cultured and infective parasite was prepared. Different doses of the parasite were used to infect BALB/c mice in different experimental groups. Mice were inspected clinically at different intervals. Cells were prepared from draining lymph nodes of mice at one week, one month, and four months after infection. Cells were cultured in presence of *Leishmania tropica* antigens and Interleukin-10 was assayed.**Results**: Our results showed:Results obtained from *Leishmania*

major were concordant with previous reported results. These results show the validity of our experimental settings.Low dose as well as high dose of *Leishmania tropica* did not resulted in production of Interleukin-10 at all the intervals studied.**Conclusion**: Our study showed that the dose of *Leishmania tropica* does not affect Interleukin-10 response, because Interleukin-10 was not produced in any dose used.

Keywords: *Leishmania tropica*, cytokine, Interleukin-10, BALB/c mouse

2526P

Diversity of partial COI *Dicrocoelium dendriticum* from Iran isolates

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Background: *Dicrocoelium* species as the digenean trematode that live in bile ducts of mammalian animals, especially small and large ruminants. The three species commonly distinguished as the causative agents of dicrocoeliasis in livestock animals, are *D.dendriticum*, *D.hospes* and *D.chinensis* with the world distribution. In this study, intra-species genetic variation of *D.dendriticum* were evaluated based on COI genemarker. **Methods:** Adults *D.dendriticum* were collected from the liver of naturally infected ovine at necropsy at local slaughterhouses. Fresh worms were washed extensively in phosphate-buffered salin. The samples were labeled and preserved immediatly in 70% ethanol solution until use.Isolation of total genomic DNA from *D.dendriticum* was performed following the manufacturer's instructions. The PCR amplification for mitochondrial gene, the cytochrome c oxidase subunit I, (cox1) gene for determination strains was performed in 50 µl volumes. Genomic DNA sequencing based on Sanger's method was performed in both directions for. **Results:** DNA amplification of the COI-mtDNA revealed a 400 bp band for all of the examined speciments. The sequences were compared against those registered in GenBank. Sequence Translation is used to translate nucleic acid sequence to corresponding peptide sequences. Amino acids sequence alignment searched against biological databases. In contrast, the simple fact that there are 12 different amino acids lined up but this diversity per sequence would not lead to different species. **Conclusion:** This study shows molecular variation of *D.dendriticum* is a multifactorial phenomenon including complexity of life cycle, plurality and diversity of intermediate hosts and worm adaptation with various conditions.

Keywords: *Dicrocoelium dendriticum*, COI, Amino acid Sequence, Iran.

2721P

An Approach for Detection of Hypohosphatemia Disease in Malaria Based on Expert System

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Malaria is the most important parasitic disease and one of the important hygienic problems of

some countries, especially the tropical countries of the world. This disease manifests itself as a severe infection (which in most cases is critical, and sometimes along with the features of intermittent fever and shivers), anemia, enlargement of the spleen, and sometimes as other minor or fatal characteristics. The importance of this disease results from its extensive prevalence and the considerable number of fatalities it causes. Every year, more than 225 million new cases of malaria are added to those already present; and the World Health Organization reported in 2010 that every year about 781000 people lose their lives due to this disease. This figure accounts for 2.23% of the total number of yearly deaths in the world. With this in mind, we have designed an expert system which, depending on the values of the inputs given to the deduction engine, can be very effective in recognizing hypophosphatemia in malaria.

Keywords: Expert system, Malaria, falciparum, vivax, malariae, Hypophasphatemia

2821P

Synthesis and compare in vitro leishmanicidal activity of 5-(nitroheteroaryl)-1,3,4-thiadiazols containing cyclic amine at C-2 with acyclic amine analogues

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Background: Leishmaniasis is an endemic parasitic disease and a major public health problem in the new world. All currently available chemotherapeutic agents are toxic, expensive, cause serious side effects and required long-term treatment. In addition, the development of the clinical resistance and increase of co-infected leishmaniasis with AIDS in some regions is a serious problem. Thus, the development of new, efficient, cheap and safe drug for the treatment of leishmaniasis is imperative. **Methods:** The compounds were obtained by treatment of 2-chloro-1,3,4-thiadiazole and appropriate acyclic and cyclic amines in ethanol. The anti promastigote activity of these compounds was performed using MTT assay. Also, anti amastigote activity and toxicity of these compounds was assessed against mouse peritoneal macrophages. **Results:** Active compounds of 5-(5-nitrofuran-2-yl)- and 5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazole bearing acyclic amine of 3-aminopropan-1-ol at C-2 position of thiadiazole ring showed excellent activity in vitro against promastigote form of *L. major* with IC_{50} values of 18 and 3 µM, respectively and good antiamastigote activity. But cyclic amine analogues exhibited less antileishmanial activity against promastigote form of *L. major*. In general, thiophen analogues exhibited better anti promastigote and anti amastigote activity in compared with furan analogues. **Conclusion:** Attachment of cyclic amines to thiadiazole ring exhibited lower anti-promastigote activity in compared with linear amines. This substitution cause steric hindrance of around the C-2 position of thiadiazole ring that may prevent binding of the compounds to the macromolecular target in the parasite.

Keywords: Leishmania, Thiadiazole, 5-Nitrofuran, 5-Nitrothiophen

3309P

The polymorphisms within promoter region of IL-4 gene areassociated with visceral leishmaniasis

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Background: Immune responses play important roles to determine outcome of leishmaniasis. The polymorphisms within promoter region of IL-4 gene are associated with level of the cytokine production. Therefore, the main aim of this study was to study the relation between visceral leishmaniasis (VL) and the polymorphisms at -589 and -590 position of the IL-4 gene in a selected Iranian population. **Methods:** In this cross-sectional study, participants were divided to three separated groups including; patients with clinical presentation of VL and seropositive for the leishmania (group 1), individuals without clinical presentation but seropositive (group 2) and healthy controls (group 3). The IL-4 -589 and -590 polymorphisms were examined using PCR-RFLP technique. The immunoflorescence techniquewas also used for analysis of anti-leishmania antibody titration. **Results:** Our results demonstrated that the polymorphism at IL-10 -33 (C/T) position was significantly associated with VL and C/T as well as T/T genotypes were significantly higher in VL patients and controls, respectively. When compared to group 2 and 3 ($p< 0.001$). The results demonstrated that the C allele was associated with VL ($p< 0.001$). **Conclusion:** Based on the presented results, it may be hypothesized that the IL-4 -33C/T and T/T genotype can be considered as risk and protective factors, respectively, for VL.

Keywords: IL-4, Polymorphism, Visceral leishmaniasis

Immunopharmacology & Medicinal Plants

Oral Presentations:

1759O

Cell cycle arrest and induction of apoptosis by scrophularia striata in human breast cancer cell line

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Background: Scrophularia striata Boiss (Scrophulariaceae) is a plant growing in the northeastern part of Iran and being used as a traditional herb for various inflammatory disorders. This study was designed to investigate the cytotoxic effects of the Scrophularia striata (S. striata) extracton MCF-7 human Breast Cancer cell line. **Methods**: Phytochemical assay by thin layer chromatography (TLC) and the 2, 2 diphenyl-1-picryl-hydrazyl (DPPH) were used to evaluate the main compounds and the antioxidant capacity of the plant extract, respectively. The inhibitory effect of the extract on MCF-7 cells was evaluated by MTT assay. In addition, cell cycle distribution and apoptotic cell death were evaluated by PI (propidum iodide) and Annexin V-FITC/PI staining, respectively. **Results:** The results showed that the main components; including flavonoids, phenolic compounds and phenyl propanoids were presented in the S. striata extract. The treatment with extract significantly showed significant cytotoxicity effect on MCF-7 cancer cell line. In addition, flow cytometry analysis indicated that S. striata extract induced cell cycle arrest in G2/M phase and apoptosis on MCF-7 cell. **Conclusion:** The results in this study indicated that S. striata extract could inhibit human Breast cancer cell line (MCF-7) growth through inducing G2/M phase arrest and cell apoptosis.

Keywords: Scrophularia striata, Cell Cycle, Apoptosis, Breast cancer cell line

1421O

Immunomodulatory potential of tretinoin is not absolutely depend on the expansion of FoxP3^{+}Treg cells

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Background: Recent evidence has proposed that Tretinoin produced in the gut preferentially promotes differentiation of FoxP3^{+}Treg cells but inhibits Th17 lymphocytes, and this may be the main immunomdulatory mechanism of Tretinoin in vivo. The present study was done to investigate the effects of Tretinoin in NRMI-mice after challenge with sheep red blood cells (SRBC).Methods: Twenty male NMRI-mice randomly allocated in two equal groups. Mice were intraperitoneally treated twice with one weak interval by 1×10^{9} SRBCs emulsified in CFA. Animals were bled 5 days after last injection. Moreover, 48 h before bleeding time, 1×109 SRBCs were injected into the left hind foot pad of mice. Tretinoin (25mg/kg-every other day) were intraperitoneally injected into the treatment group from the beginning of the study and continued thought the study. The levels of anti-SRBC antibody and the specific cellular immune responses were measured by plaque forming cell assay and Footpad thickness, respectively. Moreover, splenocytes were checked for proliferation rate, respiratory burst, cytokine production and FoxP3^{+}Treg cells frequency. **Results:** Tretinoin significantly alleviated cellular immunity and concurrently potentiated humoral immunity after mice challenge with SRBCs. Furthermore, aside from reducing NBT reduction and lymphocyte proliferation, Tretinoin significantly suppressed the secretion of interleukin-17 and conversely, increased the production of interleukin-10. However, the level of IFN-γ and the frequency of FoxP3^{+}Treg cells did not alter significantly. **Conclusion:** The in vivo immunomudlatoty effects of Tretinoin may be partly due to immune deviation from pro-inflammatory cytokine interleukin-17 to anti-inflammatory cytokine interleukin-10, but not absolutely depend on the expansion of FoxP3^{+}Treg cells.

Keywords: Tretinoin, Immunomodulator, NMRI-mice

1513O

Hydrocortisone reduces Toll-like receptor 4 expression on peripheral CD14^{+} monocytes in patients undergoing percutanoues coronary intervention

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Background: Evidence from several lines of investigations suggests that Toll-like receptor 4 (TLR4) is involved in atherosclerosis as a bridge between innate and acquired immunity. Percutaneous Coronary Intervention (PCI) can trigger inflammation through activation of TLR4 on monocytes. Hydrocortisone as an anti-inflammatory and immunosuppressant agent has

multiple mechanisms of action. In this study we aimed at assessing the effects of hydrocortisone on monocyte expression and activity of TLR4 in patients underwent PCI. **Methods:** Blood samples were taken from a total of 71 patients with chronic stable angina who scheduled for a PCI, before the intervention. Test group was composed of 30 patients received 100 mg hydrocortisone prior to the procedure. Control group was composed of 41 patients underwent PCI without receiving hydrocortisone. Blood collection was repeated 2 hours and 4 hours after PCI. The expression of TLR4 on the surface of $CD14^+$ monocytes and the serum levels of TNF-α and IL-1β were measured using flowcytometry and ELISA. The study was done in Shahid Madani Heart Hospital, Tabriz, Iran. **Results:** Compared with controls, hydrocortisone significantly reduced monocyte expression of TLR4 in the test group ($p< 0.01$). In addition, it had a significant effect on reduction of serum concentrations of TNF-α and IL-1β in the test group in a time dependent manner ($p< 0.01$).**Conclusion:** Hydrocortisone was able to reduce the monocytes expression of hTLR4 and its related pro-inflammatory cytokines and can be used to decrease inflammatory responses following PCI.

Keywords: Toll-like receptor 4, Inflammatory cytokines, Hydrocortisone, Percutanous Coronary Intervention

1815O

Cimetidine restores the serum cytokine levels after burn injury in an animal model

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Background: The burn-induced immunosuppression causes an increase in susceptibility to infection. The aim was to investigate time-related alterations in various cytokines following thermal injury and to modulate cytokines by use of an immunomodulant, cimetidine. **Methods:** Male Balb/c mice anesthetized and given a 10% total body surface area full-thickness burn by submerging in 90°C water for 9 sec. Time-dependent changes in serum levels of the cytokines IL-2, IL-10, IL-12 and IL-17 were then assessed at various post-burn day (PBD) timepoints. Effects of 10 mg/kg of cimetidine on cytokine levels were evaluated up to PBD 14.**Results:** In comparison to healthy non-burned control mice, levels of IL-2 and IL-17 significantly decreased at PBD 3, 5, 10, and 14, those of IL-10 at PBD 1, 3, 5, and 10, and those of IL-12 at PBD 1, 3, 5, 10, and 14. Administration of cimetidine significantly augmented the levels of IL-2 (at PBD 3, 5, and 10), IL-10 (at PBD 1 and 5), IL-12 (at PBD 3, 5, 10, and 14), and IL-17 (at PBD 3 and 14) as compared to those in burned counterparts who did not receive drug. **Conclusion:** These results showed significant time-dependent changes in serum cytokines levels after burn injury and that cimetidine was able to significantly augment IL-2, IL-10, IL-12 and IL-17 levels that are diminished following thermal trauma.

Keywords: Burn, Cimetidine, IL-2, IL-10, IL-12, IL-17, Mice

2064O

Methanolic extract of Scrophularia atropatana induce apoptosis

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Background: Cancer is one of the leading causes of mortality in both developed and developing countries. Recent studies show that, herbal medicines are potential remedies for cancer treatment. The present study was aimed to assess the anti proliferative and apoptosis inducing effects of Scrophularia atropatana methanolic extract on WEHI -164 (mouse fibrosarcoma cell line) in comparison to non-malignant (L929) cells. **Methods:** WEHI -164 and L929 cells were cultured in RPMI 1640 and treated by 100, 150, 200, 300, 400, 500, 600 µg/ml concentrations of the extract for 24, 36 and 48 h. The cells were incubated under humidified condition at 37 °C and 5% of CO2. Cytotoxic effects were evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. TUNEL test and Cell Death Detection ELISA were employed to demonstrate apoptosis by herbal extract included in this study. **Results:** In this study, methanolic extract of Scrophularia atropatana exhibited a noticeable cytotoxic effect on WEHI-164 cells in a dose and time-dependent manner in 24, 36 and 48 h with the IC50 600, 521 and 465µg/ml respectively. This extract did not show cytotoxic impacts on L929 cells at the same dose. According to the results of TUNEL and ELISA methanolic extract of Scrophularia atropatana induce apoptosis in WEHI-164 cells. Conclusion: The whole data indicates that methanolic extract of Scrophularia atropatana has a proapoptotic effects on WEHI-164 cell line and could be effectively utilized in cancer treatment.

Keywords: Cytotoxic, Scrophularia atropatana, WEHI-164 cell line, MTT assay, Apoptosis, Methanolic extract

1920O

Methanolic extract of Scrophularia atropatana promotes P53 gene expression and apoptosis in MCF7 cells by non- epigenetic mechanisms

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Background: Nowadays, Epigenetic modifications have been widely considered in cancer study and treatment. The aberrant methylation of DNA is one of the most important epigenetic changes which could result in cancer. On the other hand, herbal medicines are attracted a great deal of interest in this field. Methods: In this research, MCF7 breast cancer cells grown in RPMI medium were exposed to different concentrations of S. atropatanamethanolic extract. Cell viability was evaluated by MTT assay. In order to assess the apoptotic effects, ELISA and TUNNEL tests were employed. Furthermore, Methylation Specific PCR (MSP) on P53 gene promoter was exploited for detection of possible methylation changes. Then, P53 gene expression level was analyzed by Real Time-PCR. **Results:** Apoptosis was demonstrated by ELISA and TUNNEL tests in MCF7 cell line treated with the extract. MSP did not reveal a considerable methylation change. However, Real time -PCR proved that, there was an obvious increase in P53 gene expression. **Conclusion:** Methanolic extract of S. atropatana led

to apoptosis in MCF7 cell line. There was no detectable methylation change in P53 promoter region. According to our findings, the higher expression of P53 gene and apoptosis caused by this extract was not caused by reverted methylation of the promoter region and other mechanisms may be involved.

Keywords: MCF7 breast cancer cell line, Methylation Specific PCR (MSP), DNA Methylation; Epigenetics, Scrophularia atropatana

1740O

Effect of aquatic and ethanolic extracts of Licorice on bleomycin induced pulmonary fibrosis in mice

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Background: Idiopathic pulmonary Fibrosis is characterized by deposition of collagen and accumulation of extracellular matrix. Increasing levels of IL6 and IL1β caused of leukocytes recruitment to the lung tissue. Leukocytes release free radicals of oxygen (ROS) and nitrogen (RNS) which exacerbate the process of epithelium damage. Studies showed that Licorice has anti-oxidative and anti-inflammatory effects. So in this study the effect of aquatic and ethanolic extracts of Licorice on the pulmonary fibrosis was evaluated. **Methods:** Pulmonary fibrosis model was induced by intratracheal injection of bleomycin (2mg/kg) and mice were divided in three groups. The positive control, were not treated by Licorice. The second and third groups were treated with Licorice aquatic and ethanol extracts (300 mg/kg daily for 21 days). On day 21, the mice were euthanized and lung tissues were collected for histopathological investigation using hematoxylin-eosin (H&E) and trichrome staining. Moreover, real time PCR was performedfor evaluation of α-SMA mRNA expression. **Results:** In bleomycin injected mice, destruction of lung structure and accumulation of inflammatory cells were observed in H&E staining sections. Trichrome staining showed collagen deposition. A significant elevation in α-SMA expression was detected in positive control. However, in mice that had received aquatic extract, those pathological changes were decreased significantly ($p<0.05$). In contrast, in group that had received ethanol extract no reduction was observed. **Conclusion:** Aquatic extract of Licoriceameliorates bleomycin induced injury in mice, but ethanolic extract of Licorice cannot reduce these damages, therefore aquatic Licorice can prevent progression of pulmonary fibrosis, which may be clinically significant.

Keywords: Pulmonary fibrosis, Licorice, Intratracheal, Bleomycin.

1792O

Effect of ethanol extract of Hypericum perforatumon the candida keratitis in immunosuppressed rabbits

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Background: Hypericum perforatum is a sprawling, leafy herb that grows in open, disturbed

areas throughout much of the world's temperate regions, such as Iran, and is used in folk medicine to treat a variety of internal and external ailments. The present study was set out to investigate the effects of Ethanol Extract of Hypericum perforatumon the experimentally induced Candidial keratitis in rabbits. **Methods:** The alcoholic extract of H. perforatum was prepared by 80% ethyl alcohol. After suppressing the immune system of 24 male rabbits byazathioprine , experimental Candida albicans keratitis was induced in the animals under local anesthesia and sterile conditions. The animals were later divided into four groups including the control or glycerin group and a nystatin and two 250 and 500µg/ml Ethanol Extract of H. perforatumgroups. Treatment continued for 21 days and after sacrificing the animals by humane methods, histopathological samples of the rabbits' eyes were prepared. **Results:** Keratitis was developed in the eyes of all rabbits a week after the yeast inoculation. In the control group in which animals received glycerin, keratitis persisted until day 21. Clinical signs of keratitis disappeared in the Nystatin and 500µg/ml Ethanol Extract of H. perforatumgroups after 14 and 21 days, respectively. The clinical signs of keratitis partially ameliorated in the animals receiving 250µg/ml Ethanol Extract of H. perforatum. Histopathological examination revealed no differences between groups receiving nystatin or 500µg/ml Ethanol Extract of H.perforatum. **Conclusion:** It is concluded that, H. perforatumessential oils could completely treat Candida albicans keratitis in 500 µg/ml concentrations. This extract can be used as a safe antifungal agent against Candida albicans and it is a good substitute for synthetic antifungal agents like nystatin.

Keywords: Candida albicans,H. perforatum essential oils, Keratitis, Nystatin, rabbit.

2171O
Medicinal plants with adjuvant activity

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Background: There has been a tendency toward using traditional medicine to treat different diseases especially when the host defense mechanism has to be activated under conditions of impaired immune response. There are various medicinal plants that have been widely used in traditional medicine for their adjuvant activity on immune system. Examples are Aloe vera, Angelica species, Astragalus membranaceus , Ganoderma lucidum, Panax ginseng, Scutellaria species and Echinace purpurea. In this presentation, we will have a brief discussion on medicinal plants with the capacity to enhance immune function and will present a result of our recent study on a native medicinal plant, Euphorbia cheiradenia, with immunostimulatory effects. The crude extract of this plant has shown immunomodulatory effects on human peripheral blood lymphocytes. Bioassay-guided fractionation using lymphocyte proliferation assay was performed in order to purify and identify the responsible compounds. Fractionation using different solvents including ethyl acetate, water, butanol, methanol, and hexane, resulted in six fractions.Interphase fraction appearing between water and butanol fractions, showed the highest immunostimulatory effect at concentration of 10 The preliminary data on NMR spectra of the compound showed the possibility of a saccharide structure.

The successful derivation of pure bioactive compounds of these plants supports the traditional practice of using these plants to stimulate the immune system.

1999O

Anti-tumor effects of DNA vaccine expressing HPV E7-NT (gp96) fusion protein combined with saffron and its ingredients as a chemotherapeutic agent

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Background: Human papillomaviruses (HPVs) are associated with a majority of genital cancers. Recently, the multimodality treatments using DNA vaccination in combination with chemotherapeutic agent have emerged as a potent approach in inhibiting large tumor growth. A larger number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Among them, saffron and its major components (crocin and picrocrocin) have been proposed as a promising candidate for cancer chemoprevention. In the present study, we evaluated the therapeutic effects of saffron extract and its ingredientsalong with HPVE7-NT (gp96) DNA vaccine in tumor mice model. **Methods:** The purification of two main components of Iranian saffron (crocin and picrocrocin) was performed by adsorption column chromatography. Large-scale purification of plasmid DNA encoding E7-NT (gp96) was conducted by ion-exchange chromatography. Mice were challenged with TC-1 tumor cells. Three days later, the mice were vaccinated with E7-NT (gp96) DNA and received a booster with the same DNA, two weeks later. Crocin, picrocrocin and saffron extract were given orally at the time of initial DNA treatment and continued for 16 days. Tumor volumes were measured twice a week for 55 days following tumor challenge. **Results:** Regarding to our data, crocin could demonstrate 100% tumor free mice compared to other groups indicating the high potency of crocin carotenoid as a chemotherapeutic agent. Moreover, the combination of picrocrocin with DNA vaccine could augment the antitumor effects of picrocrocin. **Conclusion:** The combination of DNA vaccine with picrocrocin could potentiate the antitumor effects compared with monotherapy alone, while crocin was significantly effective without immunotherapy.

Keywords: Immunotherapy, Chemotherapy, Saffron, Human papillomavirus, E7

1545O

The Effect of Silymarin on the Expression of Chemokine Receptors in T cells

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Background: Silymarin, apolyphenolic flavnoid derived from milk thistle (Silybum marianum), is known to have anti-inflammatory, hepatoprotective, and anticarcinogenic effects. The aim of this study was to investigate the effect of Silymarin on the Chemokine Receptors of Th1 and Th2 cells. **Methods:** Peripheral blood mononuclear cells (PBMCs) from healthy individuals were activated with Conconavalin ‘A’ and treated with silymarin (100 µM) and DMSO (negative control), in a standard condition (RT: 37 and CO_2: 5%).

Cells were incubated (72 h) and then examined for the cytometric evaluation of Chemokine Receptors (CCR5, CXCR3, CCR4 and CCR3) expression on Th1 and Th2 cells. Peripheral blood lymphocyte subpopulations were identified and evaluated by two color flow cytometric analysis. A nonparametric paired samples Wilcoxon test was applied to comparisons of grouped data. Results were expressed as the mean ± standard deviation (S.D.). P-values<0·05 were considered to indicate significant differences. **Results:** Our results showed that the expression of CXCR3 on Th cells was increased (8.94±3.40 vs. 16.22±15.25, P= 0.018). In other hand, a change for the expression of CCR5 was not significant. Fluctuations in the expression of CCR3 on Th2 cells were significant increased (0.52±0.43 vs. 1.91±1.75, P=0.001). Additionally, CCR4 showed significant decreased (10.32±3.45 vs. 8.06±3.17, P=0.025).**Conclusion:** This study provided evidences of effectiveness for Silymarine on the expression of CXCR3, CCR3 and CCR4. Therefore, in future, silymarin could be used instead of other drugs such as immunosuppressive with fewer side effects in autoimmune diseases.
Keywords: Silymarine, Chemokine Receptor, T helper Cells, DMSO

2848O

Evaluation of Resveratrol and Prednisolone effects on Bax and Bcl-2 gene expresstion in Acute Lymphoblastic Leukemia cell lines: CCRF-CEM

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Background: Experimental data show that Resveratrol, a compound found in grapes and other fruits may influence cell proliferation and apoptosis. The aim of our experiments was to study the effect of Resveratrol and resveratrol & prednisolone combination on acute lymphoblastic leukemia cell line (CCRF_CEM) to examine the effect of various doses of this compounds on expression of Bax and Bcl-2 genes. **Methods:** MTT assay was used to determine the subtoxic doses of resveratrol and prednisolone for treatment of cells. Human acute lymphoblastic leukemia cell line (CCRF-CEM) were treated with various doses (10, 50 and 100 µM) of Resveratrol and 700 µM of prednisolone in vitro. Total RNA was extracted from cells after 12, 24 and 48 hours of treatment. Real Time PCR was used to detect mRNA expression of Bax and Bcl-2 genes. **Results:** Resveratrol influences dose and time dependently on Bax and Bcl-2 expression. Dose 50µM of Resveratrol after 24 hour upregulated both Bax and Bcl2, and Bax expression was higher than Bcl-2 expression. In addition, a combination of resveratrol (100 µM) and 700 µM of prednisolone were also unable to induce significant changes in Bax /Bcl-2 expression in compared with control group. **Conclusions:** our study demonstrated that Resveratrol by differential regulation of pro- and anti-apoptotic Bcl-2 family members may play an important role in ALL cell lines apoptosis.
Keywords: Resveratrol, Prednisolone, Bax, Bcl-2, Real time PCR

2861O

A newly synthesized platinum-based compound with potent cytotoxic activity against breast, ovary, and prostate cancers

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Background: Cisplatin as a platinum-based chemotherapeutic agent is commonly used to treat several cancer types. Nonetheless, drug resistance as well as severe side effects has been reported upon cisplatin therapy. Here, we assessed the in vitro cytotoxicity of newly synthetized platinum-based drugs on various cancer cell lines. **Methods:** Fiveplatinum compounds were synthesized and their cytotoxic effects were investigated in reference to cisplatin on cell linesoriginated from prostate, ovary and breast cancers at various time points and concentrations using XTT assay. Additionally, the rate of apoptosis induction in the cell lines was determined by Annexin V flow cytometry. Human amniotic epithelial cells (hAECs) served as the control normal cells. **Results:** Synthetic platinum compounds were categorized based on their cytotoxic effects on prostate cell lines, DU-145 and PC3, and having minimal effect on normal epithelial cells, hAEC. With the exception of one compound, all the synthetic platinum-complexes could effectively kill prostate cancer cells. One of the compounds, $[Pt(dpyam)Cl_4]$.DMF, was selected as the most potent drug for further evaluationsdue to its low toxic effects on normal hAECs. Next, we compared this compound with cisplatin in terms of cytotoxic activity against a panel of tumor cell lines: SKO-3, Caov-4, MDA-MB, MCF-7, DU-145, and PC3.$[Pt(dpyam)Cl_4]$.DMF was shown to have significantly superior selective cytotoxic effects compared to cisplatin at higher concentrations and longer cultures periods. Moreover, with respect to apoptosis induction, treatment with this compound was significantly more effective than cisplatin in the case of five out of six examined cell lines. **Conclusion:** This study introduced a novel platinum-based compound with highly selective and potent in vitro antitumor effects superior to those of cisplatin, a commonly used chemotherapeutic agent.

Keywords: Tumor, Chemotherapy, Cisplatin, Platinum-based compound, Cytotoxicity

1649O

Garlic (Allium Sativum) extract's effect on RAGE expression and pro-inflammatory cytokines by PBMCs of Diabetes Mellitus type II patients

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Background: Receptor for advanced glycation end products (RAGE) has been shown to play a causative role in diabetes. Garlic (Allium Sativum) belongs to compounds with anti-glycation

activity that may offer therapeutic potential in delaying or preventing the onset of diabetic complications. This study was conducted to examine whether garlic extract has impact on RAGE expression and pro-inflammatory cytokines secretion by PBMCs of type 2 diabetic patients. **Methods:** Peripheral blood mononuclear cells (PBMCs) of 20 diabetic patients with fasting blood sugar level above 126 mg/dl were treated with R10 fraction and whole garlic extract in presence or absence of glycated albumin. The expression of RAGE was detected with flow cytometry and reverse transcription-PCR. Pro-inflammatory cytokines secretion was detected by ELISA method. **Results:** Treatment with whole garlic extract significantly reduced TNF-α and IL-1β secretion and RAGE expression by PBMCs but R10 fraction augmented the pro-inflammatory cytokines and RAGE expression in absence or presence of glycated albumin. **Conclusion:** As we expected, glycated albumin increased RAGE expression and pro-inflammatory cytokines secretion. Downregulation of RAGE expression was associated with decreased secretion of IL-1β and TNF-α by PBMCs after treatment with whole garlic extract but R10 fraction of garlic had augmenting effect on RAGE expression and pro-inflammatory cytokines secretion. These data indicates that modulation of RAGE expression could be one reason of the effect of garlic on pro-inflammatory cytokines secretion. Although whole garlic extract had beneficial effect as an inhibitor of inflammatory responses to AGEs but there are some components like R10 fraction in it that need to be depleted.

Keywords: Diabetes, Garlic, RAGE, Pro-inflammatory Cytokines

2895O

Effect of Aqueous Extract of *Trachyspermum copticum (L.) Link* (Ajwan) on Transforming Gowth Fctor-beta2 (TGF-beta2) Concentrations and Cell Proliferation in Cancer Cell Lines

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Background: The use of natural substances in cancer treatment is a rapidly devolving aspect of cancer research. In this study, effect of different non-toxic concentrations of aqueous extract of dried seeds of *Trachyspermum copticum (L.) Link* (Ajwan) on concentrations of transforming gowth fctor-beta2 (TGF-beta2) in cancer cell lines and its antiproliferative effect were evaluated. **Methods:** Non-toxic concentrations of the extract were determined using MTT test. Cancer cell lines (human breast cancer: MCF-7, human myeloid Leukemia: K-562, mouse colorectal cancer: CT-26) were treated with different non-toxic concentrations of the extract for 24 and 48 hours. TGF-beta2 concentrations in supernatant of cell cultures were determined using ELISA test. Nitric oxide (Griess Method), LDH cytotoxicity assay, and Apoptosis test (staining with Ethidium Bromide) were performed for further investigations of cytotoxicity. P values less than 0.05 were considered significant. **Results:** The concentrations between 4000 to 7000 µg/mL of the extract inhibited the growth of all three cell lines in a time-

and dose-dependent manners (IC50=2000 to 7000 µg/mL) [p<0.05]; but this growth inhibitory effect was not related to apoptotic effects of the extract (p<0.05). Concentrations between 600 to 1000 µg/mL increased the cell proliferation rates (p<0.05) and concentrations of TGF-beta2 (p<0.05). **Conclusion:** The aqueous extract of dried seeds of *Trachyspermum copticum (L.) Link* may be an effective antitumor compound *in vitro*, and has considerable potential to be developed as a new anticancer drug. Further studies are required to provide evidences for the effectiveness of this extract in *vitro* and *vivo* and its possible mechanisms.

Keywords: Antineoplastic agents; *Trachyspermum Copticum (L.) Link* (Ajwan); Cell proliferation and Cytotoxicity; Cancer cell lines; transforming gowth fctor-beta2 (TGF-beta2).

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2892O

Anti-inflammatory and anti-apoptotic bioactivities of Berberis vulgaris L. var asperma on human enterocytes infected with Enteropathogenic E. coli

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Background: Enterocytes, crucial sentinels of host defense lining the intestinal tract, are able to distinguish pathogens from non-pathogens. Such mechanisms are currently being elucidated involving the initiation of defensive responses like NF-kB and JNK activation, leading to inflammation and apoptosis, respectively. Activation of the inflammatory cascade and apoptosis is an effective primary mechanism in eradicating infection albeit leading to massive destruction of tissue which may be detrimental to the host. Currently, traditional medicinal plants are targeted for novel bioactive molecules with potential anti-bacterial and anti-inflammatory properties capable of eliminating pathogens while providing modulating and protective effects to host cells. Berberis vulgaris L. var. asperma is an indigenous seedless Iranian variety documented to possess several pharmacological properties of medical importance. **Methods:** This study investigated the anti-inflammatory and anti-apoptotic ability of B. vulgaris fruit extract (BFE) on human enterocyte (ATCC CRL-1831) culture infected with enteropathogenic E. coli (EPEC). **Results:** Pre-treatment with BFE showed a significant level of biocompatibility with CRL-1831 and was able to significantly downregulate the expression of pro-inflammatory cytokines, IL-8 and TNF-α, in the presence of EPEC compared to control without BFE. Consequentially, significant upregulation of the anti-apoptotic factor A20 was observed. Likewise, there was also a significant positive induction of galanin-1 receptor expression believed to be involved in enhanced fluid secretion which facilitates clearance of pathogens in vivo. These results were observed at an effective BFE concentration of 7.5–30 µg/mL. **Conclusion:** These are pioneer findings suggesting the pharmacognostic application

of BFE as anti-inflammatory and anti-apoptotic during enterocyte-EPEC pathogenesis in vitro.
Keywords: Berberis vulgaris, Anti-inflammatory, Anti-apoptosis, Enterocytes, EPEC

3197O

A comparison of the effects of silymarin and rapamycin on the generation of regulatory T cells from naïve CD4+ T cells in vitr

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Background: Many studies showed that regulatory T cells (Tregs) with CD4+CD25+CD127-/low markers have immunosuppressive effects on immune responses in transplantation and autoimmune disease. Furthermore, Tregs have main role in the induction and maintenance of immunological tolerance. Among immunosuppressive drugs, rapamycin can inhibit mammalian target of rapamycin (mTOR), results in Foxp3 expression, Tregs expansion and effector T cells inhibition. Silymarin (isolated from milk thistle or silybum marianum plant) is a flavolignan complex with anti-inflammatory, hepatoprotective, antioxidant and immunomodulatory activities. Previous studies by our group revealed inhibition effect of silymarin on mTOR activity in activated T cells. In this study the effect of silymarin on in vitro generation of Tregs was evaluated in comparison with rapamycin. **Methods**: Naïve CD4+ T cells were separated from healthy individuals' peripheral blood mononuclear cells (PBMCs) and activated with monoclonal antibody anti-CD3 (5µg/ml) and anti-CD28 (2µg/ml) or employed as mixed lymphocyte reaction (MLR) for 18h in RPMI complete medium. Then incubation was continued with addition of 10ng/ml IL-2 and 100µM silymarin or its control, DMSO, or cultured in present or absent of rapamycin (100nM) for 6 and 12 days. Cells were harvested and stained with anti-CD4, anti-CD25 and anti-CD127 antibodies or with anti CD4 and anti-FoxP3 antibodies for flow cytometry. Moreover, Foxp3 gene expression in activated T cells in the presence of silymarin or rapamycin was determined by Real-time PCR. **Results**: A significant generation of CD4+CD25+CD127-/low Tregs was observed in naïve T cells treated with silymarin for 6 and 12 days. Silymarin also significantly increased Foxp3 gene and protein expression at 6 days in compare with rapamycin. **Conclusion**: Silymarin was stronger than rapamycin in generation of Treg from naïve T cells in-vitro. This result indicates the potential therapeutic effect of silymarin in autoimmune disease.

Key word: Silymarin, Treg, T naïve, Rapamycin.

Poster Presentations:

1516P

Inhibitory effect of isosorbide on IL-13 production in human PBMCs

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Background: Isosorbide dinitrate (ISDN), as a nitric oxide donor, is one of the most effective and broadly used drugs in treatment of many ischemic heart diseases such as angina pectoris. Anti-inflammatory effects of isosorbide have also been reported.Interleukin-13 (IL-13) (a T-helper type 2 cytokine) is a mediator of airway inflammation and increases in immediate-type allergic diseases such as asthma. In the present study the isosorbide effect on IL-13 secretion in human peripheral blood mononuclear cells (PBMCs) has been evaluated in vitro. **Methods:** Human PBMCs were cultured in complete RPMI medium. Then the cells at the exponential growth phase were incubated with different concentrations of isosorbide (0.0004 -0.4 mM) for 24 hours. Afterward the level of IL-13 secreted in the cell culture supernatants was measured with the enzyme-linked immunosorbent assay (ELISA) standard kits. **Results:** Isosorbide dinitrate significantly decreased the IL-13 production in hPBMCs dose-dependently. **Conclusion**: The results of this study indicate that isosorbide down-regulates the production of IL-13 in human PBMCs. Thus, the anti-inflammatory properties of isosorbide may be partly due to its inhibitory effects on IL-13 production. Therefore isosorbide may be useful in alleviating the IL-13- induced respiratory inflammation in related diseases such as chronic obstructive pulmonary disease (COPD) and asthma. So isosorbide along with its chronic long-term usage in cardiac problems might have potential implication in treatment of airway inflammatory disorders.

Keywords: PBMCs, Isosorbide, IL-13

2011P

The efficacy of Zataria multiflora and Lemon shell essential oils on immune system function in rabbits

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Background: Little is known about the influence of Zataria multiflora and Lemon shell essential oils (EOs) on the monocytic/macrophagic system, one of the primary cellular effectors of the immune response and cell-mediated immunity against pathogen attacks. We investigated the effects of Zataria multiflora and Lemon shell EOs on the function of immune system using animal model.**Methods:** Rabbits were divided into 3 groups of 5 rabbits. Groups 1, 2 and 3 were administrated subcutaneously by Z. multiflora, Lemon shell EOs and normal saline, 6 times with 6 days of interval. Five days after the last injection of the EOs, Candida antigens were injected subcutaneously to all animals. Phagocytosis and killing assays and lymphocyte transformation test (LTT) were carried out on blood samples. **Results:** Lymphocytic responses were significantly stimulated with C. albicans antigen (mean: 619.3±12.1) and Concanavalin-A (mean: 712.3±10.7) mitogen in rabbits injected subcutaneously by Z. multiflora EO when

compared to control group, whereas Lemon shell EO suppressed the cellular responses. Both Z. multiflora and Lemon shell EOs stimulated phagocytosis and killing of monocytes from rabbits. Humoral response (IgG) to Candida antigens was significantly decreased in animals injected by Lemon shell (OD: 0.10 nm) when compared to Z. multiflora (OD: 1.88 nm) oil ($P \leq 0.05$). **Conclusion:** Our data, demonstrating that Z. multiflora has more immuno-stimulatory effect than Lemon shell EO, and it may be used in exact immunocompromised patients.
Keywords: Candida albicans, Zataria multiflora, Lemon, Essence, Innate immunity.

2155P

Scrophularia megalantha Extract have Inhibitory Effect on Pro-Inflammatory Cytokines Production and Analgesic Effect

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Background: This study designed for the analgesic and anti-inflammatory activities of Scrophularia megalantha in male rats. **Methods:** Ethanolic extract of Scrophularia megalantha was prepared. In order to determine qualitatively the chemical components of the extract, thin layer chromatography (TLC) was used. The analgesic activity of the extract at various doses (25, 50, 100 and 200 mg/kg, i.p) was assessed using formalin test while pro-inflammatory cytokines were measured by enzyme-linked immunosorbent assay (ELISA), respectively. Diclofenac (5 mg/kg) was used as positive control. **Results:** Our finding in this study indicated that phenolic compounds, flavonoids and phenyl propanoid were present in the extract. Moreover, at doses of 200 and 400 mg/kg, the extract showed significant analgesic effects ($p< 0.01$) in the first phases of formalin test. At 25, 100 and 200 mg/kg doses, the extract reduced significantly ($p< 0.05$, $p< 0.001$, $p< 0.001$) pain score in the chronic phases of the formalin test. In addition, at 50 - 200 µg/mL of the extract both TNF-a and IL-6 pro-inflammatory cytokines were inhibited significantly ($p< 0.001$) on LPS-stimulated macrophages. **Conclusion:** The extract of S. megalantha exerts analgesic and anti-inflammatory activities by inhibition of pro-inflammatory cytokines production. This extract could be used as an analgesic in traditional medicine but future study is recommended.
Keywords: Scrophulariamegalantha, Analgesic, Inflammation, Pro-inflammatory cytokines, Phenolics, Flavonoids

1475P

Chronic caffeine treatmentattunates multiple-low-dose streptozotocin-induced type 1 diabetes in Balb/c mice

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Background: Multiple low doses of streptozotocin (STZ) can be used as an animal model for type 1 diabetes. On the other hand, recent evidence has suggested that caffeine may modulate inflammatory reactions. The present study was set out to investigate the effects of chronic treatment of caffeine on type 1 diabetes. **Methods:** Type 1 diabetes was induced in male BALB/c mice by Intraperitoneal injection of STZ at 40mg/kg/day for 5 consecutive days. The mice in treatment group were pretreated with caffeine (30 mg/Kg) in their drinking water for 7 days prior to STZ injection and again afterwards for the duration of the experiment. Blood glucose levels were measured on days 0, 7, 14 and 21 (in relation to the first STZ dose). Mice were bled on day 21 and insulin level of plasma was measured. Moreover, pancreas tissue samples were removed .A half of each pancreas was fixed with 10% formalin, embedded in paraffin and stained with hematoxylin and eosin (H&E). The other half of pancreas tissues were homogenized and were checked for cytokine production by ELISA method. **Results:** Chronic caffeine treatment significantly reduced hyperglycemia and incidence of diabetes, and restored pancreatic insulin secretion. Also, methadone treatment decreased the proinflammatory Th1 and Th17 cytokines (IFN-γ and IL-17, respectively) and conversely, increased anti-inflammatory Th2 cytokines (IL-4 and IL-10). Histopathological observations indicated that STZ-mediated destruction of β cells was attenuated by chronic caffeine treatment. **Conclusion:** It seems that chronic caffeine treatment may have a protective effect against destruction of β cells and insulitis in the mouse model of type 1 diabetes.

Keyword: Caffeine treatment, Streptozotocin, Type 1 diabetes, Mice

1420P

Immunomudulatory and anti-inflammatory benefits of hydroalcoholic extract of Hypericum perforatum

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Background: Hypericum perforatum(St. John's Wort) is a sprawling, leafy herb that grows in open, disturbed areas throughout much of the world's temperate regions, such as Iran, and is used in folk medicine to treat a variety of internal and external ailments. This study reports on the immumodulatory and anti-inflammatory potentials of Hypericum perforatum. **Methods:** Twenty male BALB/c-mice randomly allocated in two equal groups and immunized with sheep red blood cells (SRBCs). Mice in treatment group were orally received hydroalcoholic extract of H. perforatum (110 mg/Kg-daily) from the beginning of the study and continued for 2 weeks. In different exam, the anti-inflammatory capacities of orally and topically administrated experiment were evaluated by croton oil-induced ear edema assay in BALB/c mice. **Results:** The results indicated a significant increase in the level of anti-SRBC antibody and simultaneously a significant decrease in the level of DTH in treatment group compared to control group. The level of respiratory burst in phagocytic cells of splenocytes was significantly increased in the of treatment groups, while the level of lymphocyte proliferation was significantly decreased in treatment group compared to control group. Moreover, extract caused a significant reduction in production of pro-inflammatory IL-17 as well as IFN-γ, parallel to increasing FoxP3^{+}Treg cells. The level of inflammatory IL-6 was significantly

decreased .Also, the extract significantly inhibited edema when applied topically or orally in croton oil-induced ear edema assay in mice. **Conclusions**: The hydroalcoholicextract of H. perforatum may be used as a natural source for treatment of immunopathologic condition.
Keywords: Hypericum perforatum, Humoral immunity, Cellular immunity, Lymphocyte response.

1728
Antibacterial and anti inflammatory effect of Punica granatum peel extract against Mycobacterium tuberculosis

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Background: Tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis (Mtb). It is estimated to maintain latent infection in approximately one-third of the world's population and kill 1.7– 1.8 million people each year. Efforts to treat the disease have been made much more difficult due to development of drug resistant TB strains (MDR and XDR TB). There is an urgent need, therefore, to search for and develop new, inexpensive and effective anti-TB drugs. Natural products isolated from plants offer hope of developing alternative drugs against Mtb. The present study was done to evaluate in vitro anti-tubercular activity of pomegranate fruit peels from Golestan province, north of Iran. **Methods:** Hydro-alcoholic extract of pomegranate fruit peels was prepared by cold percolation method. The extract was screened for anti-tubercular activity against 9 strains of Mtb by Disc Diffusion (DD) method. The extract was also evaluated for Anti-inflammatory effect in a model of phagocytized intracellular Mtb in phorbol-differentiated U937 cells (dU937). The Anti-inflammatory effect was assessed by cytokines measurement using Elisa method. **Results:** The peel extract of Punica granatum L. showed inhibitory effect on all strains even on MDR strains with 15-22 mm inhibitory zone. TNFα secretion was increased significantly in Mtb-infected dU937 cells as compared to the uninfected cells and decreased significantly after treating with extract. The amount of IL-6 and IL-10 was decreased to undetectable level in Mtb-infected cells and increased after treating with extract. **Conclusion:** The findings of this investigation illustrate the anti- tubercular and immunomodulatory effect of Punica granatum peel which offer a hope for developing alternative drugs against tuberculosis.
Keywords: Herbal plants, Mycobacterium tuberculosis, Punica granatum, Immunomodulatory

2337P
Increased lymphocytes activation by the extracts of some native Euphorbia species and induction of a Th2 immune response

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Background: Various studies have shown that plants have a number of beneficial immunomodulatory properties. In the present study the possible in vitro immunomodulatory effect of three species of Euphorbia, an important genus of Euphorbiaceae, including E. osyridea, E. microciadia, and E. heteradenia were investigated. **Methods:** Cell proliferation assay and ELISA assay for cytokine secretion including IL-4, IL-10 and IFN-γ in culture supernatants were used in this study. **Results:** Butanolic extracts of E. microciadia, E. osyridea and hexan extracts of E. heteradenia, showed stimulatory effects on the proliferation and activation of the phytohemmagglutinin-treated lymphocytes. Treatment of the lymphocytes with the extracts in the absence of mitogen resulted in an increased proliferation of the cells indicating the lymphocyte mitogenic activity of the three extracts. In this activity E. heteradenia was weaker than other extracts. The level of T cell cytokines in the supernatant of the cells was determined by ELISA assay to find the dominant T cell subsets involved in the immune response. Our data indicated that the extract of E. microciadia and E. osyridea could increase IL-4 and IL-10 secretion but not IFN-γ production showing its capacity to deviate immune response toward a TH2 pattern. E. heteradenia did not change the release of IL-4 and IFN-γ cytokines but increased IL-10 production which suggests its possible regulatory effects. **Conclusion:** these plants had the ability to modulate T cell responses suggesting their possible usefulness in the treatment of immune-mediated diseases or certain infections.

Keywords: Euphorbia species, IL-4, IL-10, ELISA, Immunoregulatory

1724P

Commiphora myrrha extract enhances pulmonary fibrosis in mice

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Background: Idiopathic pulmonary Fibrosis is characterized by inflammation, deposition of collagen and accumulation of extracellular matrix. This disease is the end stage of many of interstitial lung disease. In this stage, many of patients have severe hypoxia. Decrease in the prostaglandian-E2 level is one of the reasons of hypoxia that reduces blood flow. Studies showed that Commiphora myrrha gum used in traditional medicine for the treatment of various ailments and has anti-inflammatory, anti-tumor and wound healing properties. In this study we investigated effects of Commiphora myrrha extract on pulmonary fibrosis. **Methods:** Pulmonary fibrosis was induced in mice via intratracheal injection of 2mg/kg bleomycin. Then in a group of mice bleomycin was injected without treatment by Commiphora myrrha. In another groups following bleomycin injection, Commiphora myrrha extract (150mg/kg) was injected subcutaneously and orally. After 21 days lung tissues were collected for evaluation of α-SMA mRNA expression using real time PCR, also trichrome and hematoxylin-eosin staining for pathologic analysis of lung tissue. **Results:** In the mice that had received bleomycin, destruction of lung structure, accumulation of inflammatory cells, collagen deposition and increase in α-SMA mRNA expression were observed. In groups that was received Commiphora myrrha

extract orally and subcutaneously, these pathological changes were increased. **Conclusion:** This study shows that Commiphora myrrha extract increases bleomycin-induced injury, and thus accelerates process of pulmonary fibrosis.

Keywords: Pulmonary fibrosis, Commiphora myrrha, intratracheal,bleomycin.

2295P

Inhibitory effects of Ferula hezarlalehzaricaon the growth of various cell lines

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Background: Medicinal plants have been used as a remedy for various diseases in folk medicine. Ferula genus grows all over the world but some of them are endemic to Iran. In the past, people used this plant to reduced hypertension and as a treatment for some neurological disorders. Several investigations have proved different biological activity of these plants. It has been demonstrated that some Ferula species have cytotoxic activity. In the present study the effects of the methanolic extract of Ferula hezarlalehzaricaspecies on different tumor cell lines were examined. **Methods:** Methanolic extract of the plant was prepared and its inhibitory effect on some leukemia cell lines and solid tumor cell lines including Hela, Raji, K562, HepG2 and EL-4 was examined. Cells were treated by different concentration of the extract and after 48 hour growth inhibitory effect was measured by MTT assay. **Results:** The methanolic extract showed an inhibitory effect on the growth of all cell lines. The strongest effect of the extract was on the growth of EL-4 lymphoma cell line. IC50 values obtained from MTT assay describing 50% inhibitory effect of this extract on each cell line was 88 µg/ml for EL-4 followed by 125 µg/ml for K562 and Raji cells. **Conclusion:** The results of this study showed anti tumor activity of Ferula hezarlalehzarica extract. Further studies are needed to identify the compound(s) responsible for this effect.

Keyword: Ferula hezarlalehzarica, Growth, Cell lines

1850P

Glycyrrhizin down-regulates MCP-1 and IL-8 expression in cerulein-stimulated pancreatic acinar cells

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Background: Many inflammatory chemokines release from pancreatic acinar cells (PAC) and leukocytes which play important roles in pathophysiology of acute pancreatitis (AP). IL-8 and monocyte chemoattractant protein -1 (MCP-1) have been shown elevated in the plasma of patients with AP. Of interests, MCP-1 and IL-8 is increased in the early stage of human AP. Recently, glycyrrhizin (GRZ) as one of the main active components found in the licorice root extract, have expected to mediate many of licorice root–induced anti-inflammatory and

immunomodulatory effects. Accordingly, we have recently found that GRZ attenuates AP in mice model. In this study, we aimed to investigate the direct effect of GRZ on expression levels of IL-8 and MCP-1 in isolated PAC. **Methods:** PAC cells were isolated from the pancreas of healthy C57BL/6mice. As cerulein-induced AP is the most resemble animal model of AP to human, we stimulated PAC with cerulein (10^{-7}M), and then treated with either PBS or different doses of GRZ. The levels of IL-8 and MCP-1 expression at mRNA were assessed by Real-Time RT-PCR. Conditioned media from supernatants of each cells culture condition were collected for detection of IL-8 and MCP-1 levels by ELISA. **Results**: First, we observed that cerulein upregulates significantly both IL-8 and MCP-1 expression in PAC. Moreover, when we treat the PAC cells with GRZ, we found that GRZ significantly downregulates IL-8 and MCP-1 expression at mRNA levels in a dose-dependent manner. Consistently, the conditioned media of TRZ-treated cells contained a significant lower levels ofIL-8 and MCP-1 ($p<0.05$). **Conclusion:** Our data demonstrate for the first time that GRZ directly downregulates IL-8 and MCP-1 levels in cerulein-stimulated PAC cells which may explain the therapeutic effects of GRZ in cerulein-induced AP in mice.

Keywords: Cerulein-induced acute pancreatitis, glycyrrhizin, IL-8, MCP-1

1723P

Study of curcumin immunomodulatory effects on astrocyte functions: relevance to multiple sclerosis

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Background: Recent evidence suggests that activated astrocytes play a functional and dual role in CNS inflammatory disorders such as Multiple Sclerosis (MS). These astrocytes are capable of producing divergent pro and anti-inflammatory compounds such as IL-6, IL-10, MMP-9 and MCP-1. In addition, growth factors are synthesized by reactive astrocytes to support neurons. **Methods:** In this study, we examined the effects of Curcumin, the principal active component of Turmeric, on human astrocyte cell line (U373-MG) induced by lipopolysaccharide (LPS) in vitro. MMP-9 activity was assessed by gelatin zymography. Cytokines levels were evaluated by quantitative ELISA method and mRNAs expression were measured by real-time PCR. **Results:** We found that Curcumin decreased the released of IL-6 and reduced MMP-9 enzyme activity. It down-regulated MCP-1 mRNA expression too. However, Curcumin had not inhibitory effects on the release of IL-10 protein and the expression of neurotrophin-3 (NT-3) and insulin-like growth factor-1 (IGF-1) mRNAs.**Conclusion:** Our results suggest that Curcumin can beneficially affect astrocyte population in CNS neuroinflammatory environment lean to anti-inflammatory response and help to components in respects of CNS repair. Our findings offers Curcumin as new therapeutic agents with the potential of regulating astrocyte-mediated inflammatory diseases in the CNS.

Keywords: Curcumin, Multiple Sclerosis, Astrocyte

2259P

A comparison of the suppressive effects of Silymarin with Rapamycin and FK506 on the proliferation of human T cells

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Background: Transplantation medicine is one of the most challenging and complex areas of modern medicine. Transplant rejection can be reduced through the use of immunosuppressant drugs such as Rapamycin but with many side effects. Silymarin, an extract from the seeds of the milk thistle plant Silybum marianum, is known to have anti-inflammatory, hepatoprotective and anticarcinogenic effects. Therefore, the goal of this study was to determine immunosuppressive effect of silymarin on human T cells. **Methods:** Peripheral blood mononuclear cells (PBMC) from healthy individuals were activated with Con A (10 µg/ml) and treated with silymarin, FK506 and Rapamycin in different concentrations (0.001, 0.01, 0.1, 1, 10, 100 and 200 µM). Then, cells were incubated 5 days (37◦C in 5% CO2) for proliferation assay using CFSE and for viability analysis using flow cytometry. We analyzed data by using Graphpad prism (version 6).**Results:** This study showed that silymarin had the ability to inhibit T cell proliferation in vitro. Moreover, our results indicated that 100 µM and 200 µM of silymarin ($P<0.001$) had more inhibitory effect on T cells in comparison with FK506 ($P=0.002$) and Rapamycin ($P<0.001$).Conclusions: We conclude that Silymarin exerts immunosuppression effects, and it could be use in therapeutic situations with fewer side effects in compare with other immunosuppressive drugs.

Keywords: Silymarin, CFSE, Immunosuppressive effect

1739P

In vivo effect of glycritinic acid on bleomycin-induced pulmonary fibrosis

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Background: Interstitial pulmonary fibrosis (IPF) is a progressive inflammatory disease of lung characterized by epithelium damage and extra cellular matrix deposition. Previous studies have shown that cytokines such as TGF-β1 play crucial role in pathogenesis of fibrosis. Glycritinic acid is one of the main components of Licorice which has anti-inflammatory properties. In this study we investigated effect of glycritinic acid on pulmonary fibrosis in mice. **Methods:** Pulmonary fibrosis was induced by intratracheal injection of bleomycin (2mg/kg) in C57BL/6 mice. Subsequently the mice were placed in three groups. In the first group of mice, only bleomycin was injected. In the second group after induction of fibrosis, glycritinic acid (150 mg/kg) was injected subcutaneously, for 7 days. The third group was treated by glycritinic acid (10, 25, 50 mg/kg) intratracheally for three times (0, 24, and 72 h) following bleomycin injection. Then lung tissues were taken after 21 days and hematoxylin-eosin and trichrome staining for histopathological studies were performed. In addition, α-SMA mRNA expression in lung tissue was evaluated by real time PCR. **Results:** Mice that received

bleomycin showed destruction of lung structure and accumulation of inflammatory cells, and collagen deposition was shown by trichrome staining. In addition, significant elevation in α-SMA mRNA expression in the control positive group was observed. In group that had received glycritinic acid, subcutaneously and intratracheally, these pathological changes were decreased significantly ($p<0.05$).**Conclusion:** Glycritinic acid exhibited a potency ameliorate bleomycin-induced lung injury in mice. So this result may be indicating its significant potency to be applied in IPF treatment.

Keywords: Pulmonary fibrosis, Glycritinic acid, Intratracheal, Bleomycin.

2092P

The evalution of urtica dioica extract on neutrophilfunction

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Background: Urtica dioica is a herbaceous perennial flowering plant, native to Europe, Asia and… .This plant is very much in north of Iran, guillan .**Methods:** In this research we obtain neutrophils from normal human by dextran. We incubate neutrophils with Urtica dioica extract in different dilution in benmari at 37for 24h and performed NBT test .**Result** : We showed that in some dilution ,the extract can induce neutrophiln for reduction of NBT .**Conclusion** :In this research we showed that this extract can induce PMNs for reduction NBT but in all dilution .When the extract is not diluted cannot conduce NBT reduction .But when diluted in some dilution can induce PMNs for reduction of NBT without exist of PMA .

Keywords: Urtica dioica, Neutrophil, NBT

2363P

The effect of glycyrrhetinic acid &glycyrrhizic on the expression of CXCR4 in epithelial cells of gastric carcinoma

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Background: Gastric cancer is one of the most common cancers and the second common cause of cancer death worldwide. Overall surgery is only treatment for gastric cancerbut half of patients have inoperable tumors. For these patients, combined chemotherapy as a primary treatment is used, which this has a high incidence of side effects. Since licorice compounds have anticancer properties thus the present study is aimed to investigate the effect of licorice compounds on the expression of CXCR4 receptor in gastric cancer AGS cells. **Methods**: After preparing AGS cell line from the Pasteur Institute, 10^6 cells were poured in per well 6 pieces plates. Then the cells with various concentrations of compound Glycyrrhetinic acid and

glycyrrhizic acid (1, 10µm) were exposed for 24 hours. The CXCR4 gene expression levels were determined by Real time-PCR method. We investigated CXCR4 gene expression levels using kruskalwallis statistical test and Dunn's post hoc test in Spss 16 software. P <0.05 were considered as statistically significant. **Results**: The results show that CXCR4 gene expression in cells were exposure to acid glycyrrhizic and acid Glycyrrhetinic at 24 hours compared with control group was not statistically significant. **Conclusion**: Since mentioned compounds reduced of CXCR4 gene expression, may be used in the treatment of gastric cancer.
Keywords: Gastric cancer, Glycyrrhetinic Acid, Glycyrrhizic Acid, CXCR4 gene

1798P

Immunomodulatory potential of Echinacea pupurea: from promise to fact

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Background: The present study was set out to investigate the effects of Echinacea pupureaon immunity system in NRMI-mice after challenge with sheep red blood cells (SRBC). **Methods**: The study population was consisting of 24 male mice that randomly allocated in two equal groups and immunized with SRBC. Hydroalcoholic extract of Echinacea pupurea were administered to treatment group mice per os in daily doses of 0.025 mg from the beginning of the study and continued for 2 weeks. **Results**: The results of the present study indicated a significant increase in the level of anti-SRBC antibody and lymphocyte proliferation in treatment group compared to control group.while The level of respiratory burst in phagocytic cells of splenocytes and DTH was significantly decreased in the of treatment groups. **Conclusion**: While, humoral immunity was increased after administration of Echinacea pupurea but respiratory burst of phagocytes was dramatically decreased. Therefore, the possible therapeutic effectiveness of Echinacea pupurea in the treatment of infection is doubtful. However, this data suggest that the hydroalcoholicextract of Echinacea pupurea may be used for purposes of modulating the immune system.
Keywords: Echinacea pupurea, Humoral immunity, Cellular immunity.

1406P

Comparative histopathological Study between Amikacin and Tobramycin given alone and in combination with Calcium Gluconate in rabbits

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Background: Explore a histopathological comparison between tobramycin sulphate and amikacinsulphate, and to explore the role of calcium gluconate in antagonizingnephrotoxicity of tobramycin and amikacin. **Method**: Group (1): G1treated with distilled water
Group (2): G2 treated with tobramycin (I/M) (5 mg /kg /day).

Group (3): G3 pretreated S/C with therapeutic dose of calcium, and then injected by tobramycin (5 mg /kg /day).
Group (4): G4 treated with triple dose of tobramycin (15 mg/kg) /day
Group (5): G5 pretreated S/C with therapeutic dose of calcium and then injected with tobramycin (15 mg/kg)/day
Group (6): G6 injected with therapeutic dose of amikacin (15 mg / kg /day)/day.
Group (7):G7 givenamikacin(15 mg/kg) in to rabbits pretreated with calcium (22.5 mg/kg).
Group (8): G8triple dose of amikacin (45 mg/kg) /day by
Group (9):G9 amikacin at (45 mg/kg) in to rabbits pretreated with calcium (22.5 mg/kg).
Results: Showed that tobramycin and amikacin differ in toxic effects potential at therapeutic and triple doses.Tobramycin caused more pathological changes than that caused by amikacin. Pretreatment with calcium significantly improved the study to the levels almost similar to that of control group for both drugs, and gave complete protection from toxic effects of amikacin, and complete to partial protection with tobramycin accordingly with doses **Conclusion:** From this study it appears that tobramycin is more potent as nephrotoxic accordingly with the dose and treatment periods in rabbits while pretreatments with calcium was completely or partly ameliorate those toxic effects of both drugs.
Keywords: Amikacin, Tobramycin, Nephrotoxicity effect of aminoglycoside

1794P

New approach to the immunomodulatory benefits of calcitriol in NMRI-mouse

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Background: Recent evidence demonstrated an important role for Th-17 and FoxP3$^+$Treg lymphocytes in immunity system .Although, previous reports have determined the immunomodulatory potential of calcitriol, but this study was mostly done before the discovery of recent lymphocytes. The present study was set out to investigate the effects of calcitriol on immunity system in NRMI-mice after challenge with sheep red blood cells (SRBC).**Methods:** The study population was consist of 14 male mice that randomly allocated in two equal groups and immunized with SRBC. Mice in treatment group were intraperitoneally received 5 µg/ Kg calcitriol every other day from the beginning of the study and continued for 2 weeks. **Results:** The results of the present study indicated a significant increase in the level of anti-SRBC antibody and simultaneously a significant decrease in the level of DTH in treatment group compared to control group. The level of respiratory burst in phagocytic cells of splenocytes was significantly increased in the of treatment groups, while the level of lymphocyte proliferation was significantly decreased in treatment group compared to control group. Moreover, calcitriol caused a significant reduction in production of pro-inflammatory IL-17 as well as IFN-γ, parallel to increasing FoxP3$^+$Treg cells.Also the level of anti-inflammatory IL-10 and TGF-β was significantly increased and conversely, the level of inflammatory IL-6 was dramatically decreased. **Conclusion:** The major immunomudlatoty effects of calciteriol may be due to a significant decrease in Th17 cells activity and concurrently a significant decrease in the expansion of FoxP3$^+$Treg lymphocytes.
Keywords: Calcitriol, Humoral immunity, Cellular immunity, Lymphocyte response.

1791P

New insights to the immunomodulatory effects of β-esteradiol in NMRI-mouse

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Background: The present study was set out to investigate the effects of β-esteradiol on immunity system in NRMI-mice after challenge with sheep red blood cells (SRBC).**Methods:** The study population was consist of 14 male mice that randomly allocated in two equal groups and immunized with SRBC. Mice in treatment group were intraperitoneally received 10 mg/Kg β-esteradiol every other day from the beginning of the study and continued for 2 weeks. **Results:** The results of the present study indicated a significant increase in the level of anti-SRBC antibody and simultaneously a significant decrease in the level of DTH in treatment group compared to control group. The level of respiratory burst in phagocytic cells of splenocytes was significantly increased in the of treatment groups, while the level of lymphocyte proliferation was significantly decreased in treatment group compared to control group. Moreover, β-esteradiol caused a significant reduction in production of pro-inflammatory IL-17, parallel to increasing FoxP3^{+}Treg cells. **Conclusion:** The major immunomudlatoty effects of β-esteradiol may be due to a significant decrease in Th17 cells activity and concurrently a significant decrease in the expansion of FoxP3^{+}Treg lymphocytes.

Keywords: β-esteradiol, Humoral immunity, Cellular immunity, Lymphocyte response.

1471P

Immunomudulatory potential of Liquorice roots extract

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Background: Glycyrrhiza glabra (Liquorice),is an age-old plant used in traditional medicine to treat a variety of ailments from simple cough to hepatitis to more complex like cancers. This study was conducted to check the immunomodulatory properties of roots of Glycyrrhiza glabra in mice challenged with sheep red blood cells (SRBCs).**Methods**: Twenty male NMRI-mice randomly allocated in two equal groups and immunized with sheep SRBCs. Mice in treatment group were orally received aqueous liquorice extract (0.75 g/Kg-daily) from the beginning of the study and continued for 2 weeks. Immunomodulatory potential of extract was assumed by the effect on cellular immune response, haemagglutination antibody titre, leukocyte count, spleen weight and respiratory burst intensity in phagocytic cells of splenocytes. **Results:** The results indicated a significant increase inthe level of cellular immune response study, an enhancement in foot pad thickness, and simultaneously a significant decrease in the level of anti-SRBC antibody in treatment group compared to control group.The level of respiratory burst in phagocytic cells of splenocytes was significantly increased in the of treatment groups, while the level of lymphocyte proliferation was significantly decreased in treatment group compared to control group. Moreover, spleen weight and leukocyte count was significantly increased in the treatment compared to control mice. **Conclusions**: Aqueous root extract of Glycyrrhiza glabra may be used as a natural source to intervene in immunity system.

Keywords: Glycyrrhiza glabra, Humoral immunity, Cellular immunity, Lymphocyte response, phagocytosis.

1974P

The effect of achilleatalagonica alcoholic extract on cutaneous burn wounds healing in rabbit

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Background: Many herbal compounds are among natural remedies that have been used for healing of wounds. Achillatalagonica is one of such plants which due to its anti-inflammatory, antimicrobial and anti-oxidant properties have been applied from ancient times for this purpose. So, this study aimed to assess the efficiency of the Achillatalagonica alcoholic extract for burn wounds healing. **Methods**: It was carried out on 10 adult male New Zealand rabbits. After induction of anesthesia, six burn wounds of deep second degree were made on the back (three on each side) of each animal. The wounds were washed with normal saline and covered immediately after burning procedure by 0.5g of one of the following products: Vaseline gel (control group), Silver Sulfonamide 1% (experimental group 1), and 5% Achillatalagonica alcoholic extract mixed with Vaseline gel(experimental group 2). The treatments were repeated twice daily until complete healing. To measure the wound area, photographs have been taken from the wounds that have been made in the right side of the back on days 0, 3, 7, 14 and 21 and finally processed using Auto CAD software and average of healing percentage wase calculated. Tissue samples were taken on the same days from the wounds of the left side of the back. The wound healing was analyzed and scored histopathologically based on the reconstruction of epithelium, fibroblast maturation and collagen deposition. Results: **Results** indicated that average of healing percentage and the reconstruction of epithelium in the experimental groups were significantly higher than control in the days of 7 and 14. ($p< 0.05$). Furthermore, fibroblast maturation and collagen deposition significantly increased in the experimental groups compared to control group in the day 7 ($p<0.05$). **Conclusion**: It could be concluded that Achillatalagonica alcoholic extract promotes wound contraction and ameliorate wound healing process.

Keywords: Achillatalagonica, Burn, Rabbits, Wound healing.

1650P

The Effect Of Portulaca Oleracea Seeds On Inflammation In Asthmatic Patients

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Background: Asthma is a disease with chronic inflammation of airway. Portulaca Oleracea is a plant that grows in many areas in the world. Some studies reported anti-inflammation effect for extract of Portulaca Oleracea. The experiment is designed to study the effect of Portulaca oleracea seeds on inflammation of airway in asthmatic patients. **Methods:** In a randomized

clinical trial, 59 patients with asthma disease that refer to clinics of Tabriz University of Medical Sciences divided to intervention and control groups. First group (n=31) received protocol treatment and 10g/day Portulaca oleracea seeds in two doses, while participants in second group (n=28) had only their protocol treatment for 8weeks. Interleukin 4, TNF_α, Nitrite and Nitrate in induced sputum were evaluated at the baseline and end of study.Sputum was induced with normal saline. **Results:** The mean age of the participants at baseline was 43.68 ± 10.81 years. Median duration of asthma estimated 8(3to15) years. Administration of seeds indicated significant increment in the level of IL-4(P=0.03) in intervention group comparing with control. However, the levels of TNF_α (P>0.29), Nitrite and Nitrate (P>0.18) showed no significant difference between two groups. **Conclusion:** Administration of Portulaca oleracea seeds as adjunctive therapy resulted in increase inflammation in asthmatic patients. Apparently, further investigations about this topic are needed.

Keywords: Asthma, Portulaca oleracea seed, Inflammation

1508P

Evaluation of crocin effects on human B lymphocyte (U266)

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Background: Among various naturally occurring compound which have been extracted from Crocus sativus, crocin has shown different pharmacological effect i.e. neuroprotection and anti-tumor activity. Here, crocin effects on human B cell myeloma cell line, U266 were evaluated. **Methods**: In this study, we examined the effects of 24 and 48 hours of crocin treatment (50,250 and 500µM) on the viability of U266cell line. Moreover, probable apoptic/necrotic outcomes, reactive oxygen species (ROS) production variations along with crocin treatment consequences on DNA were investigated. **Results:** Results demonstrated that 48-hour crocin treatment at 500 µM, significantly reduced cell viability ($p< 0.05$).DNA fragmentation was recorded to be significantly increased at higher doses of crocin following 24 and 48 hours ($p< 0.01$). According to our result while apoptosis was detected at all concentration, necrosis detected at the highest dose only. In comparison with control, ROS production WAS reducedat 500µM.**Conclusion**:In according with previous reports, crocin exhibited mild cytotoxic effects on a myeloma cell line which might be mediated through the increase of DNA fragmentation.

Keywords: Crocin, U266, Apoptosis, Necrosis, Reactive Oxygen Species.

2019P

Effects of crocin on serum adiponectin in rats with diabetes mellitus type I

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Background: Adipose tissue secretes several bioactive proteins, or adipokines, that regulate hepatic and peripheral glucose and lipid metabolism. Secretions of these adipokines include

adiponectin changed in patients with diabetes mellitus. Crocin is the chemical constituent isolated from the Saffron and is found to be effective as anti-oxidant, attenuated hyperglycemia and improves the insulin resistance in rats. The objective of this study was to evaluate adiponectin level in rats with Diabetes mellitus type I (T1DM) and study of the effects of crocin administration on this adipkine. **Methods:** In this study, 120 male rats were divided into 2 major day20 and Day40 groups. Each major group was the divided into ten groups (6 rats in each group) as follow: Group1 received normal saline, groups 2, 3, 4 and 5 treated with crocin at doses of 12.5, 25, 50 mg/kg and insulin 5 iu/kg respectively. Group 6 administered with STZ before normal saline, groups 7, 8, 9 and 10 treated with crocin at doses of 12.5, 25, 50 mg/kg and insulin 5 iu/kg after STZ respectively. Normal saline and crocin were i.p. injected five days a week from 3rd day after i.p. injection of citrate buffer and STZ and lasted to the end days of the experiments. Diabetes was induced by STZ (50mg/kg). At the end days of the experiments (days 20 and 40), the animals were anaesthetized and blood samples were collected from the heart and glucose, insulin and adiponectin were determined. **Results:** In rats with T1DM insulin was significantly decreased ($p< 0.05$) whereas glucose and diponectin levels significantly increased ($p< 0.05$). Administration of crocin reduced adiponectin concentration in dose dependent manner. There was significant negative correlation between serum adiponectin and insulin concentration ($r=-0.53$, $P<0.01$).**Conclusion**: Adiponectin has been shown to have insulin-sensitizing properties and increases as a compensatory response in T1DM due to microvascular complications. Administration of crocin decreased adiponectin concentration in serum probably due to anti-hyperglycemic and antioxidant properties. It was demonstrated that increased generations of free radicals due to oxidative stress develop renal failure that may lead to the stimulation of adiponectin production as a physiological response to restrict endothelial damage. It may also decrease adiponectin clearance, and the kidney may develop secondary resistance to adiponectin.

Keywords: Diabetes mellitus type I, Adiponectin, Crocin, Insulin, Rat

1627P

Study of verapamil effect on MMP-9 activity in U937 and THP-1 cell lines in vitro

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Background: Gelatinases are a big group of proteolytic enzymes belong to matrix metalloproteinases (MMPs). MMPs are a wide cluster of peptidases, which proteolyses the extracellular matrix and have important role in inflammation. Verapamil is a calcium channel blocker extensively used in therapy of numerous cardiovascular diseases such as arrhythmia and hypertension. In this study, the effect of verapamil on gelatinases activity in U937 and THP-1 cell lines has been assessed in vitro. **Methods:** In this experimental study the cells were cultured in complete RPMI-1640 medium and after that incubated with different concentrations of verapamil (0.001-1000µg/ml) in the presence or absence of PHA (10 µg/ml) for24, 48 and 72 hours. The MMP-9 activity in cell-conditioned media was then evaluated by gelatin zymography. Statistical comparisons between groups were made by analysis of variance (ANOVA). **Results:** Verapamil significantly decreased the MMP-9 activity in U937 and THP-1 cells in a dose-dependent manner (1000, 100 and 10 µg/ml) compared with untreated control cells. **Conclusion**: Verapamil shows inhibitory effect on MMP-9 activity in monocytoid (U937 and

THP-1) cell lines. Thus it seems that the anti-inflammatory properties of verapamil may be in part due to its inhibitory effects on gelatinases activity. Verapamil with its inhibitory effects on gelatinases activity may be a useful MMP- inhibitor. MMP- inhibitors are used in some cancerous, inflammatory and autoimmune disorders.

Keywords: Verapamil, Gelatinase Activity, U937,THP-1

1696P

Effect of satureja khozestanica essential oil (SKEO) on cox2 gene expression in lipopolysaccharide-stimulated J774A.1 macrophage cell line

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Background: Satureja khozestanica is an indigenous plant of Iran which grows mainly in south –west part of the country. This medicinal herb contains many compounds such as Carvacrol which is a monoterpene. Previous studies have shown anti-inflammatory effect of Satureja khozestanica,but the mechanism of this effect have not yet been elucidated completely. Since prostaglandins are one of the major mediators of inflammation and Cox2 gene is involved in their synthesis, we decided to study the effects of SKEO and Carvacrol on Cox2 gene expression in macrophage cell line J774A.1.**Methods**: SKEO was prepared from fresh aerial parts of the plant. The cell line was treated by different doses (0.004%, 0.008%, and 0.016%) of SKEO and Carvacrol for 8 hours. The total RNA content of the cell line was extracted and Cox2 gene expression was measured by RT-PCR technique. **Results**: SKEO reduced Cox2 gene expression in a dose and time dependent manner in Lps-stimulated cell line, and its effect was more powerful than Carvacrol. **Conclusion**: SKEO could reduce Cox2 gene expression, thus its anti-inflammatory effect could be due to the inhibition of this pro-inflammatory gene.

Keyword: Satureja khozestanica, Carvacrol, J774A.1-Macrophage cell line, Inflammation, Cox2 gene.

1828P

Effectiveness of massage therapy in improving dyspnoea in children with allergic asthma: a randomized clinical trial

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Background: Patients with asthma suffer from dyspnoea in their daily life and this may be by anxiety. massage may promote relaxation and relieve dyspnoea. Thus, it is appropriate to explore the effectiveness of massage on dyspnoea in patients with asthma. **Methods:** This

study was a randomized-controlled trial, that was performed in the Immunology And Allergy Clinics of Tabriz University of Medical Sciences, Iran. 60 children with allergic asthma of 6-12 year-old were invited to participate in this study; only 58 of them agreed. They were divided into two groups at random. Subjects in the massage therapy group received a 30 minute acupressure and massage therapy by their parents at home before bedtime every night for 4 weeks in addition to the standard asthma treatment. The control group received the standard asthma treatment alone for 4 weeks. both groups were given a diary notes to monitor symptoms. **Results:** The results of this study showed that dyspnoea Scores massage group improved significantly compared with control group, and the rate of dyspnea in the experimental group than the control group declined by about 45 percent. **Conclusions**: The findings suggest that massage can be used as a nursing education for parents to improve dyspnoea in children with asthma.

Keywords: Massage, Dyspnoea, Asthma, Children.

1781P

Acutalchohol consumption induced brain mast cells degranulation and Csf histamine and tryptase in rat: New approuch to alchoholism patogenesis

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Background: Alchoholism is a common behavioral disorder characterized by addictive consumption of ethanol, the development of tolerance and dependence and finally ethanol withdrawal sign such as seizure and anxiety. Despite excessive investigation the main pathological couse of seizure following alchohol deprivation is not clear yet. Mast cells are the main source of histamine and tryptase and ethanol is a powerful mast cell degranulator. On the other hand histamine is a well-known anti-convulsive transmitter.We hypothesized that deprivation of brain histamine due to ethanol consumption could be a main couse of mental and behavioral sign following ethanol withdrawal. **Methods:** In this experimental study 54 male-Wistar rats were used. Animals devided in two main group: Alchoholic and non-Alchoholic Each main group had 3 subgroups witch treated with Internacerebral ventricular(ICV): saline(5 micL) as a sham group, mast cell stabilizer(sodium cromolyn 20micg/micL) or compound 48/80(10micg/micL). In alchoholic groups animals received ethanol(4g/kg) 7days(3 times per day). After 48 hours ethanol deprivation csf and serum samples collected under anesthesia (ketamine/xylazine 10 and 100 mg/kg). Serum and csf amount of histamine and tryptase measured by Eliza kits. **Results:** Our finding shows that acute ethanol consumption couses significant csfhistamine(alchoholic and non-alchoholic,$p<0.05$) and tryptase(alchoholic and non-alchoholic,$p<0.01$).but serum tryptase not significant changes. bothcsf serum histamine and tryptase amounts elevated by 48/80 and attenuated by sodium cromolyn treatments in alchoholic and non alchoholic animals. **Conclusion:** Our data show significant csf histamine and tryptase elevation and mast cell degranulation following acute ethanol consumption. This investigation present brain histamine deprivation due to mast cell degranulation as a new approach for alchoholism mental and behavioral pathogenesis.

Keywords: brain mast cell, alchoholism, histamine, tryptase

1780P

Evaluation of effects of sodium cromolynon the brain mast cells: role of mast cells on function of immune system

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Background: Functional directions of mast cell-neuronal interactions remain poorly understood. Mast cell activationand degranulation can result in the release of powerful inflammatory mediators such as histamine andcytokines. The histaminergic system stimulate immune activation, so that this modulation is done thorough a possible mechanism of ascending medullary immunesensitive projections. The effect of cromolyn sodium on hyperresponsiveness and immune system may due to its ability to stabilize mast cells. The aim of this study is evaluation ofthe effects of microinjection of cromolyn sodiumon immune system in rats. Westudied possible mechanism for these effects too. **Methods:** Subjects in this experiment were male Wistar rats. In one group of rats, microinjection of sodium cromolyn, a mast cell stabilizer (20mg/ml, 5microlitr) and in other group microinjection of saline (5microlitr) were performed in Forth ventricle. After preparating and coloring slices of hypothalamic area of brain rats, granulated and degranulated mast cells were accounted. **Results:** This study showed that there is a significant reduce of mast cell degranulating in the tissue of brains in sodium cromolyn group compared to the saline group($P < 0.05$).**Conclusion:** These findings suggesstes that sodium cromolyn is an effective drug in stabilizing of mast cells and may contribute to function of immune system.

Keywords: Sodium cromolyn ,Mast cells,Degranulate, Immune system

1821P

Comparison of effects of ethanol extract of commiphora myrrha gum on inhibition of proliferation ofthe human AGS cell line and peripheral blood mononuclear cells (MNCs) and mesenchymal stem cells (MSCs) in vitro

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Background: Gastric cancer is one of the major causes of death throughout the world. Gum of Commiphora myrrha, as a medicinal plant, is reported to have anticancer effects. **Methods:** The ethanol extract of gum of Commiphora myrrha was prepared using 85% ethanol hydroalcoholic solvent. Effects of the extract on theHuman gastric carcinoma AGS cells were assessed using staining with the trypan blue method and MTT assay. Furthermore, effects of the extract on peripheral blood mononuclear cells (MNCs) and mesenchymal stem cells (MSCs) as normal cells were evaluated. **Results:** After 24 h of treatment with the extract; AGS, MNCs, and MSCs were killed at the extract concentrations of 100, 200, and 1000 µg/ml, respectively. Cell viability of AGS cells treatment with the extract at the concentration 100 µg/ml after 24h was 28.02%. **Conclusion:** Gum of Commiphora myrrha showed significant anticancer effects in vitro. Therefore, it can possibly be considered as an appropriate candidate for treatment of gastric cancer.

Keywords: Commiphora myrrha, Anticancer; AGS, MNCs, MSCs.

2345P

The effect of herbal preparation on mononuclear cells of normal and multiple myeloma bone marrow

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Background: SIM5 is an herbal preparation with toxic effect on cancerous cell lines and the potency to activate normal lymphocytes simultaneously. In this study the effect of SIM5 was studied directly on mononuclear cells from bone marrow samples of normal and multiple myeloma individuals. **Methods:** Samples of bone marrow aspirates were obtained from 3 individuals who were diagnosed as multiple myeloma (MM) and 6 individuals pathologically considered normal and mononuclear cells were isolated. The mononuclear cells were cultured with or without SIM5 (0.4 mg/ml final concentration). After48 hincubation, MTT test was performed and toxicity or activating effect was calculated. **Result**: In culture of MM samples of bone marrow mononuclear cells including cancerous cells, SIM5 causes a significant decrease in MTT test (28% and $p<0.04$), but in a completely different manner in bone marrow samples of non-cancerous individuals, an activating effect is observed (about 17% increase in MTT test which is statistically significant with $p<0.000$). **Conclusion:** The results not only confirms previous studies e.i. SIM5 has toxic effect on cancerous but activating effect on normal lymphocytes but also intensify its discriminative potency using human bone marrow samples of patients and controls.

Keywords: SIM5, Herbal, Anti-cancer, Multiple myeloma, Bone marrow.

2059P

Carnosolof immunomodulatory effect on the humoral and cellular immune system in mice balb/ c

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Background: Carnosol a diterpene compound isolated from the herb rosemaryand has been reportedvarious biological effects. This study investigated the immunomodulatory activity of carnosolin a mouse model in with compared cyclophosphamide immunosuppressive drug. **Methods:** Delayed hypersensitivity responses in mice was induced with 1×108 sRBCs injected subcutaneously in the back on first day and were treated for 5 days with carnosol. On day 5 of sensitization, the sensitized animals were challenged with 1×108 sRBCs injected subcutaneously on the left hind foot pad and PBS on the right hind foot pad. The increase in the foot pad thickness was measured 24,48and 72 h after the antigen was challenged by vernier caliper. In the test haemagglutination (HA) assay mice were sensitized intraperitoneally with 1×108 sRBCs on the first day andafter 5 days intraperitoneal administering of carnosol, blood samples were drawn and serum was collected. Serial dilutions of serums were made and antigen (1×108 sRBC) was added. After were assessed and last dilution of the hemagglutination titer

was reported .**Results:**Delayed type hypersensitivity responses were expressed as the mean precentage increase in the foot pad thickness after 24, 48 and 72 and were calculated according to the following formula. Mice sensitized with different concentrations of carnosol and cyclophosphamide reduced foot pad challenge compared to negative control. HA test results as mean antibody titers were reported in both treatment groups. Carnosol showed a significant reducein specific antibody production compared to cyclophosphamide group(positive control). **Conclusions**: Carnosol have significant inhibitory effects on humoral immunity and cellular immunity in treated mice. This effect is comparable to the inhibitory effect of cyclophosphamide as a standard drug.

Keywords: Carnosol, Humoral immunity , Cellular immunity, Immunomodulatory

2257P

Comparative study of Thymus vulgaris and Matricaria Chamomilla extracts with Myrthuscommunis preparation in the treatment of recurrent aphthousstomatitis

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Background: Recurrent aphthous stomatitis (RAS) is a prevalent and complicated disorder and its management is directed toward treatment of symptoms. The purpose of this study was to compare the efficacy of three herbal preparations in the management of RAS. **Methods:** One hundred and one out of 115 patients with minor aphthae were selected and randomly divided into four groups. Groups A, B and C received topical preparations of Thymus vulgaris, MatricariaChamomillaand a 50 % (v/v) mixture of Thymus vulgaris and MatricariaChamomilla respectively. Groups D (positive control) received Myrthuscommunis, **Results:** which reported to be efficient in the management of RAS. The time of pain elimination and the duration of the thorough healing were recorded. Mean time of pain elimination showed significant differences ($p<0.01$) between groups A (3. 00±1.14 day), C (3.08±1.84 days) and D (4.30±2.12 days) with group B (5.20±3.11 days). The mean duration of healing also showed significant differences ($p<0.03$) between groups A (6.00±2.80 days), C (6.70±2.70 days) and D (7.60±3.10 days) with B (8.70±3.90 days). No significant differences were observed between groups A and C with group D (positive control). The result obtained for treatment with T. vulgaris(group A) was similar to that of group C, but better than M. communis (group D). **Conclusion**: Findings of this study revealed that T. vulgaris extract showed better effects than M. communis which is reported to be effective in the treatment of RAS. It is concluded that the T. vulgaris extract is an effective product for the management of minor aphthae.

Keywords: Thymus vulgaris, MatricariaChamomilla, Myrthuscommunis, aphthous stomatitis

2563P

Conjugation of genistein and Bowman-Birk trypsin inhibitor (BBI) and study of anti-inflammatory effect of the conjugate on BALB/c mice

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Background: Genistein, has been shown to have many biological activities, such as anti-cancer, anti-oxidant and anti-inflammatory actions. It has become a popular candidate for drug development because of these features. The aim of this study was to investigate if genistein conjugate to bowman-birk protease inhibitor (BBI) may its anti-inflammatory effect be increased. **Methods:** After purification of BBI protein from soybeans, the BBI-genistein conjugate was synthesized according to a standard protocol. The acute inflammation was induced in BALB/cmice after intra-peritoneal (ip) injection of lipopolysaccharide (LPS) after treatment of the animals with conjugate. Finally, the expression of cytokines TNF-α and IFN-γ was determined by real-time PCR. **Results:** Our results showed that the mRNA levels of both TNF-α and IFN-γ genes in treated mice were significantly decreased by genistein-BBI conjugate and also genistein, compared to the control mice. Besides, the anti-inflammatory effect of BBI-genistein conjugate was more than the genistein significantly. **Conclusion:** The present observations suggest that the synthesis of a BBI-genistein conjugate can increase the effectiveness of anti-inflammatory of genistein. Clarifying the structural changes and its effect on the anti-inflammatory mechanism of the conjugate would be a good idea for future research in conjugation of flavonoids and protease inhibitors in order to augment their effectiveness.

Keywords: Conjugation, Genistein, Acute inflammation, Immunomodulator agent

2330P

Topical in vivo inhibitoryeffect ofMenthapiperitaessential oil against candida albicansin infected rat

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Background: Wound infections caused by Candida albicans has grown substantially in recent years. Development of drug resistanceled to the use of biological materials to be considered as an alternative solution. Studies show that Menthapiperita contains anti-bacterial substances (flavonoids and menthol) and hence it can reduce the inflammation period. **Methods:** For this goal we used Forty-five male Wistar rats (weight 195-205 g). One square surgical wound with dimensions of 1/5×1/5 cm were performed on the back of each animal and immediately became infected whit 0.1 ml of $1/5\times10^7$ CFU Candida albicans suspension. Then the rats were divided into 3groups (control, 1.5% and 3%) each with 15 rats and randomly distributed into 5 subgroups each whit 3 rats (sample groups on different days).Wound healing activity was performed by histological studies and yeast counts on the end of 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th}days after surgery. **Results**: Neither therapeutic dose of Menthapiperita oil, has promoted significantly reduced the Candida albicans cloning in tissue yeast counts, in treatment groups

compared to control group ($P < 0.001$).Reducing the number of PMN (as main immune cells in inflammation phases of wound healing process), increasing the MNC, fibroblast cell migration and re-epithelialization in both treatment groups compared with the control group were better. **Conclusion:** With these effects and we concluded that Menthapiperita essential oil especially in 1.5% and 3% doses could be useful for healing the wounds and Reducing Inflammation.
Keywords: Menthapiperita, candida albicans, Infected wound, Rat.

2309P

Immunotoxicity effect of carbaryl: in vivo study of Th1/Th2 balance in male rats

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Background: Carbaryl is one of the most potent pesticides for controlling pests and plant diseases in agricultural use. Carbaryl can cause different toxic effects in humans through skin contact, inhalation and ingestion. Objective: This study investigated the Th1/Th2 cytokine balance change in response to carbaryl in male rats. **Methods:** In the present study, rats were randomly divided into four groups. Experimental groups were intraperitoneally injected with 10 and 30 mg/kg carbaryl, while the control groups were injected with the almond oil solvent for 28 days and sterile normal saline. One week after the last injection, blood samples were collected and IL-1β, TNF-α, IFN-γ, IL-4, and IL-10 levels were measured using ELISA method after serum separation. **Results:** The cytokine assays showed a significant increase in IL-4 and IL-10 levels in a group injected with 30 mg/kg carbaryl in comparison with control. Although, there was a decrement in IL-1β and TNF-α level in both experimental and control groups, but the decrease was not significant. Moreover, IFN-γ level revealed a significant reduction in the group injected with 30 mg/kg carbaryl as compared with control. Discussion and **Conclusion:** This study indicated that carbaryl can lead to increment in IL-4 and IL-10 levels, which is indicative of Th2 cells. In addition, this toxin could reduce IFN-γ level and suppress the Th1 deviation. The results of this study suggested that carbaryl can contribute to the development of allergic and autoimmune diseases through disturbing the balance of Th1/Th2 immune response, and potentiating the Th2 activity.
Keywords: Carbaryl, Th1/Th2 cytokines, Male rat, Immunotoxic

2319P

Immunomodulatory effects of(Citrulluscolocynthis L. Schrad)in NMRI mice immunized by SRBC

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Background: The present study was carried out to investigate the effects of the hydroalcoholic extract of Citrulluscolocynthis L. Schrad on humoral and cellular immunity in mice after challenge with sheep red blood cells (SRBC). **Methods**: In this study, 14 male mice were used. Groups immunized with SRBC. In treatment group, Mice were received 200mg/kg extract of Citrulluscolocynthis L. Schrad by oral gavage daily for 2 weeks. **Results**: The results of Investigation indicated a significant increase in the level of anti-SRBC antibody and simultaneously a significant decrease in the level of DTH in treatment group compared to control-sham group. Moreover, the level of respiratory burst of macrophages was significantly decreased in the splenocytes of treatment groups, while the level of lymphocyte proliferation and spleen weight was significantly increased in treatment group compared to control-sham group. **Conclusion**: Citrullus colocynthis may be used as a source to intervene in immunity system.

Keywords: Immunomodulatory effects, Mice immunized, SRBC

2423P

Topical application of Thymus essential oil accelerate wound healing with increase of mono nuclear immune cells in wound site (experimental in vivo study)

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Background: Candida albicans is an opportunistic pathogen and it can transform to invade form in appropriate conditions. Thymus essential oil contains as thymol and carvacrol that have been found to antibacterial activity. The aim of the present work was to investigate the in vivo antifungal activity of topical effect of Thymus Essential oil on infected skin wound with candida albicans on rats. **Methods:** In this study on 45 male Wistar-albino rats (weight 210±10 g). After general anesthesia, and a wound square with dimensions 1/5 in the 1/5 cm area between the shoulders, immediately was applied to the wound 0.1 ml of the suspension containing $1/5\times10^7$ CFU Candida albicans. Then tested in three groups of 15 rats each (control, ointment 1.5% and 3%Thymus Essential oil) were randomly distributed into 5 subgroups of 3 rats each (sample groups on different days) groups. During the project was obtained, the end of days 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} from wounds of different groups, in order to histopathology and yeast counts by a special punch biopsy specimen. **Results**: In the skin excisional wound animal model, Thymus oil at clinical relevant neither dose promoted infection wound healing with significantly reduce the number of poly morph nuclear cells, increase the mono nuclear cells; and also increase fibroblast cells ($p < 0.01$).**Conclusion:** Be considered this herbal formula, accelerate infection wound healing with candida albicans and better choice to use a topical ointment containing 3%Thymus Essential oil.

Keywords: Candida albicans, Thymus, infected wound, essential oil, rat.

2650P

Anti-inflammatory effect of pramipexole, as a dopamine agonist

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Background: Pramipexole is an aminothiazole dopamine agonist with selective actions at dopamine receptors belonging to the D_2 subfamily, where it possesses full activity similar to dopamine itself. Pramipexole currently used for treatment of Parkinson's disease. Pramipexole's preferential affinity for the D_3 receptor subtype could contribute to efficacy in the treatment of both the motor and psychiatric symptoms of Parkinson's disease. Both in vitro and in vivo studies in animals suggest that pramipexole possesses numerous neuroprotective properties, including dopamine autoreceptor agonist properties, antioxidant properties, ability to block the mitochondrial permeability transition pore and the ability to stimulate the release of trophic factors. In this study, anti-inflammatory activity of the Pramipexole was investigated.**Methods:** Intraperitoneal administration of the Pramipexole (0.5 and 1 mg kg^{-1}) was evaluated on two well-characterized animal models of inflammation, including carrageenan- and formalin-induced paw edema in rats. **Results:** Pramipexole (0.5 and 1 mg kg^{-1}) significantly inhibited carrageenan-induced paw edema 4 h after carrageenan challenge ($p < 0.001$). Pramipexole (0.5 and 1 mg kg^{-1}) also showed considerable anti-inflammatory activity against formalin-evoked paw edema over a period of 24 h ($p < 0.001$).**Conclusion:** Our findings demonstrated potent anti-inflammatory effect of Pramipexole which may be due to its similarity to dopamine and antioxidant property.

Keywords: Anti-inflammatory; Pramipexole; Formalin; Carrageenan;

2919P

The anti-oxidant activity of ginger correlates with its therapeutic effects on EAE

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Back ground: An association is reported between the development of multiple sclerosis (MS) and oxidative stress. Ginger has been observed to have anti- oxidant effects in vivo and in vitro, but there is no data regarding the effects of ginger on multiple sclerosis (MS) or EAE, as the animal model of MS. This study was done to explore the anti- inflammatory and anti-oxidant effects of ginger and its correlation with EAE clinical symptoms and degree of cell infiltration to CNS tissue. **Methods:** EAE was induced in female C57BL/6 mice on day 0 with injection of MOG peptide mixed with complete Freund's adjuvant. The mice were intra peritoneally (I.P) administered with either vehicle (PBS) in control group or hydro alcoholic extract of ginger (200mg/kg BW, every other day) from day 3 to 40 in experimental group. The EAE clinical scores were evaluated till day 40. Total Antioxidant Capacity (TAC) was assessed by Ferric Reducing-Antioxidant Power (FRAP) method. For extent of cellular infiltration analysis, mice brain was removed and thin sections were stained with hematoxylin-eosin and analyzed by

light microscopy. **Results:** The hydro alcholic extract of ginger significantly increased the total antioxidant capacity of the serum and at the same time delayed the onset, reduced the peak clinical score and cumulative disease index of EAE and prevented or significantly attenuated the relapses in experimental group compared with controls. Pathological study of CNS tissue of the mice in experimental group also showed less cell infiltration compared to controls. Our results showed a correlation between the TAC of the serum and the extent of cell infiltration as well as the mean clinical score of the EAE. **Conclusion:** According to our results ginger showed a significant anti-oxidant and anti-inflammatory activity in EAE suffering mice and its anti-oxidant activity correlated with its capacity to prevent the development and progression of EAE. Considering the long term use of ginger in different nations and its proven safety, its regular use is recommended and may be beneficial for prophylaxis and dampening the progress of MS.

Keywords: Experimental autoimmune encephalomyelitis, Multiple sclerosis, Ginger, Anti-oxidant activity

2945P

Using Echinacea powder for improvement the immune response in the birds

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Background: Echinacea is an herb. Several species of the echinacea plant are used to make medicine from its leaves, flower, and root. Echinacea is widely used to fight infections, especially the common cold , upper respiratory infections including the flu and also urinary tract infections, vaginal yeast infections, genital herpes, bloodstream infections (septicemia), gum disease, tonsillitis, streptococcus infections, syphilis, typhoid. **Methods:** In current study its immune stimulation is studied in some broiler chickens, so three pens included 72(3X24) chickens were selected and breeded for 42 days, all of the conditions were the same just in the first group 2 days before and 2 days after the oral vaccination , about 10g/kg Echinacea powder were added to diet ,in the second group 20g/kg Echinacea powder were added to diet and in the last group no any Echinacea powder were added to diet, the surveyed vaccine were 3 times oral New castle disease vaccine at 11, 19 and 28 days of old, and blood sampling for HI test were happened 1 week after each vaccination. **Results and Conclusion:** Regarding to the results the mean HI titers for ND, in 3 groups were different in which second group were most of the third and first group.

Keywords: Echinacea, Immunity, Broiler, Stimulation

3272P

The effects of alcoholic extract of Ferula Gabrielii on the proliferation of peripheral bloodmononuclear cells (PBMCs) and IL -4 secretion

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Background: Plants have been used traditionally in medicine in Iran. Ferula Gabrielii is an endemic plant of yazd that has been used for treatment of GI tract disorders. In order to

determine some of its immunological effects the present study was performed by the following procedure. **Methods:** Alcoholic extract was made using maceration method. Then it's different concentrations were added to PBMCs culture media and incubated for 48 hours at 37°C and 5% CO_2. After incubation their supernatants were extracted and their IL-4 were measured using ELISA method. **Results:** The results showed a significant increase of PBMCs proliferation and decrease of IL-4 secretion at 100µg/ml of Felura Gabrrelii extract. **Conclusion:** It can be concluded that Ferula Gabrielii extract may have immunomodulatory effects. To determine its mechanisms further studies required.

Keywords: Ferula Gabrielii, PBMCs, IL-4

3219P

Honey stimulates peritoneal macrophages in mice infected with Invasive Aspergillosis

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Background: Infectious diseases caused by opportunistic fungi have increased in recent years. Invasive aspergillosis (IA) is the most severe disease caused by the opportunistic fungus Aspergillus. It is the main reason of fungal related mortalities in high risk patients. The increase in the number of IA in non-neutropenic immunocompromised patients highlights the importance of non-neutrophil defense-related factors. Macrophages are the first line of defense against IA. In this study the effects of three Iranian honeys on macrophage activity and survival rate during invasive aspergillosis was investigated. **Methods:** Mice were divided into 10 groups (honey alone, honey and infection, negative and positive controls) each containing 10 individuals. Mice were treated with three different types of honey (Thyme, Pennyroyal, Astragalus and Mixed) (1.5g/kg BW/orally) for 10 days. At day 6,Aspergillus fumigatus conidia (5×10^5/ml) were injected intravenously to the infected groups. Mice were euthanized at day 11, peritoneal cell culture was performed. Macrophage killing and nitric oxide production was assayed. For survival rate, 10 mice from each infected group were considered and monitored for 30 days. **Results:** The results showed that honey treatment could significantly increase macrophage killing ($p<0.05$). Our results showed that LPS had no significant effect on NO production by peritoneal macrophages ($p>0.05$). Mice treated with Thyme and Pennyroyal honey had significantly lower NO concentrations than Astragalus and mixed honey groups ($p<0.05$). More over mice treated with honey had a greater survival time than infected groups. **Conclusion:** Our results suggest that honey could boost the immune system by activating macrophages and increase mice resistance to IA.

Keywords: Honey, Aspergillus fumigatus, Invasive aspergillosis, peritoneal macrophage, killing

2893P

The Effect of shallot (Allium hirtifolium) extract on the profile of Th1/Th2 cytokine in BALB/c mouse sphenic lymphocytes

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Background: Modulation of cytokine secretion offers a novel approaches for treatment of a variety of diseases. A class of herbal medicines, known as immunomodulators, alters the activity of immune function through the dynamic regulation of cytokines. **Methods:** In this study, we evaluated the immunomodulatory activity of hydro-alcoholic extract of shallot (A. hirtifolium) on the immune respons in mice. Extract of A. hirtifolium bulbs were prepared to study the effects A.hirtifulium on groups of Balb/C mice were used. Two dose of extract (80 mg/kg ,400 mg/kg) injected intrapritonealy within 10 day. The After Il-4 , IFN-ɣ and TNF-α levels assessed by Enzyme-linked Immunosorbent assay (ELISA) kit. **Results:** In vivo result showed that hydroalcholic extract of A. hirtifolium significantly reduced in Th2 and Th2 response by reduce in Il-4 and IFN-ɣ levels. And also inflammatory response by reduce in TNF-α levels. **Conclusion:** A. hirtifolium are safe and rich source of biologically active compounds with low toxicity. This data the protective potential of extract shallot against autoimmune and inflammatory disease.

Keyword: Th1, Th2, Allium hirtifollium, Immunomodulatory, Cytokine

2307P

Investigation of serum levels of INF-γ as Th1 cytokine in diabetes type I patients

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Background: Type 1 diabetes (T1D) is a polygenic autoimmune disease characterized by the destruction of insulin-secreting pancreatic β cells. Cytokines may contribute to pancreatic β cell death. IFN-γ is a Th1-type cytokine that plays an intricate role in antiviral host defense mechanisms, activation of CTLs, and enhancement of inflammatory reactions. It is involved in the up regulation of MHC on β cells and APCs in the islets. It seems in the absence of MHC over expression in β cells, T1D does not occur. Thus, in regards to probable role IFN-γ in pathogenesis of T1D, its expression in peripheral blood of patients with diabetes type I was examined. **Methods:** From spring of 2010 to the fall of 2011, peripheral blood was collected from patients with diabetic case (44 patients) and normal control volunteer (44 healthy people) as control from hospitals of Jahrom University of Medical Sciences. Then serums were isolated and assessed for IFN-γ using ELISA technique (ebioscience) and HbA1c was evaluated by cation-exchange high pressure liquid chromatography. Finally results were statistically analyzed by SPSS. **Results:** As a result, patients had high blood sugar (>120 mg/dl) ($P<0.0001$) and also increased HbA1c was found than control group ($P=0.02$). However, cytokine analysis revealed that IFN-γ serum level in patients has not significant difference than control group ($P=0.27$). Probably with increased cases, it may be significant. **Conclusion:** Depending on

stage on disease, Th1-related cytokines such as IFN-γ- as inflammatory cytokine can be used as diagnostic or following markers in autoimmune disease including diabetes type I.
Keywords: Diabetes type I, IFN-γ, T helper 1

2778P

Erythropoietin restrains immunological cascade after carbon monoxide poisoning

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Background: Erythropoietin (EPO) has a critical role in the development of nervous system. In this study, the effect of EPO in the treatment of carbon monoxide (CO) neurotoxicity and the possible mechanisms were examined. Soluble CO in the plasma causes a cascade of events which leads to lymphocytic immunologic response, microglia activity, and finally neurologic defects. **Methods**: Rats were exposed to 3000 ppm CO in air for 1 hour until they lost consciousness, and then different doses (2500, 5000 and 10000 u/kg) of EPO were administrated intraperitoneally. After 24 hours, glial fibrillary acidic protein (GFAP) levels in the serum were determined by ELISA. The effect of EPO on brain lipid peroxidation was determined by measuring malondialydehyde (MDA) using a colorimetric method. Also we evaluated the myeloperoxidase (MPO) activity in the brain tissue. We carried out western blot method for determination of MBP, BAX /BcL2 relative expression. Cation exchange chromatography was used to evaluate the MBP alteration in the brain tissue. **Results**: EPO reduced the MDA level at doses (2500, 5000 and 10000 u/kg) ($p < .05$ as compared to CO poisoned animals). Lower doses of EPO (625, 1250, 2500 u/kg) remarkably decreased the elevated serum levels of GFAP ($p < .001$). However, EPO could not reduce the water content of the edematous poisoned brains but at 5000 and 10000 u/kg it protected the blood brain barrier from the disruption as a result of CO poisoning by 30.2% ($p < .05$) and 39.2% ($p < .01$) percent, respectively. EPO could decrease the MPO activity after carbon monoxide poisoning significantly and in a dose dependent manner. In this model, CO-mediated oxidative stress causes chemical alteration in myelin basic protein (MBP), which initiates an adaptive immunological response. We found that EPO given after CO poisoning prevented this deficit, but did not eliminate all of the CO-mediated immunological alterations in MBP. MBP in EPO treated CO-exposed rats are recognized normally by a battery of antibodies, and exhibit a nearly normal charge pattern. After treating the CO exposed rats with EPO (5000u/kg), MBP density was significantly restored ($p < .001$). BAX/Bcl-2 ratio assessment showed a 38.78%

increase in CO exposed rats ($p < .05$) and 38.86%reduction by EPO treatment after carbon monoxide poisoning ($p < .01$). **Conclusion:** EPO has the potential to prevent CO poisoning immunological cascade.

Keywords: Carbon monoxide poisoning, Erythropoietin, Myelin Basic Protein, Immunological cascade

2746P

Glycyrrhetinicacidinhibitscellgrowthandinduceapoptosisinovariancancercelllinea2780

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Background: Ovarian cancer is one of the most common cancers among women. It is usually treated by a combination of cisplatin, carboplatin and paclitaxel drugs. Nowadays, plant extracts are used in research and many clinical trials. Recent studies have shown that Glycyrrhetinic acid (GA), which is one of the derivative compounds of the licorice plant, induces apoptosis and death of many cancer cells. However, its effect on ovarian cancer cells has not been yet studied. **Methods:** The cytotoxic effect of GA on A2780 cells as a cell line of ovarian cancer was measured by dye exclusion assay and XXT in vitro. The amount of Fas and Fas ligand (FasL) as well apoptosis was measured by flow cytometry. **Results:** Our data demonstrated that GA reduced the cells growth of A2780 cells in a dose –dependent manner. Moreover, we observed that GA induces apoptosis in A2780 cells as measured by annexin V and PI. Consistently, treatment of the cells with GA resulted in upregulation of apoptosis-related molecules such as Fas and FasL in dose-dependent manner. **Conclusion:** According to the results, GA reduced cell growth and induced Apoptosis in A2780 cell line.

Keywords: Glycyrrhetinic acid, Apoptosis

2800P

Olive leaf extract has potent antioxidant property with little effect on the levels of Th17 related cytokines

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Background: Olive has a protective effect against chronic inflammatory conditions. However, it is not clear weather this effect is due to its immunomodulatory or antioxidant property. The aim of this study was to investigate the effect of Olive leaf extract on serum levels of Th-17 related cytokines and its antioxidant properties. **Methods:** Forty male rats divided into 5 groups, and were treated by placebo (Control group), vitamin C (as a known & potent antioxidant) and different doses of Olive leaf extract. Four test groups, received vitamin C 10mg/kg and olive leaf extract which contained 5, 10 and 15 mg/kg Oleuropein. All treatments

were applied for 10 consecutive days orally via gavage. After this period, cardiac puncture was performed to retrieve blood from animals in order to determine interleukin 17, 23 and TGFβ levels in their serum by ELISA method. Glutathione peroxidase (GPX), Superoxide dismutase (SOD), Catalase (CAT) activities and thiobarbituric acid reactive substances (TBARS, as a lipid peroxidation marker) were assayed in right brain hemisphere of treated animals. **Results:** TBARS increased significantly in control group when compared to the other groups ($p<0.05$). GPX and SOD enzymes indicated higher activity in the animal group which was treated with 15mg/kg Oleuropein, in comparison with control group and a group who treated with 5mg/kg Oleuropein ($p<0.05$). Although there were no significant difference in IL-23 and IL-17 levels among control and test groups ($p>0.05$), TGFβ concentration was significantly lower in animals which treated by 5 and 15 mg/kg of Oleuropein. **Conclusion:** Olive leaf extract, which contains Oleuropein, had a significant antioxidant effect brain of studied animals, while it was not able to change the Th-17 cell-related cytokines significantly. Therefore, it could concluded that the protective role of Olive against chronic degenerative diseases is related to its antioxidant property rather than its effects on pathogenic cytokine profile of Th17 cells.
Keywords: Olive, Oleuropein, Th17, Antioxidant

3045P

Therapeutic potential of Citrus limon peel extract in carrageenan induced inflammation in rat

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Background: Medicinal plants and herbs have been used for many centuries for the treatment or prevention of diseases and for the promotion of good health. Plant extracts as well as their primary and secondary metabolites have served as antioxidants in phytotherapeutic medicines to protact against various diseases for centuries. Therefore, the aim of the present study was to investigate the antioxidant and anti-inflammatory potential of Citrus limon peel extract in rats. **Methods:** The antioxidant activity of Citrus limon peel extract was assessed using DPPH and hydrogen peroxide methods. Anti-inflammatory activity was assessed by measuring paw volume in rats. **Results:** In DPPH and hydrogen peroxide assay, Citrus limon peel extract showed maximum activity in dose dependent manner as compared to ascorbic acid. Further anti-inflammatory activities of Citrus limon peel extract (200, 300 and 400mg/kg) were studied on carrageeanan induced inflammation in rats. Extract (400mg/kg) produced significant decrease in paw volume as compared to diclofenac. **Conclusion:** The results of present study showed that Citrus limon peel extract may be used as a future antioxidant for the treatment of inflammation.
Keywords: Extract, Citrus limon, Antioxidant, Inflammation, Rat.

2837P

The Evaluation of antioxidant capacity of Citrus limon essential oil and its effect on serum lipid profiles in dairy Moghani sheep

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Background: The Essential Oil (EO) which is produced by different plants confirms antioxidant and anti- inflammatory activities which affects by different ways. Therefore, the aim of the present study was to investigate the antioxidant capacity of Citrus limon essential oil and its effect on serum lipid profiles in dairy Moghani sheep. **Methods:** The antioxidant capacity of EO was assessed using DPPH and ABTS methods. Six lactating dairy Moghani sheep with 45±5 lactation days, and body weight of 55±5 kg were allocated in a 3×3 Latin square (crossover) design with 3 dietary treatment including 0, 150 and 300 mg/kg Citrus limon EO per day. The experimental period was 21 days that 17d for adaptation, and 4d for sampling. **Results:** In DPPH assay, lipid peroxidation inhibitions were 6, 12, 22, 44 and 84% for concentrations of 31.25, 62.50, 125, 250 and 500 mg/ml, respectively. In ABTS assay, inhibition percentages of 59, 80, 85 and 90% were obtained for above mentioned concentrations, respectively. EO significantly decreased plasma VLDL and triglyceride concentration ($p<0.05$), but plasma cholesterol, HDL and LDL concentrations were not influenced by dietary EO. **Conclusion:** The results of present study showed that Citrus limon EO can act as natural antioxidants and have beneficial effects on the lipid profile in dairy Moghani sheep.

Keywords: Essential Oil, Citrus limon, Antioxidant, Serum Lipids, Moghani Sheep.

2828P

Ethylpyrovate ameliorate the testis in cyclophosphamide treated adult mice

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Background: Cyclophosphamide (CP), a cytotoxic alkylating agent, is extensively used as an antineoplastic agent for the treatment of various cancers, as well as an immunosuppressive agent. Side effects in CP treated men were decreased of fertility and even sterility, This study performed to evaluate the protective effects of Ethylpyrovate in oxidative stress induced by cyclophosphamide (CP) on testis. **Methods:** Three groups (6 mice in each) of adult mice were used. Control group treated with normal saline.,ip, and group 2 treated with CP 15 mg/kg/week.,ip, and group 3 treated with CP along with Ethylpyrovate 40 mg/kg/day.,ip. After 35 days samples were taken and fixed in 10% formal saline and paraffin sections were prepared and stained by TOLUIDEN BLUE method. The mast cell numbers were counted with latticed objective divice in $1mm^2$ field in region of each slide. All obtained data were analyzed by SPSS software in ANOVA and Duncan test. **Results:** Results showed that the mast cell number in CP treated group were significantly more than two other groups ($P<0.05$). **Conclusion:** This study showed that Ethylpyrovate ameliorate the oxidative stress effects of CP on male reproductive organ.

Keywords: Ethylpyrovate,Testis ,Cyclophosphamide, Mice.

2891P

Effect of aqueous extract of Gymnema sylvestre on macrophage killing activity against Candida dubliniensis, in vitro

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Background: Fungal infections are known as the fourth leading cause of nosocomial infections. Among candida spp. Candida albicans and Candida dubliniensis are known for their ability of germ-tube formation. One of the virulence factors is important in the yeast pathogenesis is their capability of transition from yeast to hypha form and germ-tube formation. This capability is characterized as one of the evasion mechanisms of yeasts from immune system response and survival of phagocytized yeasts. According to other studies the mechanism of action of Gymnemasylvestre could be based on its inhibition of transition of yeast to hypha form when the yeast is phagocytized. **Methods:** We planned 2study groups including; LPS-activated peritoneal macrophages and non-activated macrophages, each group divided in 3 sub-groups receiving10, 20 and 40 µg/ml concentrations of extract for 30 min at 37ºC, respectively.1 ml of 1×10^6 yeast/mL yeast suspension and 1 ml of pre-warmed RPMI 1640 medium containing 1×10^6macrophages of each group added to 24-well plates and incubated for 1h at 37ºC.Incubation at 37ºC was performed to simulate the host body temperature and germ-tubes formation. After staining of samples, the killing activity was assessed under direct microscopic examination. **Results:** All groups show increase in killing activity compared to control groups. Killing percentage was highest at 40 µg/ml concentration. The results were not significant at 10 µg/mlbut they show an increase in killing activity. **Conclusion:** Gymnemasylvestre could be used as potential complementary therapy in candida dubliniensis-related infections.

Keywords: Gymnemasylvestre, Macrophage, Killing, Candida dubliniensis

2885P

Evaluation of Isfahan inhabitant attitude about asthmaand common herbal remedies

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Background: Asthma is one of the most common chronic diseases in modern society and there is increasing evidence to suggest that its incidence and severity are increasing. There is a high prevalence of usage of complementary medicine for asthma in Iran. This study was undertaken to evaluate Isfahan inhabitant attitude about asthma and common herbal remedies that they use for treatment of asthma. **Method**: This evaluation was a random simple study which was carried out on a sample of 330 people in the city of Isfahan by means of questionnaires. Validity and reliability of the questionnaires Confirmed by expert and criterion Cronbach alpha level was0.89 . In this study, descriptive statistics (mean, standard deviation) and (χ^2 tests, Pearson correlation) were performed to determine whether there were statistically significant associations between People knowledge and the use of herbal medicine. The data was analyzed using SPSS for Windows (Version 21.0). **Result**: The study included 330 patients (48.4% of women and 51.6% male) with a mean age of 30.79.Method of accessing information on herbal and traditional medicinewere, radio (5.2%), books (14%), television (18.6%), Internet (35.4%), friends (10.8%), magazines and newspapers (16%). Respondents used methods was, herbal methods (38.4%), chemical methods (18.8%) and a combination

of both methods (42.8%).It was observed that the level of education and choice of therapy (p=.008), the level of familiarity with traditional medicine with the choice of therapy (P=.003) are related. **Conclusion**: According to the results, we can conclude that the use of various information portals lead to more familiarity with the symptoms of asthma and related risk factors, and thisissue encourages people to use herbs and traditional treatments.
Keywords: Herbal remedies, Asthma, Traditional medicine

3028P
Immunomodulatory effects of Cynodon dactylon in NMRI mice immunized by SRBC

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Background: The present study was carried out to investigate the effects of thehydroalcoholic extract of Cynodondactylon on humoral and cellular immunity in mice immunized with sheep red blood cells (SRBC). **Methods**: For this purpose, 14 male mice were used. The animals were immunized with SRBC. The treatment group received 200mg/kg of Cynodondactylon extract by gavage daily for 2weeks. **Results**: The results of this study indicated a significant decrease in the level of anti-SRBC antibody and a significant decrease in the level of DTH in treatment group compared to control-sham group. Moreover, the level of respiratory burst of macrophages was significantly decreased in the splenocytes of the treatment group, while the level of lymphocyte proliferation and spleen weight was significantly decreased in the treatment group compared to control-sham group. **Conclusion**: Cynodondactylon is a potential source for naturally occurring immunomodulatory compounds which might be useful in the development of drugs against auto-immune disorders due to its significant effects on the immune system. Further studies are required to precisely clarify the underlying mechanisms.
Keywords: Cynodondactylon, Immunomodulation, SRBC

3024P
Effect of Cynodon dactylon aquatic extract on angiogenesis in a carrageenan-induced inflammatory rat model

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Background: Cynodon dactylon is a traditional medicinal plant which has been widely used by traditional rural healers for the treatment of cardiovascular disease. Our aim in preparing this study was to evaluate the probable effects of this plant on angiogenesis in rats. **Methods**: For this purpose, 25 male rats were divided into 5 groups each in which 5 rats were placed. The first group was considered as the control group and in the second group inflammation was inducedusing and air pouch method. The three remaining groups orally received 100, 200 and 400 mg/kg of Cynodon dactylon aqueous extract, respectively. At the end of the experimental period the jugular vein of each animal was cannulated and 2 cc of carmine red was injected. Specimens of the granulation tissues and skin were collected and studied for angiogenesis.

Results: Angiogenesis was significantly enhanced in all treatment groups in comparison to the control group. The best effect was observed at a dosage of 400 mg/kg. **Conclusion**: The present study shows thatCynodon dactyloncontains potential angiogenic compounds which can be recruited for the development of novel therapeutic agents. Further studies are required to precisely clarify the underlying mechanisms and the main responsible compounds.
Keywords: Cynodon dactylon, Angiogenesis, Carrageenan

3032P

Immunological properties, anti-inflammatory and anti-oxidant effects of Alhagi species

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Background: Alhagi species are well known in Iran (locally known as KharShotor) and other parts of Asia as a popular folk medicine. Recent decades of research has shown extensive pharmacological effect of these species. **Methods:** This article reviews the pharmacological effects and traditional uses of Alhagi species and their active constituents with especial attention to the effective dosages and roots of administration. **Results and Conclusion:** The extract significantly reduced the thickness of paw edema induced by formalin in a dose -dependent manner. It has been shown that A. maurorum Medic is a more potent anti-inflammatory agent in comparison to diclofenac sodium (30 mg/kg), a conventional anti-inflammatory drug. Discrepancies were settled through discussion. This paper shows that Alhagi species are safe and rich sources of biologically active compounds with low toxicity. Further studies are required to confirm the safety and quality of these plants to be used by clinicians as therapeutic agents.
Keywords: anti-inflammatory, Alhagimaurorum, A. camelorum, A. persarum

2929P

Antiangiogenic and cytotoxic effects of aqueous and hydroalcoholic extract of Tribulus terrestris

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Background: Tribulus terrestris (TT) is a member of the Zygophyllaceae family. Traditional Chinese medicine recommends the use of TT for the treatment of avariety of diseases.Despite the fact that the mechanism of action of this plant as an anti-cancer drug is not known, but Eastern medicine also uses it to treat cancer. In the present study we further investigated the antiangiogenic and antimetastatic property of TT on Human umbilical vein endothelial cell(HUVEC) and Human colon carcinoma cell line ($Caco_2$). **Methods:** TT were collected, and after authentication, two different extracts including aqueous and hydroalcoholic were prepared. Anti-proliferative and cytotoxic activity of the extracts(10, 20, 40, 80, 160, 320, and 640µg/ml) was determined against HUVEC at collagen matrix and $Caco_2$ cell line by LDH

method. **Results:** TT extracts inhibited proliferation of $Caco_2$ at a dose dependet manner with an IC_{50} value of ~320µg/ml. Furthermore, the two extracts inhibited tubulogeis by HUVEC at collagen matrix. **Conclusion:** This study indicated that aqueous and hydroalcoholic extracts of TT acts as a potent antiangiogenic and anticancer agent which exert their inhibitory effect mainly through inhibition of proliferation in related cell lines.
Keywords: Tribulus terrestris, antimetastatic, antiangiogenic

3041P

Immunomodulatory effects of Artemisia dracunculus L. in NMRI mice immunized by SRBC

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Background: The present study was carried out to investigate the effects of the hydroalcoholic extract of Artemisia dracunculus on humoral and cellular immunity in mice immunized with sheep red blood cells (SRBC). **Methods**: For this purpose, 14 male mice were used. The animals were immunized with SRBC. The treatment group received 200 mg/kg of Artemisia dracunculus extract by gavage daily for 2weeks. **Results**: The results of this study indicated a significant decrease in the level of anti-SRBC antibody and a significant decrease in the level of DTH in treatment group compared to control-sham group. Moreover, the level of respiratory burst of macrophages was significantly decreased in the splenocytes of the treatment group, while the level of lymphocyte proliferation and spleen weight was significantly decreased in the treatment group compared to control-sham group. **Conclusion**: Artemisia dracunculus is a potential source for naturally occurring immunomodulatory compounds which might be useful in the development of drugs against auto-immune disorders due to its significant effects on the immune system. Further studies are required to precisely clarify the underlying mechanisms.
Keywords: Artemisia dracunculus, Immunomodulatory effect, SRBC

3108P

Influence of Ginger and Cinnamon intake on inflammation and muscle soreness endued by exercise in Iranian female athletes

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Background: Ginger rhizomes (rich in gingerols, shogaols and zingerone) have been used in Asia for the treatment of asthma, diabetes, and pain, and have shown potent antiinflammatory attributes. Common spices such as Cinnamon (including cinnamic aldehyde and cinnamyl aldehydeis) are used in food and many studies have focused on its antiinflammatory components. The efficacy of dietary ginger and cinnamon as antiinflammatory agents and their effectiveness

in reducing muscle soreness has been investigated in limited studies on humans. **Methods**: Sixty healthy, trained women, aged 13-25 years, were enrolled in the sixweek investigation and randomly categorized into three groups (cinnamon, ginger or placebo) and received 3 g of ginger, cinnamon or placebo powder each day, depending on the group they belonged to. The IL6 level and Likert Scale of Muscle Soreness were evaluated at the beginning and the end of the study and compared among the groups. **Results**: Fortynine of the participants completed the sixweeks intervention. There were no significant changes in the IL6 cinnamon and ginger group when compared with the placebo group, whereas, there was a significant fall in muscle soreness in the cinnamon group and placebo ($P < 0.1$) and ginger group and placebo ($P < 0.01$). **Conclusions**: Administration of ginger and cinnamon in athlete women for six weeks did not show any significant change in the IL6 level, but showed a decrease in muscle soreness in the cinnamon and ginger groups.
Keywords: Athletes, cinnamon, ginger, inflammation, muscle soreness

3117P

Suppression of doxorubicin-induced apoptosis in mouse embryos by simvastatin

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Background: Doxorubicin (DOX) is a broad-spectrum anthracycline antibiotic widely used as an antineoplastic agent against a variety of malignancies. However, its use in chemotherapy has been limited largely due to its diverse adverse effects, including reproductive toxicity. This study was designed to explore the possible ameliorating action of simvastatin (SIM), an antihyperlipidemic agent with antioxidant and anti-inflammatory activities, on DOX-induced apoptosis in mouse embryos. **Methods:** Male mice at 5-week of age were distributed into four groups (n=10, each). DOX was administered to two groups of mice in 5 equal intraperitoneal injections over a period of 4 weeks (accumulated dose of 20mg/kg). One of these groups received 5 equal oral doses of SIM (accumulated dose of 60 mg/kg) along with DOX. A vehicle-treated control group and a SIM control group were also included.Embryo apoptosis was identified using acridine orange (AO) staining following in vitro fertilization. **Results:** AO staining showed that mice treated with DOX alone displayed elevated apoptotic index, while embryo apoptosis was greatly attenuated by SIM co-administration as compared to the DOX-treated group. **Conclusion:** This study provides that SIM can be a promising chemoprotective agent to attenuate unwanted reproductive outcomes risks in patients receiving DOX involved treatment that reside, at least in part, in its radical scavenger activity.
Keywords: Doxorubicin, Simvastatin, Apoptosis, Embryo

3177P

Protective effect of linalool on PC12 cells injury induced by serum and glucose deprivation

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Background: Neurological disorders and neurodegenerative diseases represent an important health concern faced by society, accounting for great numbers of disabilities . Recent interests have focused on natural antioxidants and anti-inflammatory compounds as potentially useful neuroprotective agents. Linalool is a natural occurring monoterpene compound with numerous pharmacological properties like antioxidant activity. However, it's effects on ischemic damages have not been evaluated. **Method**: We used an in vitro model of cerebral ischemia and studied the effects of (±)-linalool (racemic mixture) and R-(-)-linalool on cell viability and markers of oxidative damages mainly intracellular reactive oxygen species (ROS), and oxidative DNA damage following injury induced by serum and glucose deprivation (SGD) in PC12 cells that were measured by MTT assay, fluorometry method and comet assay, respectively. **Results:** Linalool at high concentrations significantly decreased PC12 cells viability. The IC50 values of (±)-linalool and (-)-linalool in PC12 cells were 482.6 and 414.9 µg/mL (after 14 h treatment) and 472.6 and 433.2 µg/mL (after 18 h treatment), respectively. These values were not statistically different. SGD for 12 h produced significant cell death which accompanied by increased levels of ROS and DNA damages. Pretreatment with different concentrations of (±)-linalool and (-)-linalool (0.1-500 µg/ml) for 2 and 6 h, markedly restored these changes, in a concentration dependent manner. However, no significant differences were seen in the protection against ischemic insult between the enantiomers as well as the time of exposure. **Conclusion:** The experimental results suggest that linalool protects the PC12 cells from SGD-induced injury via antioxidant mechanisms. Our findings might raise the possibility of potential therapeutic application of linalool for managing cerebral ischemic and other neurodegenerative disorders.
Keywords: PC12 cells, oxidative stress, serum/glucose deprivation, neurodegenerative disorders, linalool

3224P

Immunomodulatory and anti-cancer properties of sesquiterpene lactone fraction from Artemisia khorassanica

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Background: Artemisia species are important medicinal plants throughout the world. Present study aims to investigate different properties of Artemisia khorassanica and elucidate potential underlying mechanisms. **Methods**: Sesquiterpene lactone fraction was prepared from A. khorassanica (SLAK). For anti-inflammatory assessing, induced amount of nitric oxide (NO), prostaglandin E2 (PGE2) and also expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were examined in peritoneal macrophages. Function of splenocytes was defined as stimulation index using MTT assay and secretion of IL-4 and IFN-γ using ELISA. Anti-cancer potential was evaluated by toxicity against human melanoma and fibroblast cell lines. To explore the involved pathways, pattern of any cell death was determined using Annexin-V/PI staining and also the expression of Bax and cytochrome c was investigated by Western blot. **Results**: Results showed that while SLAK caused negligible

proliferation inhibition, peritoneal macrophages displayed considerable decrease in NO and PGE2 production along with iNOS and COX-2 expression. In addition, SLAK noticeably was capable to suppress PHA/LPS stimulated splenocyte proliferation and also up-regulate Th-2 mediated cytokine IL-4 and decline Th-1 mediated cytokine IFN-γ. Moreover, SLAK selectively caused a concentration-dependent inhibition in the proliferation of melanoma cells which was associated with remarkable increase of early apoptosis and overexpression of both Bax and cytochrome c. **Conclusion**: Current experiment presents Artemisia khorasanica as a traditionally used herb with potent anti-inflammatory, immunomodulatory and anti-cancer activities. We anticipate that the ingredients may be employed as therapeutic candidates in the regulation of different immune responses implicated in various conditions and ailments.

Keywords: Artemisia khorassanica, Peritoneal macrophage, Balb/c mice splenocyte, Melanoma

3225P

In vitro screening of Berberis vulgaris extracts according to anti-inflammatory and anti-cancer properties

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Background: To confirm isolated extracts from Berberis vulgaris (Barberry, Berberidaceae) have the potential as anti-cancer and anti-inflammatory agents. **Methods**: Barberry different extracts including aqueous, methanol, butanol, ethylacetate and dichloromethane were prepared. Their toxicity against a panel of human melanoma (MM200, Mel-RM, Me4405 and A375) and fibroblast cells were studied using MTT assay. Patterns of cell death were defined using Annexin-V/PI staining. To explore the activation of caspases, cells were treated with pan-caspase inhibitor and extract and any changes in apoptosis pattern was analyzed using flowcytometry. Anti-inflammatory property was verified by assessing nitric oxide (NO) production using Griess reagent and inducible nitric oxide synthase (iNOS) expression using western blot analysis in lipopolysaccharide (LPS)-induced J774A.1 macrophages. **Results**: All isolated extracts possess selective toxicity against human melanoma cells. Me4405 cell proliferation was most potently suppressed with Barberry dichloromethane extract (BDE). BDE also caused an outstanding increase in DNA content of sub-G1 area and early apoptotic population of cells. Blockage of BDE-induced apoptosis was along with the cleavage of procaspases and the presence of their cleaved bands. BDE remarkably inhibited NO production and iNOS expression in macrophages. **Conclusion**: The results revealed for the first time anti-cancer properties of different Barberry extracts on human melanoma cells which is possibly mediated through the induction of apoptosis and accompanied by the activation of caspases. Anti-inflammatory effect of isolated extracts was showed also in stimulated macrophages. Current experiment provides useful information on anti-inflammatory and anti-tumoral properties of Berberis vulgaris, supporting the folk use of this medicinal plant.

Keywords: Berberis vulgaris, Melanoma, Caspase, Inflammation, Macrophage

3226P

Ursolic acid induced apoptotic cell death by activating caspase expression in human melanoma cells

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Background: Melanoma is an aggressive cancer with a growing incidence worldwide and high resistance to conventional drug, which is partly due to resistance to apoptosis. Ursolic acid (UA) is plentifully presents in various fruits, foods and medicinal plants and showed anti-cancer capacity on divergent of cancer cell lines. However, the effects of UA on some melanoma cells and the mechanisms of action have not yet reported. Our aim was to confirm UA have the potential as anti-cancer agent through the induction of apoptosis and involvement of caspases. **Methods:** The effect of UA against a panel of human melanoma and fibroblast cell lines was investigated using MTT assay. Cell death pattern was determined using Annexin-V/PI staining. To explore whether the activation of caspases was required for apoptosis induction, cells were treated with pan-caspase inhibitor and UA. After that, any changes in apoptosis pattern was analyzed using flowcytometry. The expression of caspases was detected using western blot analysis. **Results:** A significant suppression of cell proliferation was demonstrated after 24 and 48 hours in the presence of UA, which occurred in a concentration-dependent manner. Apoptosis was considerably increased after treatment with UA which was indicated by enhancement of Annexin-V positive population as well the enhanced sub-G1 peak. In western blotting analysis, UA induced the proteolytic processing of caspase-3. **Conclusion:** The results confirmed anti-cancer properties of ursolic acid on human melanoma cells which is possibly mediated through the induction of apoptosis and accompanied by the activation of caspases.

Keywords: Ursolic acid, Melanoma, Proliferation, Apoptosis, Caspase

1980P

Emerging role of the saffron ethanolic extract in improving age-related change in oxidant-antioxidant system in hippocampus of male rat

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Background: Using antioxidant nutrients may be a good diet strategy for the prevention of oxidative stress associated to age-related disease. Saffron is a dried stigmas of Crocus sativus L. with antioxidant properties. Oxidative damage by free radicals is one of the mechanisms underlying the aging process. This study was done to investigate the effects of saffron treatment on lipid peroxidation and antioxidant status in hippocampus of male aged rats. **Methods:** Saffron extract (5, 10, 20 mg/kg/day) was intraperitoneally administered to the rats for 4 weeks. At the end of experiments, the rats were anesthetized deeply by ether and then decapitated by guillotine. Hippocampus of animals were removed on ice immediately and

kept frozen. Tissue was homogenized in prepared buffer and after centrifugation produced supernatant was used for determination of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), glutathione peroxides (GPx), catalase (CAT) and nitric oxide (NO). **Results:** The results obtained in the present study indicated that normal aging was associated with a significant decrease in the activities of antioxidant enzymes, and an increase in MDA and nitric oxide levels in the hippocampus of aged rats. Furthermore, supplementation of saffron ethanolic extract was effective in reducing hippocampus MDA and NO levels and in increasing the antioxidant status. **Conclusion:** There is possibility that saffron extract acts as a hormetin by inducing mild oxidative damage which leads to the activation of antioxidative enzymes. Therefore, Crocus sativus L. by acting as a potent antioxidant exerted considerable neuroprotective effect and proved efficacious in protecting rat hippocampus against age-related oxidative damage.

Keywords: Saffron, Aging, Oxidant-antioxidant system, Rat hippocampus

2349P
Evaluation of immunomodulatory effects of Thymol on mouse splenocytes

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Background: Many plant products are important for the treatment and prevention of diseases. Immunomodulatory effects of medicinal herbs may be useful in reducing the risk of various diseases. This study aims to investigate the immunomodulatory properties of thymol, a major component of thyme. **Methods:** The effects of thymol at concentrations of 1,10, 25 and 50µg/ml on the proliferation and apoptosis of C57BL/6 mouse stimulated splenocytes was evaluated by Brdu cell proliferation assay and flow cytometric based AnnexinV-PI staining, respectively and the level of IFNγ, IL4 and IL10 were measured by enzyme-linked immunosorbant assay. **Results:** Thymol reduced cell proliferation at concentrations of 25 and 50µg/ml and no significant changes in early ($Annexin^+$ PI^-) and late ($Annexin^+$ PI^+) apoptosis at the above concentrations was observed. The level of IL4 in supernatant of cells containing 25 to 50µg/ml and IFNγ at concentrations of 10 to 50µg/ml of thymol was significantly decreased when compared to untreated cells but the reduction observed in IL10 production was not significant. IFNγ/IL4 ratio was lower in treated compared to untreated cells. **Conclusion:** Thymol was able to reduce proliferation of mouse splenocytes and this reducing effect was not due to induction of apoptosis or necrosis. This compound diminished both Th1 (IFNγ) and Th2 (IL4 and IL10) related cytokines but this reduction was greater in Th1 cytokine as the IFNγ/IL4 ratio showed a significant decrease.

Keywords: Thymol, Immunomodulatory effects, IFNγ/IL4 ratio

3176P

Effect of pomegranate seed oil against mercury chloride – induced hepatotoxicity in rat

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Background: Heavy metals such as mercury can induce oxidative stress which is associated with tissue injury following metal intoxication. The present study was designed to evaluate the protective effect of PSO (Pomegranate seed oil) against liver damage induced by ip injection of mercury chloride ($HgCl_2$) (5mg/kg) in rat. **Methode**: 24 rats were used in the present study and were divided into four groups : group I (control)received i.p. injection of corn oil(1ml/kg); group II rats were received $HgCl_2$ at dose of 5 mg/kg bwt (i.p.) for 3 days ; group III , IV rats were given PSO at doses of 0.4ml/kg and 0.8 ml/kg respectively then after 1 hrs rats were treated with $HgCl_2$ (5 mg/kg) . Serum levels ALT, AST and also specimens of liver were removed and prepared for histopathological study. The markers of oxidative stress including thiobarbituric acid reactive substances (TBARS), total sulfhydryl (_SH) group were also determined. **Results:** Rats injected with $HgCl_2$ showed a significant increase in levels ALT, AST ($p<0.01$, $p<0.05$) and shoewd hepatocytic vacuolization and necrotic alterations in the liver. $HgCl_2$ induced a significant increase in malondialdehyde and a significant reduction in total sulphydryl group) ($p<0.001$). Rats that were given PSO before $HgCl_2$ injection improved histopathological alteration and a significant decrease Serum ALT and AST levels ($p<0.05$). **Conclusion:** Mercury chloride intoxication induced marked hepatic deleterious effects which alleviated by PSO pretreatment.

Keywords: $HgCl_2$, Oxidative stress, Total thiol groups , PSO

2651P

Evaluation anti-inflammatory effect of Fluvoxamine on 12-O-tetradecanoylphorbol-13-acetate(TPA)-induced skin inflammation in mice

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Background: Fluvoxamine is an antidepressant which potently and specifically inhibits neuronal reuptake of serotonin. In the absence of other major pharmacological effects it appears that its antidepressant activity stems from facilitation of serotoninergic neurotransmission as a result of reuptake inhibition. In our previous studies we confirmed the anti-inflammatory systemic administration of Fluvoxamine. In line of the indicated work, topical anti-inflammatory activity of the Fluvoxamine on TPA-induced skin inflammation was investigated. **Methods:** Skin inflammation was induced in the right ear by the topical application of 2.5 µg/ear of TPA dissolved in 20 µl of acetone. Left ear was considered as control. The animals were sacrificed by cervical dislocation after 4h, and ear biopsies were obtained with a punch (a diameter of 6mm) and weighed. The increase in the weight of the right ear punch over the left indicated

the edema. Fluvoxamine (2.5 and 5 mg/ear) or indomethacin (0.5 mg/ear) dissolved in acetone were applied at the same time (20 µl). **Results:** The topical application of Fluvoxamine (2.5 and 5 mg/ear) markedly inhibited the skin inflammation induced by TPA ($p < 0.05$). Pathological studies also approved our results. **Conclusion:** The results demonstrated topical anti-inflammatory effect of fluvoxamine which may be due to its serotonin reuptake inhibitory property.

Keywords: Topical anti-inflammatory; Fluvoxamine; TPA.

2649P

Evaluation anti-inflammatory effect of hydroalcoholic extract from aerial parts of Stachys pilifera on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin inflammation in mice

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Background: Stachys pilifera has long been used in Iranian folk medicine to treat infectious, respiratory and reumathoid disorders. Moreover, anticancer, antioxidant, and antimicrobial properties of Stachys pilifera have been reported. In this study, anti-inflammatory activity of the hydro-alcoholic extract from aerial parts of Stachys pilifera on TPA-induced skin inflammation in mice was evaluated. **Methods:** Skin inflammation was induced in the right ear by the topical application of 2.5 µg/ear of TPA dissolved in 20 µl of acetone. Left ear was considered as control. The animals were sacrificed by cervical dislocation after 4 h, and ear biopsies were obtained with a punch (a diameter of 6mm) and weighed. The increase in the weight of the right ear punch over the left indicated the edema. Stachys pilifera extract (2.5 and 5 mg/ear) or indomethacin (0.5 mg/ear) dissolved in acetone were applied at the same time (20 µl). **Results:** The topical application of Stachys pilifera extract (2.5 and 5 mg/ear) markedly inhibited the skin inflammation induced by TPA ($p < 0.001$), with both doses significantly affecting ear edema. Pathological studies also approved our indicated finding. **Conclusion:** Our results demonstrated the potent topical anti-inflammatory effect of Stachys pilifera, which may be due to its phenolic compounds and antioxidant properties.

Keywords: Topical anti-inflammatory; Stachys pilifera; TPA.

2426P

The role of Sprycel in the treatment of EAE

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Background: Experimental autoimmune encephalomyelitis (EAE) is the most commonly used

experimental model for the human inflammatory demyelinating disease, multiple sclerosis (MS). MS is an autoimmune disease of the central nervous system (CNS) that leads to an inflammatory demyelination, axonal damage and progressive neurologic disability. Sprycel is a selective protein tyrosine kinase inhibitor that abrogates multiple signal transduction pathways implicated in autoimmune diseases. **Methods:** In this experiment, EAE induction was performed by Hooke Kit. The kit consists of antigen (MOG_{35-55}) in CFA emulsion, and pertussis toxin (PTX). The mice were injected subcutaneously on upper back and lower back with 0.1 ml of emulsion respectively. Within 2 hours of injection of the emulsion, the first dose of PTX (0.1 ml per mouse) was injected intraperitoneally. 22-26 hours after injection of the emulsion, the intraperitoneally injection of second dose of PTX into the mice (0.1 ml) were done. The mice were administered orally with Sprycel at the specified dose (50 mg/kg) from day 7 after immunization on five consecutive days per week for 2 week. The mice were sacrificed on day 21 post-immunization. Brains and cerebellums were removed, post-fixed in formalin, embedded in paraffin, sectioned and then stained with Luxol fast blue (LFB) and with eosin and hematoxylin. **Results:** Our results showed that treatment with Sprycel caused a significant delay in the time of onset and a significant reduction in severity of the EAE in treated animals compared with control groups. **Conclusion:** This data suggest that FDA-approved drug Sprycel has potential therapeutic effects on EAE as an autoimmune demyelinating disease.

Keywords: Sprycel, MS, EAE, MOG, Autoimmune disease

2372P

Acute anti-inflammatory effects of Salbutamol in the rat air-pouch model of inflammation

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Background: Recent studies have proven that immune and nervous systems have a bidirectional communication. The aim of the study is to investigate the effects of salbutamol on the inflammatory parameters in the rat air pouch model inflammation. **Methods:** Male Wistar rats were anesthetized; 20ml and 10ml of sterile air were injected subcutaneously on the back of animals on day 1 and 3 respectively. On day 6, inflammation was induced by injection of 1ml of carrageenan 1% into pouch. One ml of salbutamol (125, 250 & 500 µg/pouch) and salbutamol plus propranolol (500µg/pouch) in the test groups and saline in the control group were administered intra pouch at the same time as the carrageenan. After 6 hours animals were sacrificed. The pouches were flushed with PBS. Then they were opened, pouches fluid was collected in order to determine exudates volume and cells were counted. The granulation tissues formed were dissected out and the weight determined. **Results:** Leukocytes accumulation and exudates volume were decreased significantly by salbutamol 250 µg/pouch ($P<0.05$). The dose of 500 µg/pouch of salbutamol reduced significantly the number of leukocytes ($P=0$), exudates volume ($P<0.001$) and granulation tissue weight ($P<0.01$) dose dependently. In addition there were no changes in the inflammatory parameters by co-administration of salbutamol and propranolol. **Conclusion:** From this study it may be concluded that salbutamol possesses anti-inflammatory property possibly through the stimulation of β-2 adrenergic receptors. This anti-

inflammatory effect may be as important mechanism in asthma treatment, where inflammation also has role in the etiopathology.
Keywords: Salbutamol, Air pouch, Carrageenan, Acute inflammation.

2700P

Synergistic effect of curcumin and insulin on glucose Transporter Isotype-4 Translocation from Intracellular Compartments into the Cytoplasmic Membrane of C2C12 Myotubes

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Background: Curcumin, the yellow pigment in turmeric, has been shown an anti-diabetic agent for centuries but only in recent few years its mechanism of action has been under investigation. Some studies showed thatcurcumin might exert its anti-diabetic effect via increasing glucose transporter isotype-4 (GLUT4) gene and glycoprotein contents in cells. Investigate this possibility, we investigate the effects of Extract and commercial curcumin with and without insulin on enhances GLUT4 translocation from intracellular compartments of nuclear or endoplasmic reticulum membranes (N/ER) into the cytoplasmic membrane (CM). **Methods:** C2C12 myoblastic cell line were seeded in DMEM plus 20 % FBS and differentiated to myotubes using 2 % horse serum. After myotubes formation, 40 µmolar Extract and Commercial curcumin, with or without insulin as intervention, and as control 1 % DMSO were added for 3 h. Cells were washed and homogenized followed by ultracentrifuge fractionation, protein separation by SDS-PAGE and GLUT4 detection using semi-quantitative Western blotting. Data analysis was done by two-independent samples t test for comparison of mean ± SD of GLUT4 percent in categories. GLUT4 contents were higher in CM groups curcumin and curcumin with insulin in compare to 1 % DMSO treated myotubes control group. **Results:** As our results have shown Extract and commercial curcumin induces GLUT4 translocation from intra-cell into cell surface. The results have also shown synergic effect of curcumin on translocation of GLUT4 from intra-cell into cell surface in the presence of 100 nm insulin. **Discussion**: We conclude that curcumin maybe a choice of type-2 diabetes mellitus treatment because its extract and cummercial enhances GLUT4 contents in CM where it facilitates glucose entrance into the cell. However it is necessary to trace the signaling pathways which are activated by curcumin.
Keywords: Curcumin, Insulin

2731P

Everything about a well known nutritional compound; the panacea named Quercetin

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Specifically over recent ten years, in the field of clinical immunology and in line with any expedient enterprises are lending themselves to pave the way for health care policies/purposes; in terms of immunologically intervening, preventing and even, treating of several human ailments, major emphasis is being placed on the utilization of medicinal plants. In this context, numerous medicinal plants, thanks to have a variety of natural components with anti-oxidant/free radical-scavenging and anti-inflammatory/anti-allergic properties, still serve as leads for the development/progression of novel nutraceutical agents/preparations. Currently, compounds of interest to immunologists are FLAVONOIDS in general, and quercetin in particular, which are present in every day frequently-consumed nourishments in fairly, great levels. Prevalently, flavonoids exist as aglycones, glycosides and methylated derivatives. The flavonoid aglycone is composed of a benzene ring(A) condensed with a six-membered ring(C) which in the 2-position carries a phenyl ring(B) as a substituent group. The aglycone flavonoids can be allotted to various classifications on the basis of their molecular building arrangements. Quercetin, a nutritional compound belonging to "Flavonol" sub-group of the flavonoid family, is the aglycone of rutin, quercetrin and, other glycoside flavonoids. It is extensively disseminated in the plant Kingdom such as oaktrees, onions and tea, and is found in many human foods including apples, onions, teas, berries, and brassica vegetables, as well as, many seeds, flowers, barks, and leaves. In contrast to, just a few, earlier studies denoting quercetin toxicity/mutagenicity in in-vitro, several more recent reports/studies assert/indicate that quercetin has indeed "anti-mutagenic" and "immuno-protective" properties in in-vivo.

2066P

Effect of dried extract of phytolacca Americana on human macrophage

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Background: Phytolacca is a genus of perennial plants native to Caspian Sea. Some part of this plant are highly toxic to livestock and humans and some parts can be used as food if properly prepared. Phytolacca was used as a heart stimulant and treating cancer, rheumatoid arthritis, itching, and syphilis. Recent studies have been shown that phytolacca toxins can cause liver toxicity, so the aim of this study was to evaluate the toxic effects of extract of phytolacca on human macrophages. **Methods:** We separated the monocytic cell of 5 ml preipheral human blood then 10% of phytolacca extract was added to monocytic cell culture. After 2 hours we evaluate the cytotoxic effects of phytolacca on macrophages by staining them with Gimsa. **Results:** We observed that 90% of macrophages have been apoptotic by effect of phytolacca toxins. **Conclusion:** It is concluded that probably the liver cytotoxicity of phytolacca may be due to its cytotoxic effects on Kupfer cells.

Keywords: Phytolacca Americana, Macrophage, Apoptosis

Innate immunity & Inflammation

Oral Presentations:

3459O

Study the relation between serum level of interleukin-6 and short term morbidity & mortality in acute coronary syndrome

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Background: Ischemic heart disease (IHD) is the most common causes of morbidity and mortality in developed countries and causes many economic costs. Inflammatory markers, specially IL-6 has a significant role in a atherosclerosis process. The aim of this study was to find any relation between the amount of serum level of interleukin-6 and short term morbidity & mortality in acute coronary syndrome. **Methods:** This cohort study was done based on simple sampling method on patients with acute coronary syndrome in Ali-Ebne-Abitaleb and Khatam-Al-Anbia hospitals in Zahedan city in 2011-13. At the time of admission, serum level of IL-6 from patients with and without events of IHD was measured, then 6 months later first level of this factor in patients with and without new events were compared with each other. The obtained results were analyzed using SPSS software and T-test and regression logestic test. **Results**: The results of this study revealed that the amount of serum level of IL-6 was increased in acute coronary synd but there was no relationship between serum level of this factor at first admission with new coronary events in short period of time . Conclusion: This study as well as some previous studies showed that there is no independent relationship between IL-6 and short term morbidity and mortality in acute coronary syndrome . IL-6 is not a useful marker for predicting vascular risk in patient with acute coronary syndrome in a short period of time but the predicting role of IL-6 in long term can be assessed in later study. We suggest further study needs to be done in future to identify the role of other inflammatory markers in this disease.

Keywords: IL-6, Morbidity & mortality, Acute coronary syndrome

3126O

In-vitro effect of different doses of TGF-Beta on differentiation of human naïve CD4+ T cells to Th17

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Background: Appropriate differentiation of distinct human CD4+ T cell subsets is critical for manipulating these cells for using in immunity related diseases. While there are a lot of attempts to clarify the role of different factors involved in Th17 differentiation, many crucial contradictions yet remained for Th17 cell polarization conditions. Although it is well shown that the differentiation of in vitro Th17 cells culture conditions requires the presence of IL-1beta, IL-23, IL-2, IL-21, IL-6 and TGF-β, however the optimum amount of TGF-β that regulates in vitro human Th17 cell differentiation is still unclear. **Methods:** In this report we used a flow cytometric assay to evaluate the effect of different concentrations of TGF-β and a combination of IL-1beta, IL-23, IL-2 without usage of IL-6 on development of Interleukin (IL)-17–producing T helper (Th17) cells. **Results:** We found that 0.1ng/ml TGF-β significantly increase the expression of IL-17 in comparison to other concentration of the cytokine. **Conclusion:** Our results demonstrate the key role of TGF-β cytokine in human in vitro Th17 cells polarization.

Keywords: CD4+ T cells, Th17 cells, transforming growth factor-β

3127O

In vitro generation of polarized human Th17 Cell population by MicroRNA miR-326

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Background: The proliferation and differentiation of antigen-specific CD4+ T lymphocytes into different subpopulations of effectors T cells following priming of naïve cells is central in the orchestrating adaptive immune responses by virtue of differential cytokine production. Among a range of distinct cell subsets of CD4+ T cells, Interleukin 17 (IL-17)-producing T helper cells (TH-17 cells) are increasingly recognized as key participants in various autoimmune diseases. Although so far, related studies showed that gene expression during lymphocyte development is driven primarily by transcription factors, additional level of regulation is mediated by different sets of expressed non-coding RNA (miRNA) found in different immune cell types. Regarding emerging ideas that exist among different studies which have tried to clarify critical miRNA in human Th17 cell differentiation. **Methods:** Naïve CD4+ T cells were isolated from normal blood samples and cultured in X-VIVO 20 serum-free medium. Purified cells were treated with combinations of polarizing cytokines (TGF-β, IL-1β, IL-23 and IL-2) and miR-326 followed by analysis of the expression of characteristic genes and their relevant cytokines by real-time quantitative RT-PCR and flowcytometery method, respectively. **Results:** we found that cells transfected with miR-326 could produce more Il-17 compared

to cells differentiated in Th17-polarizing conditions, as determined by protein and mRNA levels detected by flow cytometry and Real Time PCR. **Conclusion:** Here we showed that in vitro transfection of human naïve CD4+ T cells by miRNA-326 resulting abrogated Th17 cell differentiation.

Keywords: Th17, MicroRNA miR-326

1734O

An investigation of innate immune response of human blood macrophage to sense and antisense dsRNA

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Background: Silencing of gene expression by siRNA (small interfering RNA) is powerful approach to study the genetic analysis and functional roles of mammalian genes. There are already no reports towards effects of mammalian two hybrid system plasmids delivery of sense and antisense strands. **Methods:** The leishmaniapteridinereductase 1 (PTR1) gene was cloned as a sense and antisense strands into mammalian two hybrid system plasmids, the constructs were transfected into human blood macrophages on the basis of 8 experimental groups. (Antisense strand ± LPS, sense strand ± LPS, dsRNA ± LPS, negative control ± LPS). After 24 hours, cytokines production was assessed by ELISA. **Results:** Transfection of sense and antisense strand RNAinto monocyte derived macrophages (MDM) was confirmed by RT-PCR. Single strands RNA expressed IL-8, IL-12, IL-1β inflammatory cytokines and dsRNA induced IL-8, IL-12 and TNF-α production in MDM. In contrast, random uptake from a mixture of two plasmids was down-regulated IL-8, IL-12, IFN-γ cytokines with significant difference $p<0.05$ in macrophage. **Conclusion**: Increased level of IL-8 in macrophage detected in single strand groups: The chemokine production as a major feature of innate immunity is a powerful tool for evaluation of sense/ antisense in experimental and therapeutic gene vaccine delivery. siRNA –based gene therapy could be great potential in cancer.

Keywords: Sense and antisense, dsRNA, Gene expression, Monocyte/macrophage

2912O

Purification and partial characterization of agglutinin lectin from heamolymph of German cockroach, *Blattella germanica*

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Background: Lectin molecules are proteins which have crucial biological role in insects' immune system. lectins involve in self and non-self recognition defense, metamorphosis,

shedding, wound healing, repairing tissue and interaction between cells to cells. **Methods:** Heamolymph of German cockroach, *Belatella germanica* was used for hemagglutination tests against different RBC including rabbit, rat, sheep, Guinea pig, syrian mouse and human RBCs (A, B, AB and O). Then the highest agglutinin blood cell was candidate for sugar inhibition assay. Consequently, the hemolymph was applied for analytic and preparative HLPC using ion-exchange or reverse phase methods. All protein fractions were tested for lectin activity and then characterized by SDS-page. **Results:** The most agglutinin activity was found against RBC of syrian mouse at titer 1/128 dilution and sugar inhibition assay showed that fucos, N-acetyglucoseamine and galactose reduced titer of agglutinin to 1/2. Among the separated proteins (HPLC fractions) tested for agglutinin activity, only fraction appeared at rotation time of 36 minute showed agglutinin activities. The molecular weight of this protein was about 120 kDa. **Conclusion:** The range of agglutinin activities against different RBC indicates that the isolated lectin is not specific for a particular carbohydrate. In addition, the isolated lectin at low concentration present in heamolymph should be an innate lactin not secreted, because we found it without any trigger immunity of the insect.

Keywords: Lectin, purification, Heamolymph, insect, *Blattella germanica*,

1646O

Interaction of *Bordetella pertussis* filamentous hemagglutinin with human TLR2: identification of the TLR2-binding domain

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Background: Filamentous hemagglutinin (FHA) is a major adhesion and virulence factor of *Bordetella pertussis* and also a main component of acellular pertussis vaccines. Interaction of FHA with different receptors on human epithelial and immune cells facilitates entrance and colonization of bacteria as well as immunomodulation of the host immune response. **Methods:** Three overlapping segments of the FHA gene were cloned in a prokaryotic expression vector and the recombinant proteins were purified and applied along with the native FHA protein to assess their potential Toll-like receptor (TLR) stimulatory effects and to localize the TLR binding region. TLR stimulation was monitored by applying HEK293-Blue cell lines cotransfected with TLR2, 4 or 5 and a NF-κB reporter gene. Culture supernatants were checked for secretion of the reporter gene product and IL-8 as indicators of TLR stimulation. **Results:** Native FHA was found to strongly stimulate TLR2, but not TLR4 or TLR5 transfected cells. Among the recombinant FHA fragments only the fragment spanning amino acid residues 1544-1917 was able to stimulate TLR2 transfected cells. **Conclusion:** Interaction of FHA with TLR2 suggests its involvement in induction of the innate immune system against *Bordetella pertussis*. The TLR2-binding domain of FHA may contribute to immunoprotection against

pertussis infection.

Keywords: Filamentous hemagglutinin, *Bordetella pertussis*, Toll-like receptor, Innate immunity, HEK293 cell line

1959O

Effects of oral probiotics feeding on Toll-like receptor (TLR) gene expression in chicken's cecal tonsil

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Background: Probiotics have been shown to influence immunity through contact with immune cells. TLRs on immune cells by identifying conserved bacterial structures can modulate immune response in many ways. In order to elucidate the immunopotentiative effects of probiotics, the changes in the TLR expression in cecal tonsil studied before and after oral feeding of L.acidophilus in chickens as an experimental model. **Methods**: Thirty one old day chicken selected and separated in 3 groups, as probiotic fed, dairy fed and control group. The cecal tonsil was removed and gene expression of TLR2, TLR4 and TLR5 was examined by real-time PCR analysis, after 14 and 21 days oral feeding of probiotic and sterile dairy milk and compared with control group. **Results**: at 14days, the expression ratio of all TLRs (TLR2, TLR4 and TLR5) was higher in those fed by probiotic and sterile dairy milk compared with control group, the increase in TLR expression was still higher in probiotic fed chicken. Instead of changes seen in 14days, expression ratio of TLRs at 21days was decreased except for TLR2. **Conclusion:** It seems that probiotics affect the increased expression of innate receptors which may potentiate the mucosal cell integrity and local immune reactivity.

Keywords: Probiotic, TLRs, Chicken

2327O

Evalution of pathologic indexes and inflammatory cytokines IL-6 and IL-8 expression in sinonasal polyposis

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Background: Sinonasal polyposis is a disease resulting from complex pathogenetic mechanisms. Many cytokines and mediators have role in polyp development. Sinonasal polyposis is mainly characterized by an accumulation of inflammatory cells in the lamina propria, edema, fibrosis, or epithelial degradation. IL-6 and IL-8 are important inflammatory cytokines that have a significant role in inflammatory diseases. In this study we investigated

the pathologic indexes and IL-6 and IL-8 expressionin polyp tissue samples. **Methods:** 130 tissue samples of patients with Sinonasal polyposis as cases, and 27 tissue samples of patients without sinonasal polyposis as control group were studied. Pathologic indexes were studied microscopically. The presence of IL-6 and IL-8 was studied using immunohistochemistry staining and their expression was evaluated especially in respiratory epithelium and lminapropria. **Results:** Inflammatory cells infiltration, severity of edema and fibrosis and layer of epithelial cells of the respiratory epithelium was significantly higher in polyp than control. IL-6 and IL-8 expression in respiratory epithelium and lminapropria were significantly higher in polyp than control. **Conclusion:** The observed increase in the expression of IL-6 and IL-8 in polyp tissue may be a result of inflammatory cells infiltration and development of pathologic events in sinonasal polyposis.

Keywords: Sinonasal polyposis, inflammation, IL-6, IL-8, Immunohistochemistry

2561O

Comparison of anti-inflammatory potentials of the soybean bowman-birk trypsin inhibitor (BBI), genistein, and mixture of BBI and genistein in BALB/c mouse model

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Background: Inflammation is a protective response that triggered in order to removing the initial agent of the cellular damage and necrotic cells. The recent studies indicate that inflammation has an important role in the development of chronic diseases and cancer. Because of anti-inflammatory potential and anti-cancer effects of soybean bowman-birk protease inhibitor (BBI) and genistein, this study was undertaken to investigate anti-inflammatory properties of BBI, genistein, and a mix of genistein and BBI in BALB/cmice induced by lipopolysaccharide (LPS).**Methods**: Before induction of inflammation by LPS (16 mg/kg), the mice (5 per groups) were administered with the given doses of BBI, genistein, and a mix of genistein and BBI. After 2 hours, the serums and peritoneal fluids were obtained. Finally, the level of tumor necrosis factor-α (TNF-α) in the serum and peritoneal fluid in treated and untreated groups was measured by enzyme linked immunosorbent assay (ELISA). **Results**: Our findings indicated a significant decrease in inflammatory cytokine TNF-α level in serum and peritoneal fluid of the animal treated with BBI, genistein, and a mix of genistein and BBI. In addition, the anti-inflammatory effect of the mix of genistein and BBI was more than the other treatments. **Conclusion**: The results showed the synergistic effect of BBI and genistein on decrease of inflammation in mice induced by LPS. Moreover, because of anti-inflammatory activity in vivo, it would be regarded as suitable candidate for the development of anti-inflammatory cases, which needs to be more investigated.

Keywords: Inflammation, Trypsin inhibitor protein, Soy, Tumor necrosis factor

2487O

The effect of insulin treatment on TLR4 gene expression and cell density in hippocampal brain and testis tissues in induced hyperglycemia conditions (diabetes type 1)

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Background: The several studies reported that the diabetic conditions induce apoptosis in various organs such as brain and testis. Recent studies have shown that the diabetes conditions can trigger male infertility and neurodegenerative diseases. Also, it is increasingly evident that there is a close relationship between diabetes, cell death, Alzheimer disease and infertility. Toll like receptors (TLRs) are the main components of innate immune system. TLRs are involved in cell death and inflammation in diabetic condition. It is clear that TLRs especially TLR4 is up regulated in diabetic mice. This study designed to define the effect of insulin treatment on mRNA expression of TLR4and cell density in the brain hippocampal tissue and testis of diabetic rats in after induction of diabetes type 1.**Methods:** Diabetes type 1 was induced in rat by single injection of streptozotocin(STZ) with dose 55 mg/kg. To assessment the direct effect of hyperglycemia on target gene expressions and morphological analysis, insulin treatment was done to reduce the blood glucose. Hippocampal and testicular tissue damage and expression of the TLR4 in various times after diabetes type 1 induction were assessed in control, diabetic and diabetic insulin treated rats.In order to histological analysis, after certain periods, the animals were killed; their hippocampi and tests were removed, and fixed in formaldehyde (37%). The fixed samples were then embedded in paraffin and sectioned (4 µm thick) on gelatin precoated slides. They were further deparaffinized, stained with Hematoxylin-Eosin (H&E) and DAPI, and observed under the microscope to evaluation the cell density of hippocampi and tests tissues section in specified times. Also, expression levels of TLR4 quantified by qPCR and statistically analyzed by one way ANOVA method. **Results:** The results showed differential pattern expression among different groups of treated (diabetic and diabetic-insulin treated) and control rats. Hyperglycemia condition after diabetes type 1 induction increased expression of TLR4 and cell density reduction in diabetic groups. The insulin treatment decreased the expression of TLR4 in diabetes-insulin treated group. Also, testicular and hippocampi cell density were improved after insulin treatment. **Conclusion:** Insummary,the results suggesting that hyperglycemia following diabetes play an important role in initiation innate immunity and cell death, and insulin treatment can reduce the expression of TLR4 and increased cell density in diabetic tests and hippocampi tissues.

Keywords: Insulin, TLR4, Testis, Hippocampus, Diabetes type 1

Poster Presentations:

2954P

The effect of Spirulina on inducible nitric oxide synthase gene expression in spleen macrophages of Balbc/ mice with systemic candidiasis

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Background: The purpose of this study was to evaluate the activity of Spirulina (*Spirulinaplatensis*), a blue-green alga used as a dietary supplement,on inducible nitric oxide synthase gene expresion(iNOS) in spleen macrophages of mice with systemic candidiasis. **Methods:** Two groups of 5 Balb/c mice received a dose of 800 mg/kg of *S. platensis (trial group) and 200 µl salin* (control group) for four days, and then they were inoculated intravenously with 1×10^6*Candida albicans* (*C. albicans*). After 24 hours, they were euthanized and their spleen cells cultured in sabouraud glucose agar and iNOSgene expression was evaluated by RT-PCR. **Results:** The results showed that the numbers of *C. albicans* colony in the spleen of Spirulina-treated mice were significantly lower than the control group (p<o/oo1), but we couldn't be able to indicate iNOS gene expression in spleen cells of these animals. **Conclusion:** a significant reduction of fungal burden in trial group spleen, supposed that other leukocytes except macrophages, such as neutrophilsare stimulated and accumulated in the spleen during the first 24 hour after infection then clearing organ from Candida yeasts.

Keywords: Spirulina, INOS gene expression, Macrophages, Spleen

1580P

Higher levels of IL-33 in sera of patients with ST Elevation Myocardial Infarction are associated with decreased ejection fraction

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Background: IL-33 is a newly discovered cytokine belonging to alarmin family of molecules, which are produced endogenously in response to tissue injury and necrosisas well as human heart and aorta. Pathophysiological changes during myocardial infarction, which are induced by mechanical overload of cardiac fibroblasts and myocytes, are regulated by IL-33/ST2 system. We studied the level of IL-33 and its correlation with clinical risk factors in sera of patients with acute myocardial infarction (AMI) in comparison with healthy age- and sex-matched controls. **Methods:** The patients group consisted of 39 patients who were admitted to the hospital with the diagnosis of AMI. Forthy-two age- and sex-matched healthy individuals were enrolled in this study. IL-33 levels in the sera were measured using a commercial ELISA

assay. **Results:** The mean levels of IL-33 in patients and controls were found to be 124.85 ± 203.57 and 78.00 ± 41.39 pg/ml, respectively (p=0.17). Patients with a history of abnormal angiography had significantly lower levels of IL-33 in their sera compared to those without (66.71 ± 19.85 vs. 133.40 ± 216.99 pg/ml, p=0.016). We observed a negative correlation between Left Ventricular Ejection Fraction (LVEF) measured by echocardiography and the level of IL-33 in sera of patients (Pearson correlation= -0.3; p=0.046). **Conclusion:** Higher IL-33 levels in patients with lower than 45 LVEF and its correlation with normal angiography results are major findings of this study. This finding is in line with the reported elevation of CRP, with a negative correlation with LVEF, and higher risk of death, recurrent MI or readmission. The secretion of IL-33 from damaged tissues of atopic dermatitis and rheumatoid arthritis patients and their benefit from IL-33 receptor administration along with the results of this study propose this cytokine pathway as a novel cardiac biomarker with prognostic and therapeutic significance.

Keywords: IL-33, ST Elevation Myocardial Infarction, Ejection fraction

1581P

IL-17A increases in patients with Atrial Fibrilation and correlates with the history of coronary artery bypass graft

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Background: Atrial Fibrillation (AF) is the most common cardiac arrhythmia among elderly. A role of inflammation in atrial remodeling along with the correlation of IL-6 and CRP levels with the development and recurrence of AF is shown. We aimed to evaluate the serum level of IL-17A in different types of AF in comparison to healthy controls. **Methods:** For this purpose, 133 patients with AF and 107 age- and sex-matched healthy controls were enrolled. The concentration of IL-17A was measured using a commercial IL-17A specific ELISA assay.**Results:** IL-17A level was significantly increased in patients with AF compared to controls (1.28 ± 3.5 vs. 0.19 ± 0.64 pg/ml, p=0.001). There was a significant difference in IL-17A positivity between patients and controls (69.6% vs. 23.4%, p<0.001). Moreover, IL-17A levels were higher in patients with a history of coronary artery bypass graft (CABG), compared to patients without a history of CABG (p=0.01). IL-17A levels showed more than two-fold increase in the persistent and permanent AF compared to paroxysmal AF. There was also a trend of increase in the IL-17A levels in patients who had moderate or severe mitral regurgitation (MR) compared to those with mild MR (p=0.059). **Conclusion:** Our report, which is the first on the IL-17A serum levels in AF patients, indicates that IL-17A plays a role in the pathogenesis of AF probably by attracting Neutrophils as the main players in the AF related inflammation. The augmented increase in IL-17A levels in the AF patients with a history of CABG may underline the common pathogenic inflammatory mechanisms in both conditions. In conclusion, there may be the opportunity of IL-17A based interventions in AF condition.

Keywords: IL-17A, Atrial Fibrilation, Coronary artery bypass graft

1579P

Soluble ST2 levels are only marginally increased in the sera of patients with Acute ST Elevated Myocardial Infarction

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Background: ST2 is a member of Toll-like/IL-1-receptor family proteins, which binds to Interleukin-33 and exists is both soluble and membranous forms in cardiomayocytes. We studied the level of sST2 in sera of patients with acute myocardial infarction (AMI) compared it with healthy age- and sex-matched controls. **Methods:** The patients group consisted of 39 patients who were admitted to the hospital with the diagnosis of AMI and all were presented with ST elevation (STEMI). Forty-two age- and sex-matched healthy individuals were enrolled in this study. Serum levels of sST2 were measured using a commercial ELISA assay. **Results:** The mean levels of sST2 in patients and controls were found to be 419.84 ± 285.22 pg/ml and 248.02 ± 181.28, respectively, p=0.08). We observed a significant negative correlation between the level of sST2 in patients and the left ventricular ejection fraction (LVEF) (p= 0.028). Patients with increased heart rate (greater than 100 bpm,) had higher levels of sST2 (397.58 ± 252.53, n=29 vs. 484.39 ± 372.63 pg/ml, n= 10, respectively); however, the difference did not reach the significant level. There was no significant difference in the level of sST2 in patients who smoked (366.64 ± 295.04 pg/ml, n=18) compared to those who did not smoke (465.44 ± 275.40 pg/ml, n=21; p=0.11). There were also no significant differences in sST2 levels between patients based on the systolic or diastolic blood pressure. **Conclusion:** Although sST2 is marginally increased in STEMI patients at the time of admission, the finding that sST2 is already elevated in the healthy individuals limits sST2 application as a diagnostic or screening biomarker. However, the negative correlation between ejection fraction and sST2 levels at the time of admission confirms the useful application of this biomarker in clinic.

Keywords: ST2, Acute ST Elevated Myocardial Infarction

2504P

Investigation of Association between GATA 2 Gene Polymorphism and Premature Coronary Artery Disease in Iranian Patients

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Background: Coronary artery disease is one of the main factors of mortality in advanced countries. Genetic and environmental factors influence heavily on coronary artery disease. Recently, some contradictory data reported on the possible role of rs2713604 polymorphism of GATA2 gene in coronary artery disease.The goal of this research was to investigate the possible relationship of rs2713604 polymorphism with premature coronary artery disease in Iranianpopulation. **Methods:** This case-controlled study was carried out on 100 patients who survived a premature CAD (below the age of 50 years) and 100 healthy volunteers. The two groups were matched according to sex and age. The rs2713604 polymorphism of GATA2

gene was determined by PCR and restrictive enzyme digestion of amplified DNA with Aval. **Results:** The rate of AA genotype prevalence in patient group is 17% and in control group is 19%. Based on this research there was no significance relationship between rs2713604 of GATA2 gene and coronary artery disease (p value>0.05). Also age shows no significant relationship (*P-Value>0/05*). Comparison of GA+AA vs GG shows that in male is 52.2% and in the female is 35.2%(P -Value=0.029 , OR=1.98). Also frequency of allelA in male group is36.2% and in female group is 24.2 % (P-Value=0.017). **Conclusion:** Findings of the study show that rs2713604 of GATA2 gene has no significant relationship on premature coronary artery disease in iranian population.

Keywords: rs2713604 polymorphism, GATA2 gene, Premature coronary artery disease, Coronary artery disease

2803P

Satureja Khozestanica essential oil (SKEO) inhibits iNOS gene expression in Lipopolysaccharide-stimulatedJ774A.1macrophage cell line

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Background: SaturejaKhozestanica is a medicinal herb indigenous to Iran which grows mainly in Lorestan and Khuzestan Provinces.The main component of this herb is a monoterpene named Carvacrol. Previous studies have shown that this herb has anti-inflammatory properties, so we aimed to investigate the effect of its essential oil (SKEO) and Carvacrol on iNOS gene expression in LPS-stimulated J774A.1macrophage cell line. **Methods**: Essential oil was prepared from fresh aerial parts of the plant. The effect of different doses of SKEO and Carvacrol (0.004%, 0.008%, and 0.016%) on iNOS gene expression in normal and LPS-stimulated macrophage cell line was assessed by RT-PCR method. **Results:** Both substances reduced the expression of iNOS gene in LPS-stimulated macrophage cell line in a dose and time-dependent manner, but SKEO was more potent than Carvacrol. **Conclusion**: Anti-inflammatory property of Saturejakhozestanica may be due to its effect on iNOS gene expression and reduction of NO as one of the mediator of inflammation.

Keywords: Saturejakhozestanica, Essential oil, Carvacrol, Inflammation, iNOS

1479P

Association between Antioxidant enzymes and hs-CRP in non-smoker patients of Vessel Heart Disease (VHD)

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Background: The oxidative stress and inflammation are cooperative events involved in

atherosclerosis development. In the present study we assessed the association of antioxidant markers and high sensitive C - reactive protein (hs-CRP) non-smoker patients with Vessel Heart Disease (VHD) or Coronary Artery Disease (CAD). Significant risk factors such as smoking were excluded from the study. **Methods:** Enzymes such as antioxidant markers including: erythrocyte superoxide dismutase (SOD), Glutathione peroxidase (GPX), Total antioxidant capacity (TAC) and The inflammation marker such as hs-CRP were measured in 160 subjects including 80 CAD patients (non-smoker) with angiographically diagnosed CAD and 80 CAD-free subjects as a control group, also patients of smoking with malignancy, renal and liver disease, and other disease were excluded from the study. **Results:** The serum hs-CRP levels were increased significantly as compared to controls. However, erythrocyte SOD, GPX activities and TAC level were reduced significantly in patients (non-smoking) ($P<0.05$ in all cases). The levels of total cholesterol, Triglyceride, LDL-C were significantly higher and that of HDL-C was meaningfully lower than those of control ($P<0.05$ in all cases). **Conclusions:** The association between antioxidant markers, the inflammation index and lipid status parameters suggest their involvement in atherosclerosis development that may lead to CAD progression.

Keywords: Total antioxidant, hs-CRP, Non-smoking, Vessel Heart Disease (VHD)

1462P

Evaluation of Nitric Oxide in Osteogenesis Prosses in Rats

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Background: Nitric oxide (NO) is a cell-signaling moleculeand has more biological functions. Recent studying suggests that its production may regulate the metabolismof the osteoblasts and osteoclasts. The aim of this search wasto evaluate levels of nitrite and nitrates (NO metabolites)during ectopic osteoinduction in rats. **Methods**: Eighteen maleSprague–Dawley rats (body weight 200–300 g) were usedin this study. All animals were anesthetized and, a muscular pouch was created in each flank: the left was filled with 20 mg of demineralized bone matrix (DBM)and the right remained empty (sham). Blood sampleswere taken before (as baseline values) and at 2, 4, and6 weeks after surgery. **Results**: The mean values of NO metabolitesafter 6 weeks were significantly higher ($p<0.05$) than baseline data and at 2 weeks post-surgery. **Conclusion**: Results from thisstudy indicate that the ectopic osteoinduction causedincreased activity of the osteoblasts which subsequentlycaused increased serum levels of NO metabolites (nitritesand nitrates)

Keywords: Nitric oxide, osteoinduction, DBM, Rat

1951P

Measuring of Chemotactic Protein -1 Receptor Function

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Background: The objective of this practical work was to determine the adenylyl cyclase activity for evaluation the role of chemotactic protein-1.It is well known that Adenylcyclase is a member bound enzyme that catalyses the conversion of ATP to c-AMP. The inhibition level of adenylyl cyclase was determined by measuring the ability of the chemotactic protein-1 to inhibit the enzyme activity. **Methods**: In this study, the experiments were carried out on rats and end point of experiments animals were killed in the condition of anesthesia and heart tissue was prepared. Measurement of adenylyl cyclase activity was performed according to the procedure described previously by Wiegn et al. method. **Results**: On the basis of our findings Adenylycyclase activity in the present chemotactic protein-1 was decreased when compared to the control, without chemotactic protein-1 (5.2 ±0.8 vs. 7.8 ± 0.8 mmol/mg/min).It will be revealed that chemotatctic protein-1 significantly reduced adenylyl cyclase activity. **Conclusion**: In this study, we have demonstrated that chemotatctic protein-1 inhibit adenylylcyclaseenzyme.

Keywords: Adenylyl cyclase, Chemotactic protein-1, Chemokine

2998P

The serum levels of IL-17a and IL-10 in patients with Osteoporosis and Healthy controls

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Background: Osteoporosis is one of the most important health problems in old people worldwide.As a chronic inflammatory disease in osteoporosis the bone density is gradually decreased somehow it ultimately results in the bone fracture.IL-17a as a pro inflammatory cytokine and IL-10 as an anti-inflammatory cytokine could play promoting or protective roles in osteoporosis respectively. **Methods**: Accordingly, as a preliminary study we determined the levels of IL-17a and IL-10 by ELISA in sera of 42 subjects with osteoporosis (20 men and 22 women, mean of age 69.4±6.73) who had the T score≤-2.5, as well as in sera of 39 healthy controls (20 men and 19 women, mean of age70.77±7.12) with T score>-1. The serum samples were prepared from the population based Amir Kola Health and Ageing Project (AHAP) cohort tissue bank which has 1616 serum specimens. Two groups were matched from sex and age viewpoints. The subjects who were using corticosteroids drugs were excluded from study. **Results**: We were not able to find any significant difference in serum levels of

IL-17a between two groups, however the patient group had a higher level of circulating IL-10 in comparison to healthy control p=0.015.we were not able to find a significant correlation between sex and age of subjects with their serum levels of IL-10. **Conclusion**: Our study showed that IL10 could not be considered as a cytokine with protective role in pathogenesis of osteoporosis. However, it could be concluded that IL-10 production may accelerated in reaction to this disease. Moreover, IL-17a neither has a significant protective nor promoting role in osteoporosis.

Keyword: osteoporosis, Inflammation, IL-10, IL-17a

1592P

Investigation of the expression of mediators of neovascularization from peripheral blood mononuclear cells in Buerger's disease

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Background: The aim of this study was to investigate the expression of the cytokines, chemokines and effective molecules of peripheral blood mononuclear cells (PBMCs) that play a role in neovascularization in Buerger's disease (BD). **Methods:** Lymphocytes from BD patients (n = 20) and control subjects (healthy smokers [n = 16] and non-smokers [n = 17]) were evaluated using realtime polymerase chain reaction in order to examine the mRNA expression of CXCL1 and interleukin 8 (IL-8; inducers of collateral development by recruitment of circulating progenitor cells [CPCs]), endothelial cell growth factor A (VEGF-A) and inducible nitric oxide synthase (iNOS; inducers of angiogenesis) and interferon gamma (IFN-γ) and vascular endothelial growth factor receptor 1 (VEGFR-1; inhibitors of angiogenesis). **Results:** CXCL1 expression was significantly higher in the BD patients than control subjects. The expressions of IL-8, VEGFR-1 and IFN-γ were significantly higher in the BD patients and smokers than in non-smokers. However, no differences in iNOS and VEGF-A expression were noted. **Conclusion:** In conclusion, PBMCs from BD patients expressed cytokines that potentially recruit CPCs and promote arteriogenesis. However, BD patients typically have low CPC levels, perhaps due to high oxidative stress. Further studies are recommended in order to investigate the efficacy of antioxidant therapy on the outcome of BD before administration of angiogenic factors.

Keywords: Angiogenesis, Vasculitis, Buerger's disease

1672P

Correlation of CCL5 and CCL18 serum levels with cardiac dysfunction in patients with acute anterior myocardial infarction

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Background: Myocardial infarction (MI) is the leading cause of morbidity and mortality

in Iran. Inflammation plays a critical role in post-MI Left ventricular (LV) remodeling and is associated with unfavorable outcomes. Chemokines are important players in myocardial healing after MI. Accordingly, low CCL5serum level is an independent predictor of cardiac mortality and CCL18 is an independent predictor of refractory ischemic symptoms in patients with unstable angina pectoris (UAP). Since, no study is performed on these chemokines in Iranian patients with acute MI (AMI)as well as CCL18 globally; we investigatedCCL5 and CCL18levels in patients with AMI. **Methods:** Five milliliter blood was collected from 50 patients who diagnosed to have acute anterior MI at the time of hospitalization. The serum levels of CCL5 and CCL18 were measured using commercial ELISA assays. **Results:** Decreased level of CCL5was significantly associated with Left atrial (LA) enlargement (46.3 ± 16.9ng/ml) compared to normal LA (72.1 ± 39.1ng/ml, P=0.003).Moreover, decreased CCL18 was observed in patients with grade 3 diastolic dysfunction (DD) (65.6 ± 9.8ng/ml) compared to the patients with normal DD (157 ± 77.9ng/ml, P=0.021). Although the level of CCL5 and CCL18 were different between groups of patients with different MI manifestations such as PR interval, axis deviation, etc., these differences were not significant (P > 0.05). **Conclusion:** Serum levels of the CCL5 and CCL18are related with structural and functional cardiac complications; however, to delineate their predictive value the follow up of the patients is ongoing.

Keywords: CCL5, CCL18, Myocardial dysfunction

2808P

Effect of polyunsaturated fatty acids on fatty acid binding protein 4 in ectopic endometrial cells

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Background: Endometriosis, a common chronic inflammatory disorder leading to infertility and pelvic pain, is defined by the atypical growth of endometrium- like -tissue outside of the uterus. The fatty-acid-binding proteins (FABPs) are a family of hydrophobic ligand-binding proteins such as fatty acids. FABP4 seems to be involved in the inflammation. Differentially expressed FABP4 gene in ectopic versus match eutopic endometrium has been reported. Potential anti-inflammatory effects of ω-3 and ω-6 fatty acids have been suggested. The objective of this study was to evaluate the effects of ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) interventions in endometrial cells culture on the level of FABP4 comparing match ectopic versus eutopic endometrial cells from endometriosis patients. **Methods:** Ectopic and eutopic endometrial tissues were obtained from 15 women were snap frozen. After thawing and tissue digestion, primary mixed stromal and endometrial gland cell culture was performed for 8 days in culture mediums supplemented with normal and high ratios of ω-3 and ω-6 PUFA.

FABP4 level was determined using enzyme immuno assay (EIA) technique. **Results:** Within ectopic endometrial cells group, the FABP4 level was considerably increased in the presence of high ω -3: ω -6 PUFA ratio compared with control medium (P=0.014). Both balanced and high ω-6 PUFAs ratio lead to increasing in FABP4 level of ectopic endometrial cells compared with control.**Conclusion:** ω -3 PUFAs by increasing the level of FABP4 in ectopic endometrial cells could contribute to prevention of endometriosis progression. Further studies are required to elucidate the precise mechanisms in this field.

Keywords: Endometriosis, Fatty acid binding protein 4, ω-3 poly unsaturated fatty acids, Cell culture

3143P

Molecular association *pks* + *E. coli* with chronic inflammation and developing cancer colon in Iran

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Background: Colon cancer has the 4thrank among cancers in men and 2nd in women in the worldwide. Enviroment and life style, dietary habits and recently intestinal bacteria identified as risk factor leading to development of cancer colon. Chronic inflammation forms of human cancer especially Colon cancer. Switching Th17 to Th1 association increased chronic inflammation response which leads to activation notrophils and production of reactive oxygen species (ROS) that causing dysfunction epithelial cell. Subsequently *Escherichia coli*contain Pks Island the ability produce colibactin to penetrate gut epithelial cell and causes DNA double-strand breaks and leading to cell cycle arrest and cell death. **Methods:** In this study, we have collected 26 biopsies from patients with Colon cancer whom referred to colonoscopy section of ShahidBeheshtiHospital (in Qom) during Aug- Dec 2013. After isolating the *E.coli* and Extraction of their DNAs, multiplex PCR performed for 2 genes clbB and clbN as a specific site for PKS Island region. **Results:** The results showed that 10% frequency of PKs genes in the *E.coli* bacteria isolated from patients and normal subjects, respectively. **Conclusion:** This result may be indicates the capacity of *E.coli* to product colibactin and cause inflammation in the gut epithelial cell. In order to confirm this idea, performing molecular experiments on a large number of patients is necessary.

Keywords: *pks*+ *E. coli*, Chronic inflammation, Colon Cancer

3054P

The effect of acute and chronic administration of morphine on the NF-κB mRNA expression level in lumbar spinal cord of male rats in the absence and presence of carrageenan-induced inflammation

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Background: NF-κB plays a key role in regulation of immune response to stimuli such as stress, cytokines, free radicals and inflammation. Morphineis one of the most potent analgesic drugs used in clinical pain management and unwanted side effects such as tolerance and

dependence can limit its effectiveness. Several studies have demonstrated opioid modulation of NF-κB activation. Studies suggested that chronic inflammatory prevents tolerance to the antinociceptive effect of morphine. The purpose of this study is assay effect of acute and chronic morphine administration on mRNA expression level of NFκB1 in lumbar section of spinal cord of male rats, in the absence and presence of inflammation. **Methods:** Morphine (10mg/kg, twice daily) for 8 days resulted in tolerance development to analgesic effect of morphine. After RNA extraction and cDNA synthesis, quantification of relative RNA expression followed established methods using Real-Time quantitative PCR (qPCR) with SYBR green reporter dye and using designed specific primers. The relative mRNA expression levels were calculated by the $2^{\Delta\Delta CT}$.**Results:**The result of molecular experiments showed that the Acute and chronic morphine administration with carrageenan-induced inflammation decreasedNFκB1 gene expression level significantly. **Conclusion:** It suggests that different expression of NF-κB is effective in various effects of opioids in inflammation state.
Keywords: Morphine, NF-κB, Inflammation, Tolerance, Gene expression

2681P

Th17 deviation of peripheral blood memory CD4+ T cells in Atherosclerosis

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Background: Atherosclerosis is the most common cause of cardiac deaths worldwide. The disease is associated with systemic immune responses and chronic inflammation. The aim of this study was to determine the functional deviation of memory CD4+ T cells in Atherosclerosis. **Methods:** Blood samples were taken from 6 patients with confirmed angiography results of carotid artery atherosclerosis who were non-smoker, non-diabetic males with hyperlipidemia and high blood pressure (age=59±1.6 yrs) as well as 3 healthy non-smoker men (age=41.3±4.2 yrs). PBMCs was isolated and stained for CD4, CD45RO, IL-17 and IFN-γ both before and after activation with anti-CD3 and anti-CD28 antibodies. The frequency of IL-17and IFN-g producing memory CD4+CD45RO+ T cells was evaluated by four-color flowcytometery. **Results:** There were no IFN-g producing cells without stimulation in the memory CD4+CD45RO+ T cells of patients and controls. A relatively high percentage of cells were producing IL-17 both before (6.74±6.97%) and after (15.69±17.49%) stimulation compared to healthy controls (0.15±0.05% and 0.5±0.5%, p=0.07 and p=0.036, respectively). There was also a significant increase in the population of IFN-γ+IL-17+CD4+CD45RO+ T cells in patients (1.07±1.99%) and controls (0.04±0.04%) after stimulation (p=0.048). **Conclusion:** We observed a clear deviation of memory CD4+CD45RO+ T cells to Th17 cells in the peripheral blood of patients both ex-vivo and in-vitro. The ex-vivo secretion of IL-17 by a subpopulation of RORγt+ Th17 cells in other conditions is already reported. Further characterization of ex-vivo Th17 memory subpopulations and the TCR-stimulated ones will reveal the functional correlation of Th17 cells with atherosclresosis pathogenic inflammation.
Keywords: Th17, Atherosclerosis, Inflammation, Flowcytometery

2572P

Modulation of ROS-induced cell death and NOTCH signaling activation by Quercetin and EUK134

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Background: There is rising evidence for impact of oxidative damage to the brain in a wide variety of human diseases including neurodegenerative diseases. It is by now well accepted that reactive oxygen species (ROS) affect a wide variety of signaling factors encompassing Notch. Thus, Study of the relevant molecular mechanisms involved in these diseases is important to find the effective therapies for Neurodegenerative diseases. Further, we studied free radical scavenging potential of Quercetin and EUK134 comparatively. **Methods:** SK-N-MC cells treated with hydrogen peroxide (H_2O_2) and menadione (superoxideanion producer) to induce oxidative stress. The free radical scavenging capabilities of antioxidants was studied through the MTT assay, antioxidant enzymes activity assay, glutathione levels andtheextent of intracellular ROS level was also evaluated. Western blot analysis was used to evaluate Notch expression. **Results:** Our results showed that H_2O_2 and menadione significantly reduced the viability of cells. Moreover, ROS led to reduction of glutathione (GSH) levels and activity of antioxidant enzymes. However, pretreatment of the cells with Quercetin and/or EUK134 decreased ROS-induced cell death by restoration of catalase and glutathione peroxidase activities. The destructive effect of H_2O_2/menadione on the GSH level of the cells was almost restored by this compound. Western blot analysis revealed that ROS activates Notch signaling. To further confirm this event we also demonstrated that Quercetin and/or EUK134 restored Notch1 signaling activation. **Conclusion:** Comparatively, our data indicated that the antioxidant potential of quercetin was more effective than the antioxidant activity of EUK134 as well as it scavenged menadione more potent than H_2O_2.

Keywords: Oxidative stress, Notch signaling, Quercetin

2571P

Inhibition of H_2O_2-induced p53 up-regulation and cell death by Resveratrol in SK-N-MC cells

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Background: There is evidence for impact of oxidative damage to the brain in a wide variety of human diseases including neurodegenerative diseases. It is by now well accepted that reactive oxygen species (ROS) affect a wide variety of signaling factors which are involved in neurodegenerative diseases. However, the molecular mechanism of the oxidative stress-associated apoptosis is far to be elucidated. Herein, we investigated whether p53, which is involved in many signaling pathways, is affected by oxidative stress. **Methods:** Cells treated with hydrogen peroxide (H_2O_2) to induce oxidative stress. The free radical scavenging capabilities of Resveratrol was studied through the MTT assay and glutathione (GSH) levels. The extent of lipid peroxidation, protein carbonyl formation and intracellular ROS level as markers of oxidative stress were also studied. Western blot analysis was used to evaluate proteins expression. **Results:** Our results revealed that H_2O_2 significantly reduced the viability of cells through up-regulation of p53 followed by increase in Bax/Bcl2 ratio. Moreover,H_2O_2

led to reduction of GSH levels. However, Resveratrol protected cells against ROS-induced cell death by down-regulation of lipid peroxidation and protein carbonyl formation as well as restoration of GSH levels. Resveratrol also increased cell content of MDM2 followed by decrease in intracellular levels of p53, p21 and Bax compared to ROS-treated cells. **Conclusion:** Our results indicated that Resveratrol can be a promising candidate in antioxidant therapy and designing of drugs for ROS-induced neurodegenerative diseases.

Keywords: Neurodegenerative disease, Reactive oxygen species, Resveratrol, oxidative stress

3020P

Peripheral Blood Mononuclear Cell (PBMC) Resistin mRNA Expression in patients with Coronary Artery Disease (CAD)and type 2 diabetes in comparison with the control group

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Background: A common cause of Coronary Artery Disease (CAD) is atherosclerosis that is a chronic inflammatory condition in which inflammatory mechanisms interact with metabolic disorder to accumulate lipid in the artery wall. Atherosclerosis has a strong association with insulin resistance and metabolic syndrome. Resistin that produced by adipose tissue and macrophage is an adipocytokine that may be acting as immune regulators to effect on inflammatory processes in atherosclerosis. This adipocytokine can be linked between metabolic disorder and inflammatory process in diabetes and atherosclerosis. For that reason, we measured serum concentration of visfatin in CAD patient with or without diabetes, diabetic patients in compression with healthy subjects. **Method:** A case-control study comprising of angiographically confirmed CAD as cases (12 with diabetes mellitus type 2 and 13 without T2DM), 15 T2DM as cases and 13 normal healthy individual as controls was undertaken. PBMC extracted from whole blood and then RNA extracted from PBMC. We compare levels of resistin gene expression in PBMC from participating subjects using real time RT-PCR. **Results:** A significant increase was observed in PBMC resistin mRNA levels in CAD patient with or without T2DM and diabetic patients in comparison control group. Also resistin mRNA levels were higher although non-significant in CAD patients with T2DM as compare to other groups. **Conclusion:** Our study suggests that, increase resistin in inflammatory condition and metabolic disorder may be having an important role in metabolic disorder and insulin resistance in T2DM with inflammation in atherosclerosis. Further studies are required to confirm this finding.

Keywords: PBMC, Resistin, Coronary Artery Disease, Type 2 diabetes

3274P

Toll-like receptor2 expression in lumbar spinal cord of rat: differential regulation by acute and chronic administration of morphine in absence or presence of inflammation

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Background: Toll-like receptor 2 (TLR2), a key immune receptor in the TLR family, is widely expressed in various systems, including the immune and nervous systems and plays a critical role in controlling innate and adaptive immune responses. There is evidence showing intracellular cross talk between MOR signaling and TLR signaling in various kinds of cells.The purpose of this study was to assess the effects of acute and chronic administration of morphine on the TLR2 gene expression levels in lumbar spinal cord of male rats in absence or presence of carrageenan induced inflammation. **Methods**: Morphine (10mg/kg, twice daily) for 8 days resulted in tolerance development to analgesic effect of morphin. Inflammation was induced by injection of 1.5% saline solution of sigma carrageenan (0.1 ml) into the plantar surface of the rat, s paw. After RNA extraction and cDNA synthesis, Quantification of relative RNA expression followed established methods using real time quantitative PCR (qPCR) with the SYBR green reporter dye and using designed specific primers. The relative mRNA expression levels were calculated by the 2ΔΔCT. **results**: The results of molecular experiments showed that the acute and chronic administration of morphine alone increased TLR2 gene expression levels in compared to controls groups. Also the results of this study showed significant increase on TLR2 mRNA expression levels in rats resistant to morphine compared to the rats taking acute morphine in presence of carrageenan induced inflammation. **Conclusions**: Taken together, the results obtained using real time RT-PCR suggested that difference in TLR2 mRNA expression levels following the chronic administration of morphine between animals with and without inflammation could influence, at least in part, opioid responsiveness.

Keyword: Morphine, TLR2, mRNA expression, Carrageenan

3094P

Evaluation of ESWL-induced renal injury: Changes in urinary level of IL-1α and IL-6

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Background: Exracorporeal Shock Wave Lithotripsy (ESWL) was established a dramatic effect on the treatment of urinary stones. According to the growing utilization of ESWL as the major method of urinary stones treatment and the reports based on following renal tissue damage, we decided to examine severity of ESWL-induced renal tissue damage and its related factors by using the monitoring of urinary levels of cytokines. **Methods:** In this study, the urinary samples of 32 patients with urolithiasis were taken before, 24 hours and 14 days after ESWL and IL-1α and IL-6 levels were measured by ELISA method. **Results:** Our findings indicated that there was significant difference in increment of urinary levels of both IL-1α and IL-6 before, until 24 hrs and after ESWL, before until 14 days after ESWL and 24 hrs until 14 days after ESWL. **Conclusion:** According to our results, ESWL leads to an inflammatory process in the urinary tract and the inflammation continues to increase up to 14 days to process.

Keywords: Lithotripsy, Interleukins, Urinary calculi, Inflammation

3125P

Serum gamma irradiation *Ichthyophthirius multifiliis* lysozyme levels in rainbow trout (*Oncorhynchus mykiss*)

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Background: The freshwater ciliate *Ichthyophthirius multifiliis*, one of the most important protozoan pathogens of farmed fish populations, causes significant economic losses to aquaculture industry. It is difficult to control this parasite using chemotherapy after penetration into fish skin and gills. Thus, vaccination against *I.multifiliis* can be considered as an alternative to chemical treatments. In this study, the effects of irradiated *I. multifiliis* trophonts on physiological and biochemical components of blood were studied for a period of 30 days. **Methods**: Healthy rainbow trout (30-40 g) prepared. *I.multifiliis* was obtained from heavily infected rainbow trout. Dose of gamma rays: 100, 150, and 200 Gray were used for irradiation of parasite samples. Two hundred and forty fish were allocated in four groups (in triplicate) of 20 fish distributed per aquarium. One group was control fish and other groups were fish exposed to gamma-irradiated *I.multifiliis* at 100, 150 and 250 Gray. On days 20 and 30, 10 fish from each aquarium were bled. Serum was isolated by centrifugation. **Results**: showed that total protein, globulin and albumin level increased in fish treated with 100 and 150 Gray gamma-irradiated trophonts. Fish exposed to 150 Gray treated parasites showed significant increase in serum lysozyme activity. Serum alkaline phosphatase level was significantly lower in treatment groups. Esetras levels were also higher in treatment groups compared to control group on days 20 and 30.**Conclusion**: This information suggests that rainbow trout appears to benefit from treatment with gamma-irradiated *I. multifiliis* trophonts especially at 150 Gray.

Keywords: Gamma-irradiation, *Ichthyophthirius multifiliis,* Trophonts, Rainbow trout

3294P

Neutrophil Phagocytosis and Killing of *Staphylococcus aureus* decreased in cattle infected with Theileria *annulata*

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Background: Theileria annulata is a tick-transmitted protozoan parasite of cattle and causes the severe lymphoproliferative disease, tropical theileriosis is endemic in Iran. **Methods:** Twenty infected heifer with theileria *annulata* as case group, and twenty healthy crossbred Holstein heifers as control group were selected. Blood samples were collected from jugular vein into heparinzed vacutainer tubes and were processed within 2 hours after collection. The percentage of parasitemia and White blood cell (WBC) counts were determined. Neutrophils were isolated and studied for the ability of neutrophils on *in vitro* phagocytosis and killing of *S. aureus*. The nitrobluetetrazolium (NBT) reduction test was used to identify the dead bacteria that were killed by neutrophils. **Results:** The results of this study revealed that phagocytosis process was affected by theileria *annulata*, and there was a significant reduction in phagocytosis

potential and also reduction in the ability of bacteria killing of neutrophils (P<0.01). There was a significant negative correlation between severity of parasitemia and the ability of neutrophils to phagocyte and killing S.*aureus* (respectively r=-0.87, r=-0.71, P<0.01). Also significant decrease in white blood cell counts was seen in case group in comparison with healthy group (P<0.05).**Conclusion:** This study showed that in addition of lymphocytes that lose their functions in producing immunological response to disease, infection by *T. annulata*also leads to the down-regulation of neutrophil activity.

Keywords: Neutrophil, Phagocytosis, *Staphylococcus aureus*, cattle, *Theileriaannulata*

3228P

Infliximab and Etanercept effect different lyon the production ofinterleukin-10 cytokine in U937 cell line

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Background: Inflammatory bowel disease (IBD) consists of two idiopathic inflammatory ulcerative colitis (UC) and Crohn's disease (CD).Crohn›s disease is an inflammatory chronic disorder of the digestive tract that is usually localised in the terminal ileum. The first known NOD2/CARD15 gene is associated with Crohn›s disease, which is located on chromosome 16. Increased CARD15 expression was detected in Crohn's disease. Infliximab is a monoclonal antibody against TNF-α used to treat Crohn's diseases. Although Etanercept is a TNF-alpha blockeras well, it does not show such an effect on Crohn's disease. The aim of this study was to evaluate the effect oftheCARD15geneover expression in U937monocytic cell linefor production of proinflammatory and anti-inflammatory cytokines after incubation with the Infliximab and Etanercept drugs separately. **Methods**: At first CARD15 gene was cloned into pBK-CMV plasmid and transfected toU937 cells with lipofectamine.CARD15geneover expression,assayed by the use of Western blotting and IL-10 production analyzed by ELISA. **Results**: Infliximab significantly increased IL-10 secretion level. However, Etanercept treated cells reduced IL-10 secretion level. **Conclusion:** We suggest that Infliximab and Etanercept effect differentlyon the production ofinterleukin-10 cytokine in U937 cell line. Infleximab increases IL-10 production, whereas Etanercept reduces IL-10 level.

Keywords: Crohn'sdisease, CARD15, Infliximab, IL-10, U937 cell line

3346P

Neutralizing effect of anti -staphylococcal enterotoxin B antibody on nitric oxide production by splenocytes

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Background: Staphylococcus enterotoxin B (SEB) is an exotoxin produced by gram positive bacteria staphylococcus aureus. SEB is asuperantigen that act through T lymphocyte

proliferation and inflammatory cytokine production that has a stimulatory effect on nitric oxide production by macrophages. One of the most important methods to neutralize toxin effect is toxin inactivation by interaction with specific antibody. In this research we determine the neutralizing potential of monoclonal antibody against SEB. **Methods:** Spleens of five Balb/c mice removed and homogenized. Splenocytes counted after RBC lysis and cultured in DMEM containing 10%FBS in 10^6cells/well. Anti-SEB antibody diluted in PBS at 10 folds serial dilution from 1:10 to 1:100000.500 µl of diluted antibodies co-cultured with 500µl of 5µg/ml SEB antigen for 2hours.After incubation, co-cultured solutions at different dilutions added to splenocytes. SEB added to splenocytes as positive control. After 72h incubation, nitric oxide measured by Griess method. **Results:** According to amounts of nitric oxide production in the supernatants of splenocytes, we found that antibody can neutralize SEB effect by reduction of nitric oxide production. Results showed that antibody at 1:10 to 1:1000 dilution can neutralize SEB function. Nitric oxide production by splenocytes reduced from 250 µM in positive control group to 30µM in 1:10 antibody dilution-SEB co-culture group. **Conclusion:** Reduction of nitric oxide production by splenocytes in antibody-SEB co-cultured groups demonstrated that if antibodies pre-incubated with SEB can neutralize SEB stimulatory effect on splenocytes.

Keywords: Staphylococcal enterotoxin B, Nitric oxide, Splenocyte

3236P

Insect immunity, an antibacterial protein from immune induced haemolymph of American cockroach, *Periplaneta americana*

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Background: Antimicrobial peptides a roleplay as effectors substances in the immunity of vertebrate and invertebrate hosts. In the current study antimicrobial peptide was isolated from the haemolymph of the American cockroach, **Periplaneta Americana**. **Methods**: **Micrococcus luteus** as Gram-positive bacteria and **Escherichia coli** as Gram-negative bacteria were candidate for injection. Induction was done by injecting both bacteria into the abdominal cavity of two groups of cockroaches separately. The haemolymphs were collected 24 hours after post injection and initially tested against both bacteria. Subsequently, the immune induced haemolymph was purified by high performance liquid chromatography (HPLC) to separate the proteins responsible for the antibacterial activity. **Results**: The non-induced haemolymph did not show any activity against both bacteria whereas induced haemolymph exhibited high activity against **M. luteus** but did less against *Escherichia* **coli**. Two fractions showed antibacterial activity against **M. Luteus**. **F**inally the molecular weight of the isolated antibacterial proteins were determined as 72 kDa and 62 kDa using SDS-PAGE. **Conclusion:** Based on this research we found that induced haemolymph of American cockroaches has the ability to produce peptides to combat against Gram-positive bacteria when an immune challenge is mounted. Further work has to be done to sequence of the protein which it would be advantageous.

Keywords: Insect immunity, Antibacterial protein, Haemolymph, American cockroach, **Periplaneta Americana**

1428P

Induction of endogenous host defense peptide, cathelicidin by interferons plus lipopolysaccharide in cell model

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Background: Cathelicidin is a host defense peptide with a wide range of immunomodulatory activities. The Cellular Expression of these peptides could be tailored by cytokines and microbial products. The present study was designed to explore the effects of different types of interferons treatments on gene expression of cathelicidin. **Methods:** After cultivation of murine macrophages J774 cell line, treatments with IFNs and LPS were carried out. Cellular RNAs were extracted by Trizole method and the first strand complementary DNAs were synthesized by Reverse Transcription method. After that, the changes in expression of the genes were studied in compared with the house-keeping gene, beta-actin by Real Time PCR method and ΔΔCT analysis. **Results:** This study showed that the cathelicidin expression was not up-regulated by Type I and II of interferons, but in synergy with LPS, cathelicidin expression significantly up-regulated ($P<0.001$). **Conclusion:** Concerning to macrophage plasticity based on its microenvironment, changing in the milieu of macrophage by induction of its endogenous cathelicidin might be useful to modulate all stages of an inflammatory process.

Keywords: Interferons, Catelicidin, Real Time-RT PCR

1867P

The association of PSGI-1 VNTR polymorphisms with coronary artery disease

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Background: Atherosclerosis is an inflammatory disease resulting from an injury that leads to an increase in the adhesiveness and permeability of the endothelium to leukocytes or platelets. P-selectin and P-selectin glycoprotein Ligand-1 (PSGL-1) regulate the initial interaction between leukocyte, activated platelets and endothelial cells. Recently, a variable number of tandem repeats (VNTR) Polymorphism in PSGL-1 gene affecting the lengths of the extracellular domain of PSGL-1 and the distance of the p-selectin binding site to the cell surface has been described. There are limited number of studies reporting PSGL-1 polymorphism might affect the inflammatory response and thrombosis. Neutrophils carrying short alleles B and C exhibit a significantly lower capacity to bind activated platelets. These alleles consistently protect against transient ischemic attack. We explored the association between PSGL-1 VNTR polymorphism and the development of coronary artery disease (CAD). **Methods:** We genotyped 173 Iranian patients who had CAD in coronary angiography (CAD positive) and 176 control subject with normal (<50% stenosis) coronary angiography (CAD negative). The role of the PSGL-1 polymorphism was also evaluated according to the additional risk factors of age, sex, smoking, hypertension hypercholesterolemia and diabetes.

Results: The frequency of the B/B genotype and short allele B was significantly lower in CAD positive than CAD negatives. (P 0.0361, OR 0.33, 95% CI 0.11-0.67) multiple regression analysis revealed that B/B genotype and B allele had an independent protective effect on the development of CAD (P 0.0145 OR 0.54, 95%CI 0.33-0.89).**Conclusion:** We found an interesting association between a functional polymorphism and the risk of CAD. According to our results, the short B PSGL-1 allele and BB genotype might protect against CAD, probably because of their lesser adhesive capacity. No association was found between pscl-1 VNTR polymorphism and severity and number of coronary artery stenosis.

Keyword: p-selectin, p-selectin glycoprotein ligand-1 VNTR, Polymorphism, Coronary artery disease (CAD)

1775P

Flow Cytometric Analysis of Inflammatory Cells in Experimental Acute Pancreatitis

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Background: Accumulating evidence indicate that inflammatory cells migrate into the pancreas tissue and play an important role in the pathogenesis of acute pancreatitis (AP). The aim of this study was to establish a flow cytometric method to enumerate these infiltrating cells in the pancreas of an experimental AP. **Methods:** Twelve hours after inducing of AP, mice pancreatic tissues were cut into small fragments and single cells were prepared by mechanical dissociation. The isolated cells were stained with either anti-mouse CD45-PerCP or isotype antibody and analyzed by flow cytometry. Using side scatter (SSC)/CD45 gating we were able to identify inflammatory cells from non-inflammatory cells. **Results:** The mean percentage of leukocytes were 5.9±1.6 in the control group whereas, it was 26.7 ±8.1 in the AP. Moreover, we found that the percentage of lymphocytes, monocytes and granulocytes were 1.1±0.2, 0.9±.04 and 2.9±1.8 of total pancreatic cells, respectively, in the control mice. In contrast to lymphocytes, the percentage of monocytes and granulocytes were significantly increased in the AP group and it was 3±1.3 and 18.2±3.2 for monocytes and granulocytes, respectively. **Conclusion:** In conclusion, quantitative flow cytometric analysis is feasible and provides a reliable and rapid assay to determine the number and percentage of inflammatory cells in experimental AP.

Keywords: Inflammatory cells, Acute pancreatitis, Flow cytometry

1833P

Glycyrrhizin attenuates tissue injury and reduces neutrophil accumulation in experimental acute pancreatitis

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Background: Leukocyte infiltration and acinar cell injury are characteristic features of acute pancreatitis (AP). However, the signaling pathways regulating inflammation and accumulation of leukocytes into pancreas tissue remains poorly elucidated. In the current study, we investigated the effects of Glycyrrhizin (GZ) on cerulein-induced AP in mice. **Methods:** AP was induced in male C57BL/6 by intraperitoneal injection of 50 **Results:** We found that GZ treatment resulted in reduction (i) both amylase and lipase activities, (ii) the serum levels of both MCP-1 and MIP-2; and (iii) markedly attenuated cerulein-induced histopathological alternations and water contents. Furthermore, we observed that GZ significantly decreased the number of infiltrated monocytes and neutrophils into the pancreas tissue. **Conclusion:** In conclusion, we demonstrate that GZ attenuates AP signs and inhibits inflammatory cell recruitments into pancreas.

Keywords: Glycyrrhizin, neutrophil accumulation, Cerulein

1731P

Evaluation of inflammatory process in testicular tissue following long term administration of ciprofloxacin in male NMRI mice

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Background: Although the therapeutic and prophylactic effects of ciprofloxacin (CPFX) on different gram-positive and –negative bacteria has been well documented, various studies reported that even short term administration of CPFX promoted male reproductive toxicity. In the present study we sought to elucidate the correlation of increased mast cells number induced by CPFX and testicular inflammatory disorders. **Methods:** Twenty four mature male NMRI mice were randomly divided into three groups. Two groups received low dose (206mg/kg body weight) and high dose (412mg/kg body weight) of CPFX and the remained group, control, received carboxymetyl cellulose p.o. for 45 consecutive days. At the end of the study the animals were sacrificed and testicular tissues were removed and prepared for histological assessment using Toluidine-blue and H&E staining methods. **Results:** Toluidine-blue staining confirmed that the number of mast cells in the testicular tissue in CPFX-treated groups was significantly higher than of the control group. Testicular damage in CPFX-treated mice was evident as obvious edema, increased interstitial space and germinal epithelium disintegration. Also CPFX caused a significant increase in the percentage of sloughing tubules. **Conclusion:** Increased amount of testicular mast cells is associated with testicular damage and spermatogenetic disorders which may be due to increased permeability of small venules resulting in tissue edema and augmentation of free radicals from degranulated mast cells after immunologic stimulus.

Keywords: Ciprofloxacin, Mice, Mast cells, Testicular tissue

1474P

Tiotropium Effects on Airway Inflammatory Events in the Cat as an Animal Model for Acute Cigarette Smoke-Induced Lung Inflammation

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Background*:* Chronic obstructive pulmonary disease is an inflammatory lung disease mainly caused by tobacco smoke inhalation. **Methods***:* Fifteen healthy adult male cats were categorized into 3 groups: I) control group, II) exposed to cigarette smoke (CS), and III) exposed to CS treated with tiotropium. **Results:** Increases in clinical signs and airway responsiveness in CS cats were found compared with control animals. These airway hyper-responsiveness and clinical signs were significantly attenuated by treatment with tiotropium. The CS-induced pulmonary release of interleukin-6, interleukin-8, monocyte chemotactic protein-1, and tumor necrosis factor alpha was reduced with tiotropium treatment. Exposure to CS significantly increased total inflammatory cell number in bronchoalveolar lavage fluid, which was significantly attenuated by treatment with tiotropium. The numbers of macrophages, eosinophils and neutrophils and lymphocytes were all increased after exposure to CS. Tiotropium significantly reduced the number of all these cells. Perivascular, peribronchiolar infiltration of inflammatory cells and Reid index increased in the CS group. Treatment with tiotropium significantly reduced these parameters to control level. Enhanced lipid peroxidation with concomitant reduction of antioxidants status was observed in the CS group. Tiotropium significantly reduced the serum, lung lavage, lung and tracheal tissue lipid peroxides to near control levels. Tiotropium also decreased lung and tracheal protein leakage, and prevented the reduction of total antioxidant status in serum, lung lavage, lung and tracheal tissue of the CS group**. Conclusion:** Cigarette smoke increases airway responsiveness and inflammation in a cat model of CS induced lung inflammation, which can effectively be reduced by treatment with tiotropium.

Keywords: Cigarette smoke, Lung inflammation. Tiotropium, Animal Model, Cat

1674P

Supernatant of bone marrow-derived mesenchymal stem cells pulsed with 17-β-esteradiol modulates the function of neutrophils

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Background: Mesenchymal stem cells (MSCs) are multipotent and may be valuable tools for cell based immunotherapy. This survey was done to investigate the interaction between17-β-esteradiol treated MSCs and Blood neutrophil functions. **Methods**: MSCs were isolated from bone marrow of male rats and pulsed with 17-β-esteradiol at concentration 20 nM for 72 h. Next, the supernatants of MSCs co-cultured with neutrophils for 4h and neutrophil

functions were assumed. **Results**: Data showed that the supernatant of MSCs treated with 17-β-esteradiol could significantly increase the phagocytosis of *C.albicans* by neutrophils and conversely, decrease the respiratory burst intensity of neutrophils. Moreover, treatment of MSCs with 17-β-esteradiol can cause a significant decrease in percent of neutrophils apoptosis. **Conclusion:** 17-β-esteradiol has important effect on interaction between MSCs and neutrophils.
Keywords: 17-β-esteradiol, Mesenchymal stem cells, Neutrophils

1705P

Investigation of the effect of Carbenoxolone in reduction of inflammation severity in a mouse model

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Background: Heat shock proteins (HSPs) inhibit production of pro- inflammatory cytokines. Carbenoxolone induces the production of HSPs. The aim of this study is the evaluation of the effect of Carbenoxolone in reduction of the severity of systemic inflammation induced by LPS in a murine model. **Methods:** 80% lethal dose of LPS was determined in balb/C mice, also appropriate dose of drug for efficient reduction in mortality was determined. LPS+D-GalN with drug were injected to different groups, in different times, after 24 hours the mortality rate was calculated and the severity of necrosis measured in the kidney and livers of survived mice. After 3 hours serum samples were collected and the concentrations of TNF-α were measured in each group. **Results and conclusion:** Administration of Carbenoxolone with LPS reduced the lethality rate from 80% to %20. The severity of necrosis in the liverand kidney of drug administrated groups were significantly decreased in compare with positive control group. The concentration of TNF-α in mice received LPS were significantly increased in compare with control group (P=0.032). Carbenoxolone significantly reduced the severity and damage due to endotoxic shock.
Keywords: LPS, Carbenoxolone disodium salt, TNF-α, Inflammation

1779P

Effect of long-chain fatty acids on secretory phospholipase A2IIa of ectopic and matched eutopic endometrial cells in the culture media

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Background: Endometriosis is a common chronic inflammatory disorder related to the presence of endometrial tissue outside the uterus. Secretory phospholipase A2 group IIa (sPLA2-IIa) plays an important role in the pathogenesis of inflammatory diseases as an acute phase

reactant. In this regards, sPLA2IIa gene is significantly up-regulated in ectopic compared with matched eutopic endometrium from endometriosis patients. Due to reported potential anti-inflammatory effects of ω-3 and ω-6 fatty acids, therefore, the purpose of the present study was to investigate the effects of ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) on sPLA2IIa level in cultured endometrial cells. **Methods:** Ectopic and eutopic endometrial biopsies were taken from 15 women enrolled at the Infertility Clinic of Avicenna Center. The specimens were immediately frozen for future culture. Following thawing and tissue digestion, primary mixed stromal and endometrial gland cells were cultured for 8 days in culture media supplemented with balanced and high ratios of ω-3 and ω-6 fatty acids. The level of secreted sPLA2IIa was detected by enzyme immunoassay (EIA) technique. **Results:** Within ectopic endometrial cells group, the level of cell secreted sPLA2IIa was remarkably increased under high ω-3 PUFA exposure compared with control condition ($P<0.05$). Both high ω-3:ω-6 and ω-6:ω-3 PUFAs ratio lead to increasing in secreted sPLAIIa of eutopic endometrial cells compared with control, although this difference was not statistically significant. **Conclusion:** ω-3 PUFAs through increasing the sPLAIIa level in ectopic endometrial cells, might prevent the progression of endometriosis, more research is encouraged to know the precise mechanisms in this area.

Keywords: Endometriosis, Secretory phospholipaseA2IIa, ω-3 poly unsaturated fatty acids, Cell culture.

2260P

Role of the eccentric exercise-induced delayed-onset muscle soreness effects on the regulation of the expression of S100A12, RAGE and NFκB

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Background: Present study was aimed to determine the regulatory impacts of delayed-onset muscle soreness (DOMS), muscle damage and inflammatory responses to eccentric exercise and other related mechanisms. **Methods:** In this study ten healthy men performed an arm exercise with left hand on a special chair designed for maximum voluntary contraction. They performed 7 sets of 10 repetitions with 80% 1RM and 1-min rest interval between sets. The DOMS was evaluated by a visual analog scale (VAS). Blood samples were collected before, immediately after, 1h, 24h and 48h after exercise and the white blood cell (WBC) count, C-reactive protein (CRP) and creatine kinase (CK) levels as plasma markers of muscle damage were analyzed. In parallel with serum S100A12 levels as pro-inflammatory marker of plasma the S100A12, RAGE and NFκB genes expression was also detected using enzyme linked-immunosorbent assay (ELISA) and real-time-PCR. **Results:** All of subjects complained muscle soreness on subsequent days and VAS which was peaked at 48h after exercise. Serum CK levels were significantly increased at 48h as compared with its pre-exercise levels which was correlated with the elevated of VAS after 48h. The circulating levels of S100A12 were also significantly enhanced at 24h due to exercise. The significant up-regulation of S100A12 and RAGE mRNA expression were also observed compared with basal levels, whereas no significant change was observed for NF-κB. **Conclusion:** These data suggested that over-expression of S100A12

mRNA and protein along with S100A12 its receptor (RAGE) in granulocytes may occur as a result of damaging eccentric exercise in arm-rise exercise. Furthermore, it appears that the transcription of S100A12 and RAGE may play a fundamental role in the amplification of the inflammatory responses post exercise muscle soreness, possibly throughout activation of NF-κB or MAP-kinase intracellular signaling pathways simultaneously with the release of inflammatory cytokines.

Keywords: Eccentric exercise, gene expression, S100A12, RAGE, NFκB, DOMS

2433P

Nicotine inhibits TNF expression in human macrophage like cell line (U937)

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Background: Tumor necrosis factor alpha (TNF-α) is a main inflammatory cytokine that plays a crucial role in many inflammatory related pathological situation. Nicotine is a cholinergic agonist with potential anti inflammatory properties. In the present study we examined the effect of nicotine on TNF expression in human macrophage like cell line. **Methods:** Human macrophage like cell line (U937) was cultured in RPMI-1640 medium with or without the presence of LPS. Then different doses of nicotine were added to cultured cells. After 48 hours incubation, the number of cultured cells was counted and after RNA extraction cDNA was synthesized. The expression of TNF-α was assayed using specific primers and probes by a real time PCR instrument. **Results:** Our results showed that nicotine inhibits the proliferation of human macrophage like cell line in a dose dependant manner. Nicotine also reduced the expression of TNF-α in stimulated and non- stimulated macrophage cells. This inhibitory effect was more significant in unstimulated cells than LPS stimulated groups. **Conclusion:** The results of the present study confirm the inhibitory effect of nicotine on human macrophage like cell line. The potential inhibitory effect of nicotine on TNF-α expression can be consider as a novel therapeutic options for a wide variety of inflammation induced pathological situations.

Keywords: Macrophage, Nicotine, Proliferation, TNF-α

2340P

The effect of Rat mesenchymal stem cells and its supernatant on peripheral blood neutrophil phagocytosis

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Background: Mesenchymal stem cells (MSCs) are a population of adult stem cells that is a promising source for therapeutic applications. MSCs are used for cell-based therapies because of their immunomodulatory properties. Present study was performed effect of Rat MSCs and

soluble factors on neutrophil phagocytic function. **Methods:** Bone marrow MSCs obtained from femurs of six two-week old Rat and were cultured in DMEM medium. MSCs and its supernatant at ratios of 1:4, 1:2 and 3:4 adjacent peripheral blood neutrophils, neutrophil phagocytosis and respiratory burst function were measured by the Nitro Blue Tetrazolium reduction (NBT) test and phagocytosis with yeast Data were analyzed by SPSSsoftware, t-test and one-way ANOVA followed by Tukey test at a significance level of $P<0.05$. **Results:** The rate of neutrophil phagocytosis treated with MSCs, decreased compared to controls, but this difference was not significant ($P<0.05$). Percent phagocytosis increased in the treatment group than the control group in all soups, 1:4, 1:2, 3:4, that the increase was significant ($P<0.05$). The respiratory burst in neutrophils treated with MSCs, significantly decreased compared to the control group. Respiratory burst was increased in the groups treated with cell supernatant, this effect was significant only in the case of 1:2 and the two other than this difference was not significant. **Discussion:** Mesenchymal cell-cell interaction with neutrophils is remarkable for therapeutic strategies in diseases associated with neutrophil function in response to physiological and pathological cell therapy with MSCs.

Keywords: Mesenchymal stem cells, Supernatants, Neutrophil phagocytosis

1737P

The effects of steroid and STZ-induced diabetic on immune system through NO during wound healing

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Background: Wound healing is a dynamic and complex immuno-biochemical processes. Impaired wound healing is a common occurrence among diabetics and patients receiving glucocorticoid therapy. Nitric Oxide (NO) is a free radical with a diverse biological roles including host defense. The role (S) of NO in both normal and chronic wound is not clear thus, this study was designed at gaining further insights into the role of NO in impaired wound healing.**Methods:** in this study 14 Sprague Dawley (SD) male rats were assigned into two treatment and control groups. Rats in diabetic group (n=8) were injected with STZ (65mg/kg i.p.) 9 days before wounding. In second study steroid-treated rats (n=6) were given cortisone acetate (CA: 10 mg i.m.). Rats received full-thickness circular dermal wounds. Wound area was measured by VIA technique. Urine samples were collected 24-hrs before and post-wounding. Urine NO was measured by NO Analyzer. Student-t-test was used for statistical analysis and $P\leq 0.05$ assigned as significant differences between groups. **Results:** Post wound increase in urinary NO3- output were significantly reduced in both diabetic (47%) and CA-treated (40%) rats compared to control group (136% , $P\leq 0.0001$). Furthermore, NO3- output in diabetic and CA-treated rats fell within pre-wound baseline levels by day 13, while that of normal controls remained elevated (54%) for the entire 30 day post-wound period. **Conclusion:** These results suggest that impaired wound lack normal levels of endogenous NO may be due to reduced activity of immune cell such as macrophages during wound healing as shown by injection of LPS and increased level of NO and subsequent decline thereafter because of impaired immune system.

Keywords: Wound healing, Nitric Oxide (NO), Diabetes

Medical Ethics

Poster Presentations:

1662P

The survey of ethical among nursing students in Urmia university of medical sciences

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Background: Ethics in nursing care is very important and professional competence. In nursing, ethics means that the nursing care is based on the principles of bioethics. However the ethical care and ethical competence in nursing students are important unanswered questions. More studies should be carried out in this regard. **Methods:** This study is a qualitative study. 35 nursing students of the faculty of Nursing & Midwifery participated in the study. The interviews were analyzed by content analysis. **Results:** The findings of the analysis were classified in four categories, including clinical environment, curriculum, teacher qualifications and academic knowledge in the field of professional ethics, motivation and interest of students to the nursing profession. **Conclusion*:*** The findings indicated that the development of moral competence in nursing students and the same areas are effective. So the moral qualification barriers should be searched and managed in different dimensions.

Keywords: Competence, Ethical competency, Nursing ethics, Nursing Students

1963P

The implementation study of student attendance in School of Medicine, Tehran University of Medical Sciences with Universities and colleges of selected Countries

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Background: Studies revealed that usually one –third of students do not attend at classes. This is why most colleges, universities and medical centers take their efforts to use of explicit policies to prohibit the problem. The purpose of the research was the implementation study of attendance policies of Tehran University Of Medical Sciences with Colleges and Universities of selected countries.**Methods:** The qualitative research was used for undergraduate medical

students in the form of practical methodology in expanding education. So written documents for example syllabuses of practical immunology and student handbooks of USA (8), Europe (2), Australia (3) and Canada (2) as well as original university websites were extracted. **Results:** There was many components such as attendance policy and rules, in the mentioned ubiquitous documents to align and familiar the students with their university. Therefore by studying the information, it was possible to match the items with the standards of the original university which are usually the standards and legislation of the Supreme Planning Council. It is noteworthy to say if the information were not in the form of legislation, certainly they couldn't have been in the website of according university, available to students. **Conclusion:** One of obvious differences is the high level of permitted absence assumed for students in each semester in original university. While the process for preventing of students absence is much clear and modified in selected universities. In addition the penalty of absence, from exam, is stricter and more inflexible in original university than the selected ones.

Keywords: Attendance policy, Medical students, Student handbook, Syllabus, Absence penalty

3174P

Ethical and Professional Issues Training for Students and Staff of Laboratory Medicine

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Background: Increasingly wide applications of clinical laboratory in medical practice, has highlighted specific topics of professional and ethical issues in the laboratory. Globally accepted ethical standards should be considered in all clinical environments including medical labs, however little has been discussed about ethical issues in the lab. This study was conducted to assess the view of laboratory science educators as the main informants of clinical lab about necessity of a professional ethics course in laboratory science curriculum. **Methods**: Study population was 28 laboratory science educators at Mashhad University of Medical Sciences. A questionnaire was designed to assess instructors' views about the importance, quantity, quality, and the need to provide a professional ethics course for medical laboratory students. **Results:** The majority of respondents (85.7%) evaluated the importance of offering an ethics course for laboratory medicine at very high levels. Most educators (75%) believed that good clinical laboratory practice is extremely related to ethical issues. Respondents (89.3%) believed that the educating professional ethics issues will enhance service quality and performance of the future laboratories. Most professors suggested offering two credit points to cover such issues for these students (85.7%). **Conclusion:** Regarding specific ethical issues in clinical labs such as modality of taking samples, interaction with patients, how to give information about genetic tests, viral and malignancies, how to store data and access to medical records, the diagnosis of infertility, prenatal diagnosis, and legal issues in poisonings, we recommend to offer a separate course in laboratory science curriculum to cover the ethical issues in a clinical lab.

Keywords: Professional ethics, Laboratory medicine, Curriculum

Monoclonal Antibody

Oral Presentations:

2626O

Selection and evaluation of a specific single-chain fragment variable (scFv) antibody against Interleukin25 Receptor

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Background: Pharmacokinetics and pharmacodynamics of scFv antibodies provide potential therapeutic advantages over intact antibody molecules and also introduce scFvs as effective alternative agents to cytokines in cancer therapy. In this study we selected a specific scFv against interleukin 25 receptor (IL25R), a novel breast cancer antigen, for its use in breast cancer immunotherapy. **Methods:** Four rounds of panning were performed to isolate specific scFvs against an immunodominant epitope of IL25R extracellular domain from a naïve phage display library. PCR and DNA fingerprinting were performed on the clones after the panning process. The soluble form of the specific scFv was produced and the reactivity of the antibody was assessed by ELISA. Cell binding capacity of the selected scFv on MCF7 breast cancer cells was analyzed by flow cytometry. **Results:** PCR products of 20 selected clones against specific epitope showed the presence of scFv genes. Using Mva-I DNA fingerprinting, the most frequent pattern of the clones was determined. Indirect ELISA showed specific reaction of the specific scFv to the corresponding peptide. The selected scFv bound to 50% of MCF7 cells which was comparable to a commercial anti IL25R antibody binding. **Conclusion:** New discovery about IL25R expression on malignant breast cells which is associated with malignant growth has projected great therapeutic potential for IL25R targeting. We selected a specific anti IL25R scFv which can bind to breast cancer cell lines specifically. Further investigations are needed to determine the functional activity of the selected scFv on breast cancer cells.

Keywords: scFv, phage display, IL25R, Breast cancer, Immunotherapy,

2858O

Isolation and screening of scFv antibodies to a CCKB-R-derived peptide using phage display technology

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Background: Gastric cancers as the most widespread and fatal cause of malignancies in the Middle East area have been frequently associated to *Helicobacter pylori that* mediates its function in carcinogenesis through triggering gastrin-cholecystokinin B receptor (CCKB-R) axis. Therefore, blockage of gastrin-CCKB-R signaling pathway by monoclonal antibodies may be a promising therapeutic strategy in treatment of gastric cancers. In the current study, we used phage display technology to isolate single-chain variable fragment (scFv) antibody against the second extracellular loop (SEL) of CCKB-R with 30 amino acids. **Methods:** Semi-synthetic phage scFv antibody library J was panned against gradually decreasing concentration of the SEL of CCKB-R (200, 100, 100 and 50 nM, respectively for rounds 1to 4) by means of solution-phase biopanning (SPB), in which specific phage binders was separated from nonspecific binders using streptavidin-coated magnetic beads. Biopanning process was monitored by phage output and polyclonal phage ELISA. From round 4, scFv clones were screened for specific binding to the given peptide by scFv ELISA. **Results:** Phage outputs for rounds 1,2,3, and 4 were 1.3×10^8, 8.5×10^7, 2×10^8, and 2.4×10^8, respectively. Polyclonal phage ELISA showed increasing optical density at 450 with positive wells (coated with the peptide) to negative wells (without peptide) ratios of 18.5, 35, 54.7, and 68.8 folds for rounds 1,2,3, and 4, respectively. Screening specific binders by monoclonal scFv ELISA was identified 38% of scFv clones after round 4 to be positive for peptide binding. **Conclusion.** ScFv antibody fragments specific to SEL of CCKB-R was successfully enriched using the SPB method.
Keywords: gastric cancer, immunotherapy, phage display technology, scFv, and CCKB-R

2916O

Production and characterization of a peptide-base monoclonal antibody against CD44 variant 6

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Background: The CD44 family includes of 20 exons, nine of which encode the standard form of the molecule, while the excess exons can be inserted in different combinations into the membrane proximal region of the extracellular domain of the protein giving rise to variant isoforms (CD44v). It was found that the CD44 variants, chiefly CD44v6, regulate tumor invasion, progression, and metastasis of carcinoma in rat experimental models. **Methods:** Producing a high affinity monoclonal antibody against CD44v6 provides an important tool to monitor and trace CD44v6 function in different biological fluids. In this study, synthetic peptide CD44v6 was conjugated to KLH and injected into mice. After immunization, mouse myeloma SP2/0 cells were fused with murine splenocytes followed by selection of antibody producing hybridoma cells. After screening of different hybridoma colonies by ELISA, a high affinity antibody was selected and purified by affinity chromatography. Western blot, and flow cytometry experiments were used to characterize the antibody. **Results:** Six stable hybridoma cell lines, designated as 1H1, 1H2, 2A12, 2G11, 3H3, and 3H7 were obtained. In Western blot analysis proved that the antibody had specific binding to its antigen. Negative cell lines of CD44v6 (HL60, Jurkat and LNCap) and positive cell line of CD44v6 (Mo7e, U937) were

used to analyze cell surface expression of CD44v6. Flow cytometry results were shown the new MAbs recognize CD44v6 on cell surface. **Conclusion:** This novel panel of anti-CD44v6 antibodies has potential for investigating the role of CD44v6 in cancer pathogenesis.
Keywords: CD44 variant 6, Monoclonal antibody, Western blot, Flow cytometry

2405O
Generation of Novel Monoclonal Antibodies against Human PLAC1

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Background: PLAC1 (Placenta Specific 1) is a newly-identified cancer-testis antigen with restricted expression in placenta. At the transcriptional level, ectopic expression of this antigen has been recently reported in a wide array of tumors and cancer cell lines including those originated from breast, ovary, prostate and colon, to list a few. No monoclonal antibody, however, is available so far to delineate the expression of this marker at the protein level. Here, we were about to produce and functionally characterize murine monoclonal antibodies against human PLAC1. **Methods:** Using *in silico* studies, a PLAC1-specific peptide in extracellular region of PLAC1 was designed, produced and conjugated with a carrier protein. Monoclonal antibodies were produced by standard method and characterized by a series of protein read out systems including ELISA, western-blotting (WB), immunohistochemistry (IHC), and immunocytochemistry (ICC). **Results:** The clones exhibited specific pattern of reactivity in IHC staining of human term placenta as positive control. Immunostaining was mainly localized to the cytoplasm of syncytiotrophoblasts. One clone appeared to have excellent staining signal in breast, ovary and prostate cancer cell lines. In line with gene expression prediction data, no reactivity was found in normal human tissues including prostate, endometrium or lymph nodes. In contrary to a polyclonal antibody with specificity toward other region of PLAC1, our monoclonal antibodies failed to detect specific band of PLAC1 in western-blot analysis. **Conclusion:** Our results clearly showed that the monoclonal antibodies presented here are specific and valuable tools for *in situ* monitoring of PLAC1 expression by IHC and ICC.
Keywords: Anti-PLAC1, Immunohistochemistry, Monoclonal antibody, Placenta, PLAC1

2498O
Investigation of Monoclonal antibodies againt hCG and subunits on the proliferation of cancer cell lines in vitro

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Background: Human chorionic gonadotropin (hCG) and its individual subunits are secreted in many cancers. Some reports have demonstrated that anti-hCG antibodies prevent the growth of cancerous cells *in vitro* and *in vivo*. In this study, effects of monoclonal antibodies (mAb) against hCG and its subunits, were investigated on the proliferation of cancer cell lines in vitro. **Methods:** mAbs, T8B12 (against complete hCG), T9B11 (against β chain),T5C4 (against the alpha chain) and T7D9 (against the carboxyl terminal peptide of beta chain) were prepared from ascetic fluids. Specificity of mAbs was analyzed by ELISA and immunobloting. The affinity constant (k_{aff}) was also determined by ELISA and the anti-cancer effects of anti-hCGmAbs on various cancer cell lines were evaluated by cell proliferation assay. **Results:** K aff of T7D9, T8B12, T5C4 and T9B11 mAbs were calculated to be 4.7×10^8, 2.7×10^8, 1.6×10^8 and 0.68×10^8 M^{-1}, respectively. Anti-hCG T9B11 and T7D9 mAbs inhibited the growth of breast (T47D and SKBR-3), prostate (LNCap), testis (TM3), ovary (C13R) and cervical (HeLa) cancer cell lines. These mAbs augmented the proliferation of MCF7, CaOV4, OVCAR3 cell lines. In addition T5C4 mAb could inhibit the growth of T47D, SKBR-3 and C13R cell lines. **Conclusion:** The results show that anti-β hCG and anti CTP- β hCG, mAbs have inhibitory effects on cancer cell lines. These data suggest that there is a correlation between the expression of membrane associated β-hCG on cancer cell lines and efficacy of anti hCGmAbs on growth of cells.

Keywords: Anti-cancer effect, hCG, mAb, Subunits of hCG

Poster Presentations:

2458P

Production and characterization of a monoclonal antibody against CD11b

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Background: CD11b is an integrin family member which pairs with CD18 to form the CR3 heterodimer. CD11b is expressed on the surface of many leukocytes including monocytes, neutrophils, natural killer cells, granulocytes and macrophages, as well as on 8% of spleen cells and 44% of bone marrow cells. The purpose of this study was to produce monoclonal antibody against CD11b as a tool for diagnostic of leukocytes and research applications. **Methods:** ALB/c mice were immunized with two peptides from extracellular domain of CD11b. Poly Ethylene Glycol (PEG) fused spleen cells of the most immune mouse with SP2/0.Supernatant of hybridoma cells were screened for detection of antibody by ELISA. The desired clones were selected for limiting dilution. Afterward, specificity and cross reactivity of these antibodies were determined by immunological assay such as ELISA and western blot analysis (WB) and

Immunofluorescence. Large scale of monoclonal antibodies was produced by ascetic fluid method. Monoclonal antibody was purified by chromatography then confirmed by SDS-PAGE. **Results:**In this study, between five positive clone wells, 3 clones were chosen for limiting dilution. Limiting dilution product was one monoclone with absorbance about 1.5. Isotype of this mAb was identified as IgG1 class with Kappa (κ) light chain. **Conclusion:** These results indicate that such monoclonal antibodies against CD11b can be used in diagnosis of CD11b in the cells surface.

Keywords: Monoclonal antibody, CD11b, ELISA, Western blotting, Immunofluorescence

2434P

The effect of application of very low concentration of IPTG in high level expression of recombinant single-chain antibody fragments in *Escherichia coli*

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Background: Recombinant antibodies have been proposed as invaluable tools for various therapeutic and diagnostic purposes, and they can be considered as one of the potential candidates for substitution of expensive mouse monoclonal antibodies. Here we described the high level expression of single-chain Fv antibody fragments (scFv) in *E .coli* cells using pET26b vector. **Methods:** Randomly 10 different clones from Tomlinson library I were selected for expression soluble scFv antibodies by applying 1, 0.1 and 0.01 mM IPTG. Subsequently, the amounts of the scFvs produced in different subcellular compartments of bacterial cells were verified. **Results and Conclusion:** Our results indicated that the best concentration of inducer was 0.01 mM and at this condition nearly 45% of the recombinant protein was translocated into the periplasmic space. Thus, for high level production of these valuable proteinsin *E. coli*, it is recommended to use micromolar concentrations of inducer (0.01mM) by pET system.

Keywords: High level expression, scFv, pET, IPTG

2456P

Production of anti-CD14 monoclonal antibody using synthetic peptide of human CD14

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Background: CD14 is a myeloid differentiation antigen expressed primarily on peripheral blood monocytes, dendritic cells and macrophages. It is a key regulator of inflammatory responses to gram-negative bacteria, oxidative burst and septic shock.The aim of this study was to produce and characterize monoclonal antibody against CD14 for use in detection and diagnosis of monocytes. **Methods:** To produce mAb against CD14 protein, mice were immunized with two KLH-conjugated CD14 peptides. The spleen cells of the immunized mice were then fused

with SP2/0 by hybridoma technique. Fused cells were grown in selective medium and cloned by limiting dilution method. The desired clones were selected and supernatants of hybridoma cells were screened by ELISA. Monoclonal antibody was purified by chromatography and confirmed by SDS-PAGE.Afterward, reactivity of these antibodies was evaluated in different immunological assays including western-blot (WB) and flowcytometry. **Results:** The generated CD14 mAbs were strongly positive with monocytes.Isotype of this mAb was identified as IgG2b class with Kappa (κ) light chain. **Conclusion:** The results show that, this antibody is highly specific and functional in biomedical applications such as flowcytometry and western-blot assays to identify monocytes.
Keywords: Monoclonal Antibody, CD14, Synthetic Peptide, Flowcytometry, Western-blotting

2560P

Assessment of in vitro co-culture for long survival of primary plasma cell: anew approach in therapeutic antibody production

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Background: in-vitro plasma cell culture has been conducted with different goals. Nowadays, it is the main part of new approach in human monoclonal antibody production, which is so-called Single B cell antibody technology. In this technology, plasma cells are isolated from blood and cultured to secret sufficient antibodies. After screening, the antibody genes are amplified from single plasma cell which is secreting desired mAb by using Single Cell RT-PCR. Unlike in-vivo long survival of plasma cells, in-vitro culture of them requires a monolayer as feeder cell. The aim of this study was isolation and culture of plasma cells by using appropriate feeder layer to survive them in-vitro for long time. **Methods:** PBMCs were isolated from vaccinated donor and stained using fluorescent mAbs against CD45, CD19 and CD38 surface markers. Plasma cells were sorted using fluorescent activated cell sorting (FACS) and collected to culture in 96 well plates with a layer of peripheral blood adherent cells as feeder cell. Ten days after co-culture, ELISA method was used to determine antibody secretion of plasma cells. **Results:** six days after donor vaccination, flowcytometric analysis has shown the presence of 0.3% plasma cells in PBMCs. Appearance of yellow color after ELISA test of plasma cells culture supernatant, were confirmed their antibody secretion and therefore plasma cell survival during in-vitro culture. **Conclusion:** Using this protocol, plasma cells can be isolate and co-culture to secrete detectable amount of antibody. Furthermore, it is possible to collect supernatants for more screening, which is first step of single B cell antibody technology.
Keywords: Monoclonal antibody, Plasma cell, PBMC, Co-culture, FACS

2583P

Designing a primer set to amplify human ScFv encoding segment

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Background: Human Monoclonal antibodies are effective tools in therapeutics. Because of the broad diversity of the human antibody repertoire, it is necessary to use degenerate primers to amplify all of the diversity of variable heavy (VH) and light (VL) antibody genes. The scFv fragment that can effectively neutralize toxins consists of the smallest functional antigen-binding domain of an antibody, in which the VH and VL chains are joined together using a flexible linker. In this study, appropriate primers were designed and used to amplify human VH and VL genes as a ScFv encoding segment. **Methods:** Primers were designed using GeneRunner program and based on IgBlast database information. To amplify ScFv, three step reactions were performed. At the firstone, human Plasma cell extracted to tRNA were used as template in RT-PCR to amplify VH and VL genes. To attach appropriate linker sequence to these amplicons, second reactions were performed using designed primer sets. After purification of VH and VL amplicons, Overlap Extension PCR was carried out to link them as a ScFv encoding segment. **Results:** In this research, during the first and second rounds of PCR experiments, VH and VL fragments of length about 400bp were amplified separately. After Overlap extension PCR, VH and VL segments were linked together as scFv encoding segment. Therefore a DNA fragment in size of about 800 bp was amplified and observed on agarose gel. **Conclusion:** Based on these results, designed primers were confirmed successfully to amplify scFv encoding segments from source of human plasma cell cDNA.

Keywords:scFv, monoclonal antibody, plasma cells, overlap extension PCR

2216P

Development of an expression system for the production of, omalizumab, an anti-IgE monoclonal antibody

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Background: Allergic diseases have become increasingly common in the world. IgE is a key mediator of allergic cascade. Therefore, anti-IgE therapy appears to be a rational approach as a clinical intervention. Omalizumab is a recombinant anti-IgE antibody that interacts with circulating IgE and interrupts the allergic cascade. Omalizumab is the only biologic agent approved for the treatment of asthma. It is not only an anti-asthmatic drug but also a promising therapeutic option for various allergic diseases. Given the high prevalence of allergic diseases in our country and the unmet need for this drug, we have developed an optimal expression system for omalizumab production. **Methods:** Antibodies should be produced in mammalian expression system to acquire biological functions. We used Chinese hamster ovary (CHO) cells, which is the most currently used cells for recombinant protein production. To express heavy and light chains at optimal stoichiometric ratios we used two separate vectors. First heavy and light chains were synthesized and cloned into their appropriate eukaryotic vectors. Then cotransfected to CHO cells via lipofection. Expression of antibody was confirmed by SDS-PAGE and western-blotting. **Results:** Cloning was confirmed by PCR, restriction

digestion and sequencing. Co-transfection efficacy was 45%inflowcytometryexamining. Omalizumabexpression confirmed by presence of 150 kD band in SDS-PAGE and western-blotting. **Conclusion:** In this investigation we have developed a mammalian expression system to produce omalizumab. Our results suggested that, this antibody is produced efficiently in our system. Also this system could be used for expression of other recombinant antibodies.
Keywords: Immunoglobulin E, Anti-IgE, allergy, Omalizumab, Mammalian expression system

2542P

Construction of human monoclonal antibody library for tetanus toxin

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Background: Fully human monoclonal antibodies are a rising category of targeted therapeutic causes. Progresses in technology to generate the molecules for study, such as transgenic mice and yeast or phage display improved the advance of human mAbs. The principle of this study was constructing an immune antibody library from a vaccinated donor with tetanus toxin. **Methods:** A person as blood donor was immunized by tetanus vaccine. After 7 days blood was taken. RNA was extracted from lymphocyte, RT_PCR was done to amplification VH and VL genes. The amplicons were combined by linker that organized by specific primers. The fragments were inserted in pCANTAB5E vector and transformed to *E. coli* XL1blue strain. After growing, matrix was provided from random colonies and then colony PCR was done. The recombinant plasmid was extracted and sequenced. **Results:** cDNA qualities were confirmed by HPRT primers. In following, transformation was applied. Positive clones were selected based on grows on LB agar. After confirmed using colony PCR and plasmid extraction was done. The mAb was confirmed by sequencing. The sequences were aligned using BLAST at NCBI. **Conclusion:** In this study, the human antibody library was constructed and confirmed using DNA sequencing. This immune library with tetanus toxin is appropriate resource to screen the high specific antibodies.
Keywords: Monoclonal antibody, Phage display, Tetanus toxin, pCANTAB5E, XL1_blue

2206P

A targeted gene integration system (phiC31 integrase) for expression of an anti-IgE monoclonal antibody chains

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Background: Expression level of recombinant protein in mammalian expression platforms is unpredictable and unstable because of random location of integration in the genome. Site-specific integration techniques, such as ΦC31 integrase, by specifically inserting a vector at a locus with specific expression trait, are capable of producing stable, high producer clonal cells. ΦC31 is a serine-recombinase that can catalyse recombination between the f31 phage attP site and the bacterial host att B site. There are several pseudo-attP sites with good sequence

similarity to the native attP sequence in mammalian genome that can act as substrates for the enzyme. The efficiency of integration by ΦC31 integrase methodhave been evaluated in this study. **Methods:** Heavy (HC) and light chain (LC) were cloned into a vector containing an attB site for a targeted integration into pseudo-attP sites in Chinese Hamster Ovary (CHO) genome. CHO cells were co-transfected with vectors containing HC, LC and the RFP reporter gene and ΦC31 integrase expression vectors. Control CHO cells were transfected similarly but without the presence of a ΦC31 integrase vector. The expressions were evaluated with SDS-PAGE and western-blotting. **Results:** Cloning steps were confirmed by digestion and sequencing. SDS-PAGEs were performed and the bands accuracy was confirmed by western-blotting. Bands densitometry confirmed that the ΦC31 integrase expression vector format has reached more mAb production compared to random integration transfectants. **Conclusion:** The site-specific non-viral vectors based on the ΦC31 integrase have been promising for increasing biopharmaceutical protein titers and decreasing the required time to achieve sufficient amounts of protein for pre-clinical evaluation.

Keywords: Site-specific recombination, Targeted integration, Serine recombinase, Chinese Hamster Ovary (CHO) stable cell lines

2410P

A fed-batch based cultivation provides high cell-density and improves yield of soluble anti-VEGF antibody fragments (Fab) in *E. coli* cytoplasm

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Background: Therapeutic antibodies and antibody fragments (Fab) comprise a major portion of biopharmaceutical market. *E. coli* has been a preferred choice for expression of non-glycosylated proteins in biotechnology industry. However, there are also some limitations such as improper folding and formation of inclusion bodies. The enzymatic glucose release system together with a well-balanced combination of mineral salts and complex medium additives provides high cell densities, high protein yields and a considerably improved proportion of soluble proteins in harvested cells. **Methods:** Anti-VEGF Fab gene was synthesized according to biological databases. The construct is bicystronic so promoter and Ribosome Binding Site for both heavy and light chain were considered. The *E. coli* expression procedure was compared in traditional batch and newly developed fed batch, EnBase® Flo system. Protein expression has been analyzed by SDS-PAGE and western-blot method. **Results:** Constructs have been confirmed by enzymatic digestions and sequencing. After transformation and optimization of conditions, the fed-batch fermentation mode, coupled with a Ni-NTA affinity purification procedure under reduced condition, resulted in higher amount of soluble protein. **Conclusion:** Considering the advantages of expression in in the prokaryotic systems, applying the novel EnBase® Flo cultivation system in shaken cultures, results the high cell densities without impairing the productivity per cell. Especially the yield of soluble (correctly folded) proteins was significantly improved in comparison to commonly used LB media. So this method showed the potential to replace miss-folded formats of proteins with proper folded, soluble ones.

Keywords: fed-batch based cultivation, *E. coli*, Folding, anti-VEGF Fab

2038P

Effect of feeding strategies on glycosylation pattern of anti-CD20 monoclonal antibody during CHO cells culturing in STR bioreactor

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Background: There have been relatively abundant studies on different feeding strategy in order to increase the expression level of recombinant protein in the stirred tank (STR) bioreactor. These strategies also may have negative effect on the structure of the target protein. Therefore, considering these effects is necessary during process development. **Methods:** In the present study a feeding strategy based on increasing osmolality of medium was applied for Anti-CD20 monoclonal antibody production in STR bioreactor. And its effect on cell density, batch duration, expression level, and product quality was investigated. **Results:** It has been shown that increasing osmolality up to 490 mOsm/kg could increase 157% in the expression titer in early days of culturing. Although, high osmolality environment reduced the viable cell count and eventually batch duration comparing the same in low osmolality medium. The protein structure was determined by peptide and carbohydrate mapping. In spite of no difference in peptide map of product in normal and high osmolality environment, the carbohydrate map was altered. The high osmolality feeding strategy also reduced the biological activity of the target protein up to 30%. **Conclusion:** In conclusion a feeding strategy based on increasing osmolality could increase the expression level however it affects the quality of anti-CD20 monoclonal antibody.

Keywords: Anti-CD20 mAb, Stirred tank bioreactor, feeding strategy, osmolality, quality

2220P

Different vector designs for mammalian expression of ananti-IgE monoclonal antibody

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Background: Monoclonal antibodies (mAbs) are among the most famous immunotherapy agents currently used for many therapeutic applications. As many other recombinant proteins, mAbs should be produced in mammalian cells like CHO. A constraint on mAbs production is the requirement of balanced expression of two different polypeptides, heavy (HC) and light chain (LC) which makes the mAbs production more complicated. There are two main strategies of vector design; the most common consisting of two vectors, one for each chain. Alternatively, a single vector carries both chains, each under the control ofan independent promoter. Here, we have evaluated the difference between these strategies for expression of an anti-IgE monoclonal antibody. **Methods:** cDNAs encoding HC and LC were cloned into separate mammalian vectors (pHC and pLC), then HC and its related promoter subcloned into

LC containing vector to make a single vector expressing both chains (pLCHC). CHO cells were transfected with entire vectors one group with a cocktail of equimolar ratio ofpLC and pHC and the other with pLCHC. Expression levelwas investigated with SDS-PAGE and western-blotting. **Results:** Cloning steps were confirmed by digestion and sequencing. SDS-PAGEs were performed and the bands' accuracy wasconfirmed by western-blots. Bands densitometry showed the single vector format (pLCHC) has reached more mAb production compared to pHC+pLC co-transfectants. **Conclusion:** Two separate vector approach showed less efficiency to obtain a balanced expression of both chains probably because of different positional effects on each chain. Hence, single vector format is more likely to assure the balanced expression.
Keywords: mAbs, Production, Expression vector

2248P

Bridging two chains of anti-IgE monoclonal antibody using 2A sequence with SOEing PCR

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Background: Monoclonal antibodies (mAbs) are an important class of therapeutic agents. Bicistronic vector design seems to be an efficient approach to assure same sub cellular localization and equimolar expression of both heavy and light chains. It has been found that introduction of foot-and-mouth disease virus (FMDV)-derived 2A linker and furin cleavage site between LC and HC chains cause equimolar expression of two chain genes from a single open reading frame. In this study bridging two chains of an anti IgE antibody, the only biologic agent approved for treatment of asthma, with 2A and furine sequences was investigated. **Methods:** To generate the construct containing the 2A self-processing sequence; furin cleavage site and FMDV-2A self-cleavage linker was introduced between light and heavy chain of the anti-IgE antibody by SOEing (Splicing by Overlap Extension) PCR. The method employs a two-step PCR strategy.Two chimeric primers that were derived partly from the sequence to be inserted (furine& 2A) and partly from the template and two outermost flanking primers were designed. Two PCR products were first prepared separately. In the second step, an overlap extension PCR was carried out, using the outermost primer pairs, to recombine the two chains. The SOEn product was cloned and its accuracy was confirmed by sequencing. **Results:** The appropriate SOEing PCR strategy showed successful results of reaching desired size which stands for LC-F2A–HC construct based on electrophoresis and sequencing results. **Conclusion:** Soeing PCR strategy described here is an accurate and efficient method for bridging two chains of mAb.
Keywords: monoclonal antibody, FMDV-derived 2A peptide, SOEing PCR

2370P

Introducing a novel method for production of monoclonal antibodies using transgenic hens

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Background: Demanding formonoclonal antibodies (mAbs) for both therapeutic and diagnostic of diseases is increasing. Many of them are produced in industrial bioreactors, but setting up such systems is time-consuming and expensive. Some antibodies are produced using

cell cultures. However, this system is an expensive and time-consumingoption. Increasing global demand for mABs has resulted in significant research being focused on development of alternative production platforms, including the use of transgenic animals as bioreactors. The use of transgenic chickens as bioreactors to synthesize mAbs, as a component of egg white, may have several advantages over other expression systems, including high protein productivity in eggs, a shorter time-scale for setup and beneficial glycosylation profile, and reduced immunogenicity of the purified product. The aim of this study is to introduce a novel method for producing transgenic hens to gain monoclonal antibodies in their eggs. **Methods:** Recently the*piggyBac* transposons have been used to insert exogenous genes into the genome of the primordial germ-cells. Therefore, this could be used as a novel method to insert genes related to mAbs into the hen genome. **Results and Conclusion:** Our prediction is that this method will have a high efficiency as it was recently reported that a chicken primordial germ-cell line was established with high efficiency of transmission to offspring. The *piggyBac* transposition into PGCs improved the efficiency of transgenic chicken production and led to high-level transgene expression. This method could be useful for producing human monoclonal antibodies at high level using transgenic chickens as bioreactors.

Keywords*:* Transgenic chicken*, piggyback transposon*, Monoclonal antibodies

1575P

Production of monoclonal antibody against nmp22

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Background: Bladder cancer is a major health problem worldwide. Diagnosis of acute and chronic bladder carcinoma is based on the detection of a number of tumor markers, so Nmp22 (Nuclear Matrix Protein22) is one of the tumor markers for detection of recurrence bladder cancer.The development of hybrid cell producing monoclonal antibodies (MAbs), specifically for Numa using hybridoma technology, is being reported in current research. **Methods:** Firstly, complete and incomplete adjuvants with Numa antigens were emulsified and injected to BALB/c mice, so the BALB/c mice were immunized. Spleen was removed and sp2 /0 myeloma cells were fused. **Results:** As a result of the fusion, hybridoma cells including high-titre secretion of antibodies were produced. Due to testing of colons based on ELISA test, 135 hybridoma were selected which eight of them involving 54D-6F-2E-36-7H-2A-8E-1D-6D were excluded.

Conclusion: Finally, according to a single fusion experiment, a type of monocolonal antibody, named as FPR92 in this research, was produced and characterized consequently. With the respect of affinity, the monocolonal antibodies were included with high affinity which were used in the development of diagnostic kits based on sandwich ELISA test systems.

Keywords: MAb, bladder cancer,

3292P

Large Scale Generation and Characterization of Anti-Human IgA Monoclonal Antibody in Ascetic Fluid of Balb/c Mice

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Background: Monoclonal antibodies are potentially powerful tools used in biomedical researches, diagnosis and treatment of the infectious diseases and cancers. The monoclonal antibody against Human IgA facilitate as a diagnostic application in detecting infectious diseases. The aim of the this study was to improve a appropriate protocol for large production of mAbs against IgA. **Methods:** For large scale production of monoclonal antibody, hybridoma cells that produce monoclonal antibody against Human IgA were injected intraperitoneally into Balb/c mice which have previously been primed with 0.5 ml Pristane. After ten days, ascetic fluid was harvested from the peritoneum of each mouse. ELISA method was carried out for evaluation of producing mAbs titration. The ascitic fluid was investigated for class and subclasses by ELISA mouse mAb isotyping Kit. mAb was purified from ascitic fluid by affinity chromatography on Protein A-Sepharose. Purity of monoclonal antibody was determined by SDS -PAGE and the purified monoclonal antibody was conjugated with HRP. **Results:** Monoclonal antibodies with high specificity and sensitivity against Human IgA by hybridoma technology were prepared. The subclass of antibody was IgG1 and its light chain was kappa. **Conclusion:** This conjugated monoclonal antibody could have application in designing Elisa kits in order to diagnosis of different infectious disease such as toxoplasmosis, and H. Pylori.

Keywords: Production, characterization, monoclonal antibody, IgA

1449P

Recombinant expression and Heavy Chain Antibody production for Human Placental Growth Factor 1

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Background: Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) Family. Unlike VEGF, PlGF is dispensable for normal cell development as well as playing various roles in pathological angiogenesis which occurs in tissue ischemia, inflammation, and malignancy. The PlGF-1 has been considered as a potential candidate for the diagnosis and targeting of pathological angiogenesis. Camelidae serum contains an important fraction of functional antibodies, called heavy-chain antibodies (HcAbs) that are naturally devoid of light chains. Camelid HcAbs recognize their cognate antigens by a single variable-domain, referred to as VHH or Nanobody. Here, we describe the expression and purification of recombinant human PlGF-1 (rhPlGF-1). This protein was subsequently used for the preparation of camel heavy chain polyclonal antibody against rhPlGF-1. **Methods:** The recombinant expression plasmid pET-26b-hPlGF-1 was introduced into *Escherichia coli* BL21 cells to express the rhPlGF-1 protein. Purified rhPlGF-1 was used to immunize camel, the specific reactivity of HcAb was determined with ELISA and western blot. **Results:** ELISA and Western blot analysis Indicated that the antiserum specifically reacted to the recombinant protein. **Conclusion**: The rhPLGF1 protein and its antibody may be used for the development

of detection assays needed for clinical research.
Keywords: PlGF, VHH, Polyclonal antibody, Angiogenesis, Heavy chain antibody

2984P

Selection of single chain antibodies against CD44 for immunotherapy of breast cancer

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Background: CD44 is a member of a large family of cell adhesion molecules that is responsible for mediating communication and adhesion between cells and the extracellular matrix. CD44 expression is highly up-regulated in breast cancer stem cells and has been implicated in tumorigenesis and metastasis. Due to several advantages of Single chain antibodies (scFv) containing high affinity and specificity, human origin, small size and faster penetration these antibodies has been introduced as useful agents for cancer immunotherapy.In this study specific scFvs were selected against CD44. **Methods:** A phage antibody display library of single chain fragment variable was used to select specific recombinant antibody against CD44 using panning process. CD44 immunodominant peptide was coated in a immunotube. Phage rescue supernatant of phage antibody was added. The bound phages were eluted by Ecoli TG1. After four rounds of panning, PCR and BstN1 fingerprinting were performed to select common patterns and isolate specific scFvs. **Results:** Two predominant patterns with frequencies 54% and 25% were selected. **Conclusion:** Immunotherapy has been selected as a new strategy for cancer therapy. An antibody in scFv is an ideal antibody in cancer treatment. Immunotherapy against CD44 can be used to target cancer stem cells in the tumor tissue. In this study we used phage display technology and selected 2 specific single chain antibodies against CD44. These specific antibodies could be helpful for immunotherapy and diagnosis of CD44 positive cancer cells specially breast cancer. Further experiments are needed to show the effects of the selected scFvs.

Keywords: CD44, scFv, Immunotherapy, panning

P2994

What is the best choice of carrier in antibody production? A new avenue toward better immunization against desired antigen

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Background: There are many molecular techniques to determine the methylation statue of various genes in different diseases or conditions. However, introducing the methods like methylated DNA immunoprecipitation (MeDIP) has exceeded in methylation studies due to there is no need to treat the DNA sample. The major obstacle to implicating these methods is the extra demand for production of the 5-methylcytosine antibody owing to very small molecular weight of 5-methylcytosine. Herein, we evaluated the potential of bovine serum albumin (BSA) and its modification or cationized BSA (cBSA) compared to other carriers in

stimulating the immune system. **Methods:** The BSA was modified according to our optimized method using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide and then lyophilized. After conjugation of all the carriers with 5-methylcytosine, they were injected intraperitoneally (IP) into separate mouses on the set 5 times. The produced antibody by each mouse was evaluated at first through immunoprecipitation test and then enzyme-linked immunosorbent assay (ELISA). **Results:** Immunogenicity of 5-methylcytosine coupled to cBSA was the most amongst all the carriers used. Cationization of BSA not only facilitates its conjugation with antigen, but also provides enhanced immune response to injected hapten. **Conclusion:** Taken together, it seems that using of alternative carriers as well as cBSA, the monoclonal antibody production industry could be extended to reach us to be self-sufficient.

Keywords: Bovine serum albumin, Cationized, 5-methylcytosine

P-3261

Purification of IgM from Hybridoma Supernatant

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Background: Protein G and protein A affinity chromatography is the most common method to isolation of IgG from hybridoma supernatants. In contrast to IgG, IgM reveal slight or no affinity to protein A and protein G. In this study we developed a simple protocol for the purification of IgM from supernatant of hybridoma by ammonium sulfate and PEG precipitation method. High strength ammonium sulfate was used to precipitate immunoglobulins and subsequently separation of IgM was performed by polyethylene glycol. **Methods:** Forty five ml of saturated ammonium sulfate (SAS) was added to 55 ml of hybridoma supernatant (SAS 45%). After centrifugation, the precipitate was washed four times by addition of SAS 45% and centrifugation. The pellet was dissolved in 2 ml of 10 mM PB, and was dialyzed against three changes of 20 mM PB at 4°C. The precipitate was mixed with equal amount of 10% PEG 8000 in 20 mM Tris buffer and centrifuged at 4000 rpm for 30 min at RT, then washed by 4 steps centrifugation and precipitation with 5% PEG 8000 in 20 mM Tris buffer. The precipitate was dissolved in 20 mM PBS. To estimate the purification, SDS-PAGE and western blotting was performed. **Results:** The purity of IgM was confirmed by SDS-PAGE and western blotting and showed that this developed procedure is a simple and cost-effective method for the isolation of IgM from the supernatant of hybridoma cells. **Conclusion:** Ammonium sulfate followed by PEG precipitation is a suitable method for purification of IgM.

Keywords: Monoclonal antibody, IgM, Purification

P3220

Preparation of monoclonal antibody against cyokeratin 19

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Background: Cytokeratins (CKs) are members of the intermediate filaments with more than

20 different types that are divided into acidic type I (CK9-CK20) and basic type II (CK1-CK8) keratins. Cytokeratin 19 is acidic type I keratins with molecular weight of 40KD. It is known that CK19 widely expressed in thyroid, breast, colon, small and non-small cell lung, and prostate cancer cells. **Methods**: Recombinant cytokeratin 19 was used for the immunization of two 6–8 weeks old female Balb/c mice. For first injection, recombinant antigen was mixed with the complete Freund′s Adjuvant and for second and third injection, mixed with the Incomplete Freund′s Adjuvant. Injections were performed with intervals of 15 days. Four days after third injection, titration of mice serum was determined using indirect ELISA and better immunized mouse selected for fusion. IV injection was performed 4 days before the fusion. The spleen cells of mouse were fused with sp2/0 cells using 50% v/v polyethylene glycol (PEG) and cells were suspended in HAT medium. Supernatants of hybridoma cells were screened for antibody secretion by indirect ELISA. **Results**: Among 78 hybridoma clones that reacted with the recombinant cytokeratin 19 by indirect ELISA, two stable hybridomas were obtained. These hybridoma cells were monocloned twice by limiting dilution. **Conclusion**: Immunization by recombinant cytokeratin 19 was led to obtain of two stable monoclonal antibodies against this antigen.
Keywords: CK19, Monoclonal antibody

P1875

Induction of Catalytic Activity of Plasminogen by Monoclonal Antibody A5E10 in the Presence of Plasminogen Activators

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Background: Conversion of plasminogen to plasmin by plasminogen activators is a key event in the fibrinolytic system. Human plasminogen is a single-chain glycoprotein of 92 kDa, consisting of 791 amino acid residues and contains five kringles. In this study, we investigated the effects of an anti-human plasminogen monoclonal antibody, A5E10 on Glu-plasminogen activation in presence of u-PA, t-PA and streptokinase.**Methods:** Producing of Hybridoma antibodies was performed by fusion of spleen cells from BALB/C mice immunized with Glu-plasminogen and NS1 myeloma cells. Antibody binding to Human Glu-plasminogen was assessed using an ELISA assay. Activation of plasminogen was determined by measuring plasmin generation using the chromogenic substrate S-2251 and the effect of A5E10 mAb, on plasminogen activation in solution was then evaluated. Initial rates and kinetic parameters of plasminogen activation in the presence of mAb were calculated. **Results:** ELISA assay showed that the antibody reacted well with antigens. Primary observations with human pooled plasma showed that in the presence of plasminogen activators (t-PA, u-PA and SK), A5E10 can enhance activation of fibrinolytic system.bD- dimer assay showed that the lytic effects of A5E10 was dose dependent. Evaluation with S-2251 synthetic substrate showed that plasminogen activation in the presence of u-PA, enhanced in the presence of A5E10 antibody. A5E10 increased the maximum velocity (Vmax) of plasminogen activation by each of the three plasminogen activators. **Conclusion:** The binding of this antibody to Glu-plasminogen increases the catalytic efficiency of plasminogen activation by plasminogen activators. Therefore, it may be useful to apply clinically A5E10 for the therapy of thromboembolic events by humanizing this antibody.
Keywords: Plasmin(ogen), Monoclonal antibody, Plasminogen activators

Nanoimmunology

Oral Presentations:

2596O

Screening the recombinant nanobody neutralizing staphylococcal enterotoxin B

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Background: *Staphylococcus aureus* is a gram-positive bacterium considered as a great pathogen in humans. Staphylococcal enterotoxin B (SEB) is the most common cause of food borne poisoning. Nanobodies are single-domain binding fragments can be isolated from sera of the camelidae. These antibodies have great characteristics such as small size, resistant to temperature and pH, and the ease of cloning and expression in *Escherichia coli*. In this research, the phage-display method was used for screening the library of nanobody genes against SEB. **Methods:** For screening the library, panning was performed by phage display technique. Helper phage (M13K07) was prepared at the titer of 10^{11}pfu/ml. The SEB toxin was coated in microtiter wells and biopanning process using phage particles was carried out. The accuracy of enrichment process was confirmed by phage ELISA. Single clones with high specificity were isolated from panning cycle with highest optical density. **Results:** To isolate the SEB specific nanobodies, five rounds of panning were done. The strongest enrichment occurred at the fifth round of panning and confirmed the presence of high affinity SEB nanobodies. A total 30 single colonies randomly picked from fifth round of panning and 8 clones with strong binding capacity were selected and sequenced. **Conclusion:** In this study, our aim was the screening of SEB nanobodies with remarkable affinity and specificity using phage display selection technique. The antibody fragments isolated from immune libraries finds their applications in the diagnostic tools and treatment.

Keywords: *Staphylococcus aureus*, Nanobody, Phage display

2133O

Development of a gold nanoparticle-based immunochromatographic test strip for rapid and sensitive detection of *Salmonella typhimurium*

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Background: Gold nanoparticles have been widely used in immunoassay techniques such as LFA, due to their long-term stability, and compatibility with antibodies. Here we used this technique for salmonella detection in contaminated food's solutions.LFA was based on a double-antibody sandwich format on a porous nitrocellulose membrane. When Salmonella-

containing samples were applied to the LFA-device, the bacterial specific antigen initially reacted with gold-conjugated mAb then reacted with the fixed polyclonal antibody on the membrane. These reactions resulted in twored lines. **Methods:** Anti-Salmonella LPS mAb–gold conjugate coated onto conjugate pad. Test and control lines, containing polyclonal pan-salmonella antibody and anti-mouse IgG respectively. For evaluating the practicability of the test strip in natural conditions, three different matrices were used as bacteria diluents to mimic actual samples. The samples were prepared by diluting *Salmonella Typhimurium* ($1x10^{11}$cfu/ml) in a stock solution with the different volume of meat extract , melted ice cream , and pasteurized cow milk(%1.5 fat). **Results:** The results showed that only one redline(control line) appeared for a negative samples, while at the positive testformed two red line (test and control line).The minimal detection limit for *S.typhimurium* in was 1.6×10^{5}cfu/ml.The cross-reactivity of *S.enteriditis*, *S.paratyphi*A ,*E.coli* K12 and *Klebsiela***p**neumoniaon the *S.typhimurium* strip was examined with the concentration of 10^{7}cfu /ml (in PBS) and all of samples displayed negative results. **Conclusion:** Here, we design a the colloidal gold-based LFA.This method can be used as an alternative screening test for Salmonella and other infecting agent instead of other immunoassay methods in diagnostic laboratories.

Keywords: Gold nanoparticles, *S.typhimurium*,LFA,Immunoassay,Rapid detection

2162O

Chitosan-dextran sulphate as a nanocarrier of Artemether against Balb/c mice bearing breast cancer and 4T1 cell line

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Background: Artemether (ARM), an oil soluble derivative of Artemisinin, has been used as an anti-malaria drug and nowadays it has received a great deal of attention as an anti-cancer agent. In order to improve half life and delivery of drug, we loaded ARM into chitosan-dextran sulphate nanoparticles. These nanoparticles can increase the circulation time of the drug. In this study we evaluated the effect of nanosystem on 4T1 cell line in vitro and in vivo. **Methods:** The nanosystem characterization was carried out by FTIR (Fourier transform infrared) spectroscopy. Size, zeta potential and morphology was measured by Malvern Zeta sizer and transmission electron microscopy. We considered the loading efficiency and release profile in pH of 5.4 and 7.4 in citrate and phosphate buffer respectively. The effect of nanosystem on 4T1 cell line was evaluated by MTT assay. We injected nanosystem into mice bearing breast cancer and the level of IL-4 and IFN-γ cytokines were measured by ELISA. **Results**: Negative zeta potential charge and high loading efficiency of ARM was detected in the nanosystem. According to release profile of drug, at ph 5.4 the drug released more as compared to that of ph 7.4, showing it is pH sensitive delivery system. This nanosystem exhibited more inhibitory effect on growth of 4T1 cell line as compared to ARM itself. It also showed increased IFNγ level but decreased IL-4 level in mice. **Conclusion:** In summary, using chitosan-dextran sulphate as a nanocarrier for a drug like ARM has significant effect on treatment of breast cancer.

Keywords: Chitosan, Dextran, Artemether, Breast cancer

2098O

Study on the immunomodulatory effects of polyurethane –arteethernanocomplex against mice bearing breast cancer and its evaluation on 4T1 cell line

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Background: Recent studies indicated the profound anti-tumor activity of arteether as an oil-soluble derivative of artemisinin. It can induce apoptosis in tumor cells but not in the normal cells. Delivery of arteether (ARE) by using nanomicelles can improve treatment of cancers. Polyurethane (PU) as a water soluble and biocompatible nanomicell is used for embedding arteether. Here, we study the effect of this nanosystem in vitro on 4T1 cell line and in vivo on BALB/C mice. **Methods:** The nanosystem was characterized by Fourier transform infrared spectroscopy (FTIR). Size and Zeta potential of the particles was measured by Malvern Zeta sizer. We considered the loading efficiency and release profile in PH of 5.4 and 7.4 respectively in citrate and phosphate buffer. The effect of synthesized nanosystem on 4T1 cell line by MTT assay was evaluated. We inject nanosystem into mice bearing breast cancer and the level of IL-4 and IFN-γ cytokines was measured by ELISA. **Results:** The ARE loaded PU nanomicelles showed negative zeta potential charge (-40) and high loading efficiency (74%). In vitro drug release profile showed a faster rate of drug liberation at pH 5.4 as compared to that of pH 7.4, implying involvement of a pH-sensitive mechanism for drug release from the nanomicelles. This nanosystem had significant inhibitory effect on the growth of 4T1 cell line and increased IFN-γ level but decreased IL-4 level in mice. **Conclusion:** Based upon these findings, polyurethane as a nanocarrier of a drug such as arteether has a significant effect in treatment of breast cancer.

Keywords: Nanomicell, Polyurethane, Arteether, Breast cancer.

2004O

In vitro evaluation of paromomycin formulated with Solid Lipid Nanoparticle on Leishmania major and Leishmania tropica

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Background: Leishmaniasis prevalent in tropical countries. Chemotherapy is among the best approaches for treatment. However, current drugs have some disadvantages. One of the most promising drugs with anti-**Leishmania** activity is paromomycin(PM), nevertheless it shows insufficient concentration at sites of action due to the physicochemical properties. One way to increased PM efficacy is utilizing delivery systems. In this study, Solid Lipid Nanoparticles (SLN) is used as delivery system and has been accessed in vitro evaluation of PM-SLN on Leishmania. **Methods:** Parasites and macrophages were co-cultured and treated with PM and PM-SLN. PM toxicity and effects on the host cell (THP-1) and promastigoteswereevaluated

by MTT assay. Macrophages were cultured in plate and chamber slide and were infected. After PM and SLN-PM treatment, the inhibitory effect of the formulations on amastigotes were investigated by Parasite-Rescue Transformation- Assay (PRTA). Chamber slide staining with Syto Green, Fluorescent microscope imaging and image analysis were used for quantification of infection and the effects of PM and SLN-PM were compared. **Results:** PM and SLN-PM CC50 and IC50 were determined by MTT assay. First, EC50 was evaluated by PRTA in regard to both L. major and L. tropica infected human macrophages. A significant inhibition of amastigote propagation observed when SLN-PM was used. The results of in vivo evaluation of SLN-PM treatment are in progress. **Conclusion:** For our country, it is crucial to re-evaluate different anti-leishmanial drugs utilizing different delivery systems and formulations in order to develop new cost-effective strategies for the treatment of the leishmaniasis.

Keywords: Paramomycin, Solid Lipid Nanoparticles

3249O

Evaluation of the toxic effects caused by the use of titanium dioxide nanoparticles (TiO2) on blood biochemical parameters in mice

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Background: Although (TiO2) nanoparticles have many applications in various industries but can cause severe toxic effects on the environment and animals. The present study aimed to examine and review the toxic effects of (TiO2) nanoparticles in mice. **Methods:** Fifty mature male mice Balb/c divided into five equal groups of 10. The first group was control, the second: groups receiving doses of 10 mg / kg of (TiO2) nanoparticles. The third: groups receiving doses of 15 mg / kg of (TiO2) nanoparticles. The fourth: groups receiving doses of 20 mg / kg of (TiO2) nanoparticles and the last group received 30 mg / kg of (TiO2) nanoparticles. All mice were treated once a day for a week, this amounts received intravenously (IV) and 24 hours after the last injection, blood samples from all animals were collected for evaluating the biochemical factors and blood cell counts. **Results:** Results showed a significant reduction in the number of RBC, WBC, hemoglobin and platelets in the groups receiving doses of 20 and 30 mg / kg of (TiO2) nanoparticles compared with the control group, However, significant changes were not observed in the other treatment groups. In addition, treatment with doses 30 mg / kg of (TiO2) nanoparticles increasing in some liver enzyme activity (AST, ALT, ALP, GGTP) and bilirubin levels were observed compared with the control while there was no significant difference in the other treatment groups. **Conclusion:** Based on our results,(TiO2) nanoparticles at doses higher than 20 mg / kg caused by severe toxic effects on the liver and blood parameters in mice.

Keywords: Mice, (TiO2) Nanoparticles, Toxic effects, Liver enzyme activity,

3231O

Comparative Evaluation of Toxicological Effects of Ionic Silver (Ag^+) and Silver Nanoparticles (AgNPs) on Goldfish (*Carassiusauratus*) non-specific immune factors

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Background: Various metallic nanoparticles are most widely used in different industries currently for which silver nanoparticles (AgNPs) are an example. We aimed to examine the toxicological effects of AgNPs and ionic silver on Goldfish (*Carassiusauratus*) immunological factors. **Methods:** 60 goldfish with the initial mean weight and height of 190-200 g and 15-18 Cm respectively, were maintained in glass aquariums supplied via ventilators and water of 20-22°C in two groups with similar conditions for two weeks before the trial start. The fish of each group were exposed to $AgNO_3$ and AgNPs covered by polyvinyl pyrrolidone (PVP) (with particle size of 80-85 nm) At the end of the treatment period, blood was taken from the caudal vein. Serum samples were analyzed for immunological parameters including complement hemolytic activity, lysozyme and peroxidase levels .It is mentionable that the fish were not fed for 24 hours before trial to maintain fixed Ag^+ concentration. Results: The trial confirmed that AgNPs and AgNO3 are capable of intoxicating Goldfish, but the suppression of non-specific immune parameters with AgNPs is more than AgNO3.**Discussion:** It was found that 92 µgr/ltr of AgNPs and 35 µgr/ltr of Ag^+ are the lethal doses causing toxicological effects on fish. The immunoloical parameters were arranged on 24 hours after exposure to evaluate both AgNPs and $AgNO_3$ effects. Significant reduction in serum lysozyme was observed in both treatment groups which was more severe in the group receiving AgNPs, while reducing serum peroxidase and complement hemolytic activity was seen only in the group receiving the AgNPs.

Keywords: Toxicological effects, non-specific immune parameters, Silver Nanoparticles (AgNPs), Goldfish, $AgNO_3$

1526O

Production of novel nanobodies against active site of tumor-associated carbonic anhydrase isoform IX by phage display technique

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Background: CA IX (carbonic anhydrase IX) is a special member of carbonic anhydrase family, which is known as a powerful diagnostic marker in a wide variety of hypoxic tumors. In addition to pH regulation, that is the typical role of carbonic anhydrases, and participation in tumorigenic processes, CA IX plays a role in cell adhesion. Many efforts have been made toward identification and inhibition of CA IX. **Methods:** a phagemid library containing VHH fragments was created from lymphocytes of immunized dromedary against active site of CA IX. Consequently, VHH linked phages with the highest affinity isolated by biopanning and one of the most adherent phages was isolated, and its VHH gene was subcloned in an expressional

vector. Resulting VHH was purified and used in an ELISA test on both recombinant CA IX and HeLa cells. **Results and discussion:** The obtained VHH was able to successfully detect CA IX molecules on HeLa cells and had no immunoreaction with control PC3 cells.
Keywords: Carbonic Anhydrase9 (CA IX), Nanobody, Phage display

3368O
Accurate sensitivity of quantum dot nanoparticles for detection of HER2 expression in breast cancer cells and tissues

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Background: Early detection of cancer requires sensitive readout systems to be employed for monitoring tumor biomarker expression. Here we introduce novel optical properties and accurate sensitivity of Quantum dot (QD) nanoparticle-based detection system for tracking the breast cancer marker, HER2. **Methods:** QD525 was used to detect HER2 using home-made HER2-specific monoclonal antibodies in fixed and living HER2+ SKBR-3 cell line and breast cancer tissues. Additionally, we compared fluorescence intensity (FI), photostability and staining index (SI) of QD525 signals at different exposure times and two excitation wavelengths with those of the conventional organic dye, FITC. **Results:** Labeling signals of QD525 in both fixed and living breast cancer cells and tissue preparations were found to be significantly higher than those of FITC at 460-495 nm excitation wavelengths. Interestingly, when excited at 330-385 nm, the superiority of QD525 was more highlighted with at least 4-5 fold higher FI and SI compared to FITC. Moreover, QDs exhibited exceptional photostability during continuous illumination of cancerous cells and tissues, while FITC signal faded very quickly. **Conclusion:** QDs can be used as sensitive reporters for *in situ* detection of tumor markers which in turn could be viewed as a novel approach for early detection of cancers. Photostability is an outstanding feature allowing pathologists to view the specified microscopic field of interest without concern of signal fading. To take comprehensive advantage of QDs, it is necessary that their optimal excitation wavelength is employed.
Keywords: Cancer, Early detection, Sensitivity, Quantum Dot, Excitation wavelength

3064O
Chitosan Nanoparticles Improve Both Th1 and Th2 Immune Responses AgainstNovel Mycobacteriumtuberculosis Fusion Protein

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Background: Tuberculosis is primarily a pulmonary infectious disease caused by Mycobacterium tuberculosis, and the leading cause of death worldwide. Therefore, a vaccine or immunization strategy, which is more potent than BCG, is desperately needed. In this regard, we examined for first time, the possibility of intranasal immunization of novel fusion protein consist of immunopotent M. tuberculosis antigens, ESAT-6 and HSP70. We started out using trimethyl chitosan (TMC) nanoparticles, which has been successfully used as a delivery system for drugs and vaccines. **Methods:** The recombinant ESAT-6 and HSP70 fusion protein (E6H70) was expressed in prokaryotic system and purified by Ni–NTA affinity chromatography. This protein was encapsulated in TMC solution (1 mg/ml) by ionic gelation method. The mean particle size, distribution and zeta potential of nanoparticles were measured and determined as 308 nanometer and +12 mV. **Results:** We observed an elevated T cell proliferative response and IFN-γ, IL-4 and IL-12 in animals who was immunized with E6H70 nanoparticles ($P<0.05$). Moreover, when the fusion protein was administered in the TMC nanoparticles, a strong level of cytotoxic T cell activity was induced in compare to other groups ($P<0.01$). The immune response induced was accompanied by high levels of protective immunity and reached the level of BCG-induced protection. Neither HSP70 nor ESAT-6 (independently as free proteins) induced significant protection in this model. **Conclusion:** Taken together, the role of chitosan nanoparticles as a suitable mucosal delivery system for induction of appropriate immune response was supported in our study. However, these data offers the potential of E6H70 fusion protein as a novel candidate for subunit vaccine against tuberculosis.

Keywords: *Mycobacterium Tuberculosis*, ESAT-6, HSP70, Chitosan, CTL, Cytokines

Poster Presentations:

3144P

Comparison of Chitosan, Alginate, and Chitosan/Alginate Nanoparticles as PharmaceuticalNanocarier for Curing of Diseases

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Background: To facilitate the delivery of genetic material, the use of appropriate carriers such as polymers is necessary. **Methods:** Nanoparticles comprising chitosan- alginate polymers were formed through pregel preparation method. Alg/chi nanoparticles had a mean Z-Average diameter of 161.8 nm and mean zeta 29.3 mV, respectively. The ability of plasmid-complex in preventing DNA migration showed chi/alg nanoparticles have great capacity to maintain plasmid. The efficiency of nanoparticles in transfection of pEGFP-N1 plasmid in the cultured HEK 293 cells was measured by flow cytometry. **Results:** Cell viability assays indicated that

nanoparticles had no toxic effect on HEK 293 cells after 4h or 24h. Alginate- chitosan- DNA complex also protects DNA from enzymatic digestion makes alginate-chitosan a suitable candidate for gene delivery.

Keywords: chitosan, alginate, nanoparticle, size, transfection efficiency, cytotoxicity

3080P

In vitro anti-tumor activities of PLA-Artemisenin loaded nanoparticles

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Background: Some plants have pharmacological activities and can be used as a source of novel treatment strategies. Artemisenin is an herbal active substance which has cytotoxic and anti-proliferative effects against cancer cells .An instrument for drug transferring in a stable form to the specific targets while preventing the immunogenic and nonspecific interactions that eliminate foreign material from the body.Inthis work we formulate Artemisenin-loaded poly (lactic acid) nanoparticlesand investigate their in-vitro activities against human breast cancer cell lines. **Methods:** Artemisenin loaded PLA- nanoparticles were synthesized by nanopercipitation method. The physicochemical properties were characterized by various analytical techniques, including scanning electron microscopy, FT-IR, zeta sizer and theiranti-cancer activities were evaluated by MTT assay.The drug encapsulation efficiency (EE) and the drug release kinetics under in vitro conditions were measured by high performance liquid chromatography (HPLC). **Results:** It was shown that PLA-Artemiseninloaded nanoparticles have spherical shapes from the results of SEM observations. The average particle size was 210±15 nanometers. The zeta potential of all the nanoparticles was negative. The encapsulation efficiency of particles was found to be 98%. PLA -artemisenin loaded nanoparticles exhibited the slowest drug release and they have shown anti proliferation effects against Breast cancer cell lines in laboratory condition. **Discussion:** Artemisenin loaded nanoparticles revealed anticancer activity against breast cancer cell lines.As these nanoparticles increased half life of drug circulation time they can be used for more efficient cancer treatment. Other drugs can also be used by these nanoparticles.

Keywords: Artemisenin, Anti-tumor activity, Nanoparticle

3070P

Computational nano Investigation of Monte carlo and Semi-empirical Methods of Alzheimer's disease amyloid beta-peptide and effect of IgG on this protein

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Background: Amyloid β-peptid is found in found in an aggregated poorly soluble in senile or nervous plaques deposited in the brain of individuals affected by alzheimers disease(AD). The major components of nervous plaques found in alzheimer disease are peptides known as amyloid β-peptid, which derive from the proteolitic cleavage of the amyloid precursor proteins. Conformational studies on these peptides in aqueous solution are complicated by their

tendency to aggregate, and only recently NMR structures of Aβ-(1-40) and Aβ-(1-42) have been determind in trifluoroethanol or in SDS micelles. All these studies hint to the presence of two helical regions, connected through a flexible kink, but it proved difficult to determine the length and position of the helical stretches with accuracy and,most of all,to ascertain whether the kink region has a preferred conformation. In the search for a medium which could allow a more accurate structure determination.We performed an exhaustive solvent scan that showed a hight propensity of Aβ-(1-42)to adopt helical conformation in aqueous solutions of fluorinated alcohols. **Methods:** β-peptid calculation in geometry optimization in thermals 250-350 and calculation in for montocarlo and Semi-empirical methods. The force fields are MM,AMBER,BIO and OPLS Normal human body temperature, temperatures below normal temperatures and fever.In this study we use chem Office software(chem3D Andchem draw) and hyper chem at the end data will be presented as tables and diagrams. **Results:** As you can see in above digrams we have maximum amount of potential energy and total energy in 250°C so with considering high amount of total energy there will be maximum amount of total energy there will be minimum stability in this temperature. **Conclusion**: Considering total energy and potential energy we will have maximum amount of stability.

Keywords: Alzheimer, amyloid beta-peptide

2371P

Development of a new nano-based rapid test for IgA deficiency screening

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Background: Selective IgA deficiency (IgAD) is the most common among other primary immune deficiencies. There is the anti-IgA antibody in some of the people's plasma. Anaphylactic reaction to transfusion of IgA-containing blood products is of more dangerous complications in such people. It has been suggested these individuals should be screened and registered until they are transfused with IgA deficient blood. The existing methods in IgAD screening are expensive and needed to experienced personnel. We have constructed a novel inexpensive nanobased immunochromatographic (ICG) rapid test for detection of IgA deficiency in screening programs. **Methods**: The (ICG) strip test was developed based on polyclonal antibody (pAb) conjugated gold nanoparticles (GNPs). The GNPs was synthesized and labeled the pAb. The antibody-GNP probe was applied on the conjugate pad, and human IgA was immobilized on a nitrocellulose membrane as the capture reagent to prepare the ICG strip test. It takes only 10 minutes to accomplish a detection of serum IgA in this assay. The ICG strip test with a detection limit of five ng/mL could distinguish human serum IgA in a highly linear range. The reliability of the test procedures was compared with the enzyme-linked immunosorbent assay. **Results:** The ICG strip was sufficiently sensitive and accurate for a rapid screening of IgA in human serum. **Conclusion:** We conclude; screening of IgA deficiency programs with our immunochromatographic rapid test strip could improve IgAD

patients' quality of life.
Keywords: Immunochromatography, Rapid test, Gold nanoparticle, IgA deficiency, Blood transfusion

1801P

Anovel therapeutic approach for breast cancer: nanoparticle coated with 2-deoxy-D-glucose and doxorubicin

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Background: Breast cancer is the most frequently diagnosed cancer in women and also is the major Cause of cancer-related deaths of women worldwide. Breast cancer treatment involves surgery, chemotherapy, radiation therapy, or combination therapy, and novel strategies are needed to boost the oncologic outcome. Doxorubicin is considered to be the most effective agentin the treatment of breast cancer patients. Unfortunately, resistance to this agent and also sever cardiotoxicity are common, representing a major obstacle to successful treatment. Also 2-deoxy-D-glucose is cytotoxic to mammalian tumor cells in vitroand inhibits tumor growth in vivo. 2-deoxy-D-glucose causes cytotoxicity in cancer cells by disrupting thiol metabolism while doxorubicin induces cytotoxicity in tumor cells by generating reactive oxygen species. On the other hand, Nanoparticles are broadly used in the diagnosis and treatment of cancer. Nanoparticles are emerging as promising agents for cancer therapy and are being investigated as drug carriers, as photo thermal agents, contrast agents and also as radio sensitizers. **Methods:** Here we examined the combined cytotoxic action of 2-deoxy-D-glucose and doxorubicin and also nanoparticles as drug carrier and photo sensitizer in rapidly dividing T47D and SKBR3 breast cancer cell lines in vitro. **Results:** So far we succeed in loading 2-deoxy-D-glucose and doxorubicin on hydroxyapatite nanoparticles and obtained a suitable loading efficiency for the drugs. **Conclusion:** This study suggests the possibility of increased treatment ratio for the combined therapy, also by reducing the common dose of each treatment modality, and consequently the reduction of related side effects.
Keywords: Breast cancer, Chemodrug, Doxorubicin, Nanoparticle, Radiation therapy, 2DG

2590P

Evaluation of the adjuvanticity of trimethyl chitosan nanospheres encapsulated with tetanus toxoid in nasal and subcutaneous administration

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Background: Vaccines have been fundamental in the control and elimination of many debilitating and lethal diseases, and more diseases are currently targeted for eradication by vaccination. Nanotechnology has had a great influence in various medical and biological disciplines such as, vaccination, pharmaceutical sciences, tissue engineering, and biotechnology and drug delivery. Physiochemical and immunological properties of the material can radically change at the nanometer size level, which can be exploited to deliver the drug or gene to desired location efficiently and also make more drug available to elicit the effect precisely at the site of action.Moreover, nanostructures can also be used as an adjuvant. Novel adjuvants are being developed to enhance the immune response elicited by a vaccine and at the same time maintain high levels of tolerability. Developments in adjuvant field are expected to provide stronger immune priming and enhance immune responses. Chitosan nanoparticles, which are non-toxic, biodegradable, biocompatible, and bioadhesive natural polysaccharide has shown a good adjuvanticity in combination with subunit vaccines. **Methods:** In this research trimethyl chitosan (TMC) nanospheres loaded with tetanus toxoid (TT) were prepared by Ionic gelation method. Then, these nanoparticles analyzed by DLS, SEM and FTIR then titers of antibody were determined by ELIZA. **Results:** Effectiveness of the vaccine is measured by antibody assessments and challenging tests. **Conclusion:** Due to its obvious benefits, pharmaceutical nanotechnology is a trust research area and vaccines could be developed with chitosan nanoparticles.

Keywords: Adjuvant, Vaccine, Trimethyl chitosan,

2702P

Production of nano dendrimer containing HPV E 16d candidate vaccine and evaluation of its immune response in murine model

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Background: Cervical cancer is the second most common cancer in women worldwide. More than 99% of cervical cancers contain human papiloma virus (HPV) and HPV type 16 is the most common type in all countries. Papillomavirus–induced carcinogenesis is mainly related to two proteins E6 and E7 that are consistently expressed in HPV positive cervical carcinomas and are considered substantially for therapeutic implications. Although the size of these proteins is small, they can attach to the regulatory proteins in host cells, eliminate cell-mediated immunity, and causes malignancy in the target tissue. Up to now, different methods have been used for production of therapeutic vaccines against human papilloma virus. These vaccines have some advantages and disadvantages. Today, researchers are seeking for carriers that can be loaded with vaccines and enhance the therapeutic effectiveness of the vaccine, so they have gone into production of dendrimers and nano technology. Due to their interesting abilities for carrying DNA, passing through the membrane and their appropriate size, dendrimers have been used extensively in vaccine delivery. In terms of size, shape, length and functional surface group's nanodendrimers are very similar. They can place the molecules among their branches and

protect them against external factors and release them in target tissues. **Methods**: In this study, nanoDendrimr based E7d protein as a vaccine candidate was made and then at the dose of 10mg was administered to the experimental groups. Two groups were vaccinated with Ed proteins; frond and alum adjutant and controls were injected with PBS buffer and Dendrimer. Mice were vaccinated subcutaneously three times at two weeks interval .Two weeks after the last injection the immune responses were evaluated. Lymphocyte proliferative responses by Brdu method and the cytokines IL-4, IFN-g, and total antibody IgG1, IgG2a were evaluated with ELISA. **Results**: Results are shown that dendrimernano vaccine candidate based on E7d-protein provoke the cellular and humeral immune responses and could be a good candidate for study in humans.

Keywords: Human papilloma virus, E7d – Protein, Nanodendrimer

2006P

Evaluation of the effects of goldnanoparticles conjugated with gamma interferon and methionin on cancer cells

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Background: Hyperthermia is usually recruited with other forms of cancer therapy, such as radiation or chemotherapy. In this study we developed a Gold nanoparticles (GNPs) complex conjugated with interferon gamma and methionin to increase its cytotoxicity effects. The complex was then used together with application of hyperthermia in cells using near-infrared (NIR) laser beams. **Methods:** GNPs was conjugated using carbodiimide family and characterized after purification by dialysis bags. The size and charge of GNPs were measured before and after conjugation by a zetasizer machine. Breast cancer cell line (MCF-7) were cultured and incubated with nanorods (10nm) at different concentration followed by irradiation with NIR laser beam. Samples were then evaluated for their viability in order to determine the effect of treatment and variables by MTT assy. **Results:** The median percentage of cell viability in 0.61µg/ml concentration of GNPs was %92. The cell viability reached to 94% at the concentration of 0.16µg/ml of GNPs, which existed in the assayed complex. The results of MTT assay showed that the 0.64µg/ml concentration of GNPs complex had toxic effects on tumor cells ($P<0.05$), whereas, by increasing the concentration to 2.55µg/ml, the complex could show toxic effects on normal cells ($P<0.05$). After exposure to hyperthermia, the viability of cells decreased significantly. **Conclusion:** The size and concentration of GNPs used in this study did not show any toxicity effect on MCF-7 cells. However, their presence during irradiation NIR laser increased the number of dead cells during this process.

Keywords: Gold nanoparticles, MCF-7, Gamma interferon, near-infrared laser

3420P

Study on the effects of Fe3O4 – Chitosan- acid folic Arteether nanocomplex against mice bearing breast cancer and its evaluation on 4T1 cell line

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Background: Recent studies indicated the profound anti-tumor activity of arteether as an oil-soluble derivative of artemisinin. It can induce apoptosis in tumor cells but not in the normal cells. Delivery of arteether(ARE) by using nanoparticles can improve treatment of cancers. Here, we studied on combination of ARE with Fe3O4 and chitosan modified by folic acid nanocarriers, then by using folic acid we targeted the drug to tumor. **Methods:** The nanoparticle Fe3O4 and the nanosystem were synthesized by using mechanical stirrer. The nanosystem and nanoparticles were characterized by Fourier transform infrared spectroscopy (FTIR). Size and Zeta potential of the particles was measured by Malvern Zeta sizer. We considered the loading efficiency and release profile in Ph of 5.4 and 7.4 respectively in citrate and phosphate buffer. The effect of the synthesized nano system on 4T1 cell line by MTT assay was evaluated. Nano system was injected into Balb/c mice bearing breast cancer. **Results:** The nano system showed negative zeta potential charge and high loading efficiency. In vitro drug release profile showed a faster rate of drug liberation at pH 5.4 as compared to that of pH 7.4. The nano system had significant inhibitory effect on the growth of 4T1 cell line and tumor size. **Conclusion:** Based upon these findings, Fe3O4 and chitosan along with folic acid for targeting drugs such as ARE has a significant effect on treatment of breast cancer.

Keywords: Arteether, Fe3O4, chitosan, breast cancer

2694P

Invitro analysis the effect of the copper oxide nanoparticles on the erythroleukemia cell line K562

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Background: The present study was set out to investigate the effects of Copper oxide nanoparticles on peripheral blood mononuclear cells (PBMCs) and K562 cell line. **Methods:** K562 cells were incubated with different concentrations of Copper oxide nanoparticles (2µg/ml, 5µg/ml, 10µg/ml, and 25µg/ml) at 24 hour. Subsequently, cytotoxic effect of Copper oxide nanoparticles on K562 cells was evaluated by Tetrazolium Dye-Reduction (MTT) Assay test. Also, after 24h viability of PBMCs pulsed with different concentrations of Copper oxide nanoparticles was evaluated by Trypan blue Statistical comparisons between groups were made by analysis of variance. **Results:** The data indicated that Copper oxide nanoparticles showed cytotoxic effect on K562 at 10µg/ml and 25µg/ml concentrations after 24h post incubation. However, Copper oxide nanoparticles didn't have any cytotoxicity effect on PBMCs at different concentrations. **Conclusion:** Since Copper oxide nanoparticles showed cytotoxicity effect on K562 cells, but hadn't any cytotoxicity effect on human PBMCs, this compound can be used as a useful strategy to control cancer.

Keywords: Copper oxide nanoparticles, K562, peripheral blood mononuclear cell

2593P

Preparation and stabilization analysis of chitosannanospheresencapsulated with tetanus toxoid for vaccination

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Background: Vaccines have a fundamentalrole in the control and elimination of many diseases,and more diseases are currently targeted for eradication byvaccination. There is a great concern over the unreliability of the cold chain for vaccines, which strongly reduces their immunogenic activity. Vaccines are also susceptible to damage by hightemperatures and bacterial and fungal contamination.This concern is, not only in developing but also in developed countries. Therefore, distribution, storage, and use of vaccines hasplaced many challenges in front of the manufacturers of vaccines. These concerns could be reduced by additive compounds such as preservative and stabilizing materials. Unfortunately usual preservatives and stabilizers have some problems, for example they could cause allergic reactions, nerve damages etc. nanotechnology could help in this case. Chitosan nanoparticles, which are non-toxic biodegradable, biocompatible and bioadhesive natural polysaccharide, has been shown to be promising both as a delivery system and an adjuvant for mucosal vaccination. du to these properties of chitosan, this study attempts to use it as a vaccine stabilizer and preservative. **Methods:** In this research, nanocapsules of chitosan were prepared by Ionic gelation method. Then, these nanoparticlesanalyzedby DLS, SEM and FTIR. **Results:** Effectiveness of the vaccine ismeasured by antibody assessments and challenging tests. **Conclusion:** with this approach we would modify vaccine formulations toincrease tolerance to temperaturefluctuations and it is likely to increase theshelf-life of the product and reducetransport and wastage issues.

Keywords: Vaccine, stabilisation, Chitosan

2097P

Immunization against Leishmaniasis by PMMA nanoparticles loaded with TSA recombinant plasmid of *Leishmania major*

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Background: Leishmaniasis is a major infectious disease caused by protozoan parasites of the genus *Leishmania*. Up to now different adjuvants and delivery systems with many candidate DNA vaccines base on immunogenic antigens of *Leishmania* have been evaluated, but most of them have been inefficient. Study PMMA nanoparticleswere utilized in this as an adjuvant for increasing immune responses candidate vaccine against leishmaniasis. **Methods:** To prepare a new nano-vaccine for leishmaniasis, Thiol-specific-antioxidant (TSA) recombinant plasmid of *Leishmania major* was loaded on to PMMA poly(methyl methacrylate) nanoparticles.

Size of nanoparticles was characterized and optimized. For immunization study the BALB/c mice received 100 µg nano-vaccines 3 times with three weeks interval via intramuscular immunization. As a challenge animals were infected by *Leishmania major*. All immunologic parameters comprising, lymphocyte proliferation was evaluated with Brdu method. IL-4, IFN-g cytokines and also total antibody, were evaluated with ELISA method. Finally lesion size was measured 6 weeks after challenge. **Results:** Immunological analysis demonstrated that TSA recombinant plasmid with PMMA adjuvant induced proliferation activity as compared with control groups. Result of cytokine assay shows that the candidate vaccine induced both cytokines but IFN-g was dominant. Moreover, results revealed that nano-vaccine was effective in reduction of lesion size produced by cutaneous leishmaniasis. **Conclusion:** The results showed that nano-vaccine containing TSA plasmid of *Leishmania major* can increase the immunogenicity toward cell-mediated immunity that is effective in *Leishmania* eradication.

Keywords: PMMA poly(methyl methacrylate), Thiol–specific Antioxidant (TSA), Vaccine, Leishmaniasis

2665P

Preparation of N-trimethylchitosan nanoparticles as a vaccine preservative in order to reduce allergic reactions and enhance its immunogenicity

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Background: Vaccine against infectious disease save an estimated 3 million lives each year. Beside its benefits, some adverse reactions are seen in injected persons suchas redness and hardness, diarrhea and vomiting andin some cases nerve damages or more intensive reactions like anaphylaxes.Most of adverse reactions came from additives used to protection and maintenance of vaccine against environmental circumstances like physical (temperature, lights), chemical (pH) and biological agents (microorganism's contamination). For instance, preservative like thimerosal, phenol and antibiotics have side effect and cause allergicreaction. Developed countries are going to replace these additives. Nanotechnology methods have been wide progress in development of novel nanobased compounds with wideranging applications in medicine and biology.With exception of antigens that are main part vaccines, other allergicor harmful vaccine components could be substitute with safe nanobased ingredients. Chitosan is native nanoparticle which is a nontoxic biodegradable, biocompatible and bioadhesive natural polysaccharide has been shown to be promising both as a delivery system and an adjuvantfor mucosal vaccination. Additionally, other features of chitosan such as non-allergic and anti micobial properties, candidate chitosan as vaccine preservative. **Methods:** In this study, TMC nanospheres encapsulated with tetanus toxoidwere prepared by ionic gelation method .Then, these nanoparticles assessed by DLS, SEM and FTIR. **Result:** Effectiveness of vaccine by antibody assessments ismeasured. **Conclusion:** With use of nanoscience method could enhance the stabilizing of vaccines, improve its productivity and decrease the allergic reactions.

Keywords: Vaccine, chitosan, preservation

Oral Immunology

Oral Presentations:

2376O

Interleukin-17 and iInterleukin-23 Levels in gingival crevicular fluid of patients with chronic and aggressive periodontitis

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Background: Th-17 is a novel subtype of lymphocytes that seems to play an important role in pathogenesis of cell-mediated tissue damage caused either by autoimmunity or immune responses against microbial infection. They produce IL-17 which is a pro-inflammatory cytokine. The role of IL-23 is stabilization and expansion of Th-17. Since periodontitis is an inflammatory disease of tooth supporting tissues, the aims of this study was to assess 1) the concentrations of IL-17 and IL-23 in gingival crevicular fluid (GCF) of chronic and aggressive periodontitis and 2) their correlation with clinical parameters. **Materials:** After periodontal examination and supra-gingival prophylaxis, GCF samples were collected with periopaper strips from 32 individuals (10 moderate to severe chronic periodontitis, 12 aggressive periodontitis and 10 healthy). Then samples were transferred to an airtight micro tube and stored at -20˚C. IL-23 and IL-17 concentration was measured using enzyme – linked immunosorbant assay (ELISA). Comparison of study groups was performed by ANOVA and Tukey HSD test. Spearman correlation coefficient was used to assess correlation between the variables. **Results:** IL-17 and IL-23 concentrations were significantly higher in the healthy group than the periodontitis group, but there was no significant difference between chronic and aggressive periodontitis. Cytokine concentrations were not significantly correlated with probing depth and clinical attachment level. **Conclusion:** With respect to the result of this study, IL-17 & IL-23 concentration may play a role in early destructive phase of periodontal disease. Yet, exact role of Th-17 in pathogenesis of periodontal disease is not clear.

Keywords: Interleukin-17, Interleukin-23, Gingival Crevicular Fluid, Chronic Periodontitis, Aggressive Periodontitis

2028O

Correlation between CXCR4 and TLR2 and aggressive periodontitis

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Background: Periodontal diseases are among the most prevalent infections in humans; they are characterized by the classic hallmarks of the inflammatory response, including erythema and edema. A wide variety of cytokines, chemokines and their receptors are synthesized by gingival fibroblasts, epithelial cells, endothelial cells and inflammatory cells. CXCR4 is involved in the trafficking of leukocytes into and out of extravascular tissues.CXCR4 may serve a homeostatic role to prevent excessive TLR2-induced inflammation or, alternatively, CXCR4 may be exploited by P.gingivalis for suppressing TLR2-mediated innate immunity. However, the interaction of P. gingivalis with CXCR4 impairs antimicrobial host defense and it may be one mechanisms of immune evasion.So the aim of this study was to evaluate the relationship between gingival expression of CXCR4 and TLR2 and aggressive periodontitis. **Methods:** For this purpose, gingival tissue samples were collected from 20 individuals with clinically healthy gingiva and 25 patients with aggressive periodontitis. After homogenizing the gingival tissues, RNA was extracted by RNA isolation kit. After synthesis of cDNA, expression of CXCR4 and TLR2 was evaluated by Real-time PCR. **Results:** We found significant difference between aggressive periodontis and healthy gingival regarding CXCR4 and TLR2 expression ($P<0.05$). It was higher expression of them in aggressive periodontitis. **Conclusion:** Higher expression of CXCR4 may be related to the stimulation of leukocyte transendothelial migration toward gingiva which leads to space creating (degradation of collagen and apoptosis of gingival fibroblasts), which in turn leads to the impairment of cell-to-cell communication in periodontitis.

Keyword: CXCR4, TLR2, Periodontal disease

2037O

Comparison of aggressive and chronic periodontitis regarding gingival expression of HSP70

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Background: Heat shock proteins (HSP) are a group of highly conserved proteins found in eukaryotic and prokaryotic cells. It has been widely thought that heat shock protein (HSP) might be involved in autoimmune disease mechanisms in humans because of molecular mimicry between the microbial and self HSP. In several studies have been shown that HSP60 has pathologic role in periodontitis, but there is no study about the relationship of HSP70 with aggressive or chronic periodontitis. So. the aim of this study was to compare aggressive and chronic periodontitis regarding gingival expression of HSP70. **Methods:** Gingival tissue samples were collected from 25 patients with aggressive periodontitis and 25 patients with chronic periodontitis. After homogenizing the gingival tissues, RNA was extracted by RNA isolation kit. Then cDNA was synthesized and after designing the specific primers, the

expression of HSP-70 was evaluated by Real-time PCR. **Results:** There was higher expression of HSP70 in aggressive periodontitis (P<0.05). It was also significant correlation between CAL (Cinical Attachment Loss) and HSP70 expression (P<0.05).**Conclusion:** Based on the data of this study, it is suggested that the expression of HSP70 may be a possible pathogenic mechanism for aggressive periodontitis which may indicate that probably autoimmune reactions may have some role in pathogenesis of aggressive periodontitis, of course, more studies are needed in order to prove the above mentioned hypothesis.

Keywords: HSP70, Periodontitis

2086O

Evaluation of Bax and Bcl-2 immunoexpression in patients with Oral Lichen Planus

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Background: Lichen planus (LP) is a chronic mucocutaneous immunologically-mediated disease which frequently involves the oral mucosa. Infiltration of inflammatory cells and apoptotic changes in epithelial cells are seen in Lichen planus. Several proteins have role in cell proliferation and apoptotic processes but Bcl2 and Bax are more important because of their participation in human carcinogenesis process especially in oral cavity. The aim of this study was to compare the degree of Bcl2 and Bax expression in OLP and normal mucosa. **Methods:** A total of fifty paraffin-embedded biopsy samples,30with diagnosis of OLP and 20 samples of normal mucosa, were included in this study.The OLP tissues were further classified as: (a) erosive type (n=15) and (b)reticular type (n=15).4 µm thick sections from formalin-fixed and paraffin-embedded samples were prepared. Then these samples subjected to immunohistochemical analysis by using avidin-biotin, peroxidase-anti-peroxidase(PAP) technique. **Results:** Significant differences in Bax expression were observed among OLP compared to normal mucosa (P=0.008). There was no significant difference in Bax expression between OLP-E and OLP-R (P=0.91). Bax expression in OLP was significantly higher than normal oral mucosa (P=0.007).No expression of bcl-2 was seen in OLP and normal mucosa samples. **Conclusion:** In this study we found a significant difference in Bax expression between OLP and normal mucosa, with no significant difference in Bax expression between OLP-E and OLP-R.This show that atrophic erosive lesions would not have more potential to undergo malignant transformation. In our study Bcl2 was not expressed in any of OLP samples.aAnd there was no significant difference in Bcl2 expression between OLP and normal mucosa. Despite our expectance to find a large number of apoptotic cells in OLP, this wasn't found.That may be the reason of Bax bounding with other anti-apoptotic proteins such as Bad or Bak which may contribute to OLP's malignant potential.

Keywords: Oral Lichen Planus , Apoptosis

2555O

Gene expression of osteopontin and symptomatic irreversible pulpitis

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Background: Osteopontin (OPN) , a kind of human genum glycoprotein , plays an important role in immune system modulation. OPN can activate osteoclasts, thus causing resorption. Also it may have a protective function against polymicrobial endodontic infection. Because different isoforms of OPN might cause their diverse role,so the purpose of the present study was to evaluate gene expression of osteopontin in symptomatic irreversible pulpitis. **Methods:** Pulps were taken from20teeth with symptomatic irreversible pulpitis as the case group and 20 teeth from intact premolars scheduled for extraction as control group. RNAs were collected from homogenized pulps and after cDNA synthesis, appropriate primers were designed by Beacon designer and were blasted to avoid cross homology. Then, quantitative real time PCR were done in order to define the expression of OPN. Mann-Whitney U test was used to analyze the possible difference between groups. **Results:** Mean expression of OPN in normal pulps and pulps with symptomatic irreversible pulpitis were 0.695+0.295, 2.52+1.82, respectively. Although there was a higher expression of OPN in symptomatic irreversible pulpitis but it was not statistically significant. **Conclusion:** It is concluded that that OPN probably does not act as an inflammatory mediator in pulpal pathogenesis.

Keywords: Osteopontin, Irreversible pulpitis

2268O

Correlation between gingival expression of TLR2 and TLR4 and periodontal diseases

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Background: Periodontal disease is a bacterial infection of periodontal tissue which can lead to bone resorption and in severe cases, causes tooth loss. Both innate and adaptive immune responses develop during the disease process. Most of the periodontopathic bacteria are gram negative, which through TLR_4 can activate both periodontal and innate immune cells; of course, LPS of P.gingivalis can also activate the cells through TLR_2. Since, there is not sufficient data about the correlation between TLR_2or TLR_4 and various kinds of periodontal diseases, the aim of this study was to determine the correlation between gingival expression of "TLR_2 and TLR_4" and "periodontal diseases". **Methods:** For this purpose, gingival tissue samples were collected from 10 individuals with clinically healthy gingiva, 20 patients with moderate to severe chronic periodontitis and 20 patients with aggressive periodontitis. After homogenizing the gingival tissues, RNA was extracted by RNA isolation kit. The expression of TLR_2 and TLR_4 were evaluated by Real-time PCR. **Results:** Higher expression of TLR_4 was found in chronic periodontitis in comparison with healthy cases especially in the cases with severe destruction ($P<0.05$), while in aggressive periodontitis, lower expression of both TLR_2 and TLR_4 were found ($P<0.05$). **Conclusion:** It is concluded that during periodontitis, the expression of TLR_4 could be increased but it depends on the kind and the number of

periodontopathic bacteria.
Keywords: Periodontal disease, chronicperiodontitis, Aggressive periodontitis, Inflammation, TLR_2, TLR_4

2040O
Pulpal expression of NLRP3 and dental caries

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Background: One of the best-characterized Nod-like receptor (NLR) family members is pyrin domain containing 3 (NLRP3). On activation, NLRP3 assembles into inflammasome, which regulates the secretion and bioactivity of interleukin-1 family cytokines. NLRP3 has broad specificity for mediating an immune response to a wide range of microbial stimuli or danger signals. Recently it was detected in normal human dental pulp cells. Therefore, we hypothesize that NLRP3 plays an important role in the detection of bacterial pathogens or their by-products within the dental pulp. Thus, the aim of this study was to compare the dental pulps of teeth with different degrees of caries regarding the expression of NLRP3. **Methods:** Sample collection was done from individuals with healthy teeth, teeth with superficial carious lesions and teeth with deep carious lesions. Twenty samples were collected for each group. After RNA extraction and synthesis of cDNA from each sample, the specific primer was designed. The expression of NLRP3 was measured by Real-time PCR technique. **Results:** There was high expression of NLRP3 in all groups although its expression in deep carious lesions was higher than the other groups ($P<0.05$).We can not find significant difference between superficial caries and normal teeth regarding the pulpal expression of NLRP3. **Conclusion:** It is concluded that NLRP3-mediated signaling pathways may play an important role in the immune responses of dental pulps which can trigger the inflammation of pulp. Of course, more studies are needed in order to define the precise role of NLRP3 in dental pulp.
Keywords: NLRP3, Dental caries

1517O
Moesin and chronic periodontitis

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Background: Untreated gingivitis can advance to periodontitis. Periodontopathic bacteria stimulate a chronic inflammatory response which resulted to synthesis different kinds of inflammatory mediatores. Since most of these bacteria are gram negative so the main pathway for inserting their inflammatory effects via innate immune receptors by their LPSs. It is well understood that TLR4 is the most important receptor for recognizing LPS but recent studies suggest that moesin can also function as a LPS receptor. Moesin first described as a cytoskeleton

protein. So the aim of this study was to evaluate the moesin expression in chronic periodontitis. **Methods:** Gingival tissue samples were collected from 20 individuals with clinically healthy gingiva and 25 patients with chronic periodontitis. After homogenizing the gingival tissues, RNA was extracted by RNA isolation kit. Then cDNA was synthesized and the expression of moesin was evaluated by Real-time PCR. **Results:** We found significant higher expression of moesin in chronic periodontitis in comparison with healthy samples ($P<0.05$) and also found positive correlation between moesin expression and CAL. It was a positive correlation between expression of CXCR4and CAL and PD in periodontitis ($P<0.05$). **Conclusion:** Based on our results, It is concluded that moesin may function as a LPS recognizing receptor in chronic periodontitis, of course more studies are needed in order to define its precise role in this regard.

Keywords: Moesin, Chronic periodontitis

Poster Presentations:

2761 P

Correlation between gingival expression of different isoforms of osteopontin (OPN) and periodontal disease.

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Background: Periodontal disease is a bacterial infection of periodontal tissue which can lead to bone resorption and in severe cases, causes tooth loss. Both innate and adaptive immune responses develop during the disease process. Osteopontin is one of the soluble factors which increased at the site of inflammation such as periodontitis. Since there is no study which was done on the correlation between the expression or the level of osteopontin and periodontitis, the aim of this study was to compare the gingival expression of OPN between chronic and aggressive periodontitis. **Methods:** For this purpose, gingival tissue samples were collected from 20 patients with chronic periodontitis and 20 patients with aggressive periodontitis. After homogenizing the gingival tissues, RNA was extracted by RNA isolation kit. The expression of OPN was evaluated by Real-time PCR. **Results:** Lower expression of OPN was found in aggressive periodontitis in comparison with chronic periodontitis ($P<0.05$). In addition we found lower expression of OPN in cases with higher tissue destruction in both groups ($P<0.05$). **Conclusion:** It is concluded that OPN probably has some protective role against periodontopathic bacteria, but with progression of the disease its expression can be more decreased due to the death of the cells which produce it.

Keywords: Periodontal disease, Chronic periodontitis, Aggressive periodontitis, Control of inflammation, Osteopontin

3058P

Comparison of symptomatic and asymptomatic irreversible pulpitis regarding the pulpal levels of CGRP and SP

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Background: Dental pulp is highly innervated with a specific type of sensory neurons containing neuropeptides, The purpose of this study was to compare substance P (SP) and calcitonin gene-related peptide (CGRP) expression in pulp tissue s with clinically diagnosed symptomatic and asymptomatic irreversible pulpitis. **Methods:** For this purpose, 40 teeth with irreversible pulpitis were collected consisted of 20 symptomatic and 20 asymptomatic irreversible pulpitis were obtained from 40 patients. SP and CGRP levels were determined by ELISA. Statistical analysis were made by Mann–Whitney U tests. **Results:** Both neuropeptides were found in all pulp samples. The highest levels for SP and CGRP were found in symptomatic irreversible pulpitis. Mann–Whitney U test showed statistically significant differences in CGRP expression between two pulpitis groups ($P < 0.05$), differences in SP expression between symptomatic and asymptomatic irreversible pulpitis groups were not significant. **Conclusion:** Regarding the immunomodulatory role of CGRP, we suggest a regulatory role for CGRP against the invasion of microorganisms or their by-products to dental pulps in order to control the inflammation.

Keywords: Irreversible pulpitis, CGRP

3337 P

Substance P and CGRP expression in dental pulps with irreversible pulpitis

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Background: The purpose of this study was to compare substance P (SP) and calcitonin gene-related peptide (CGRP) expression in pulp tissue with clinically diagnosed symptomatic and asymptomatic irreversible pulpitis. **Methods:** Healthy pulps acted as controls. Five normal pulps and 40 with irreversible pulpitis (20 symptomatic and 20 asymptomatic) were obtained from 45 different patients. SP and CGRP expression was determined by competition binding assays using enzyme immunoassay. ANOVA and Mann–Whitney tests were used to ascertain if there were statistically significant differences between the groups. **Results:**The results showed that neuropeptides were found in all pulp samples. The highest and the lowest expressions for SP and CGRP were found in symptomatic irreversible pulpitis and healthy pulps groups, respectively. The differences between healthy pulps and the groups of pulps having irreversible pulpitis were significant ($P < 0.001$). Although Mann–Whitney's post-hoc tests showed statistically significant differences in CGRP expression between two pulpitis groups ($P < 0.05$), differences in SP expression between symptomatic and asymptomatic irreversible pulpitis groups were not significant. **Conclusion:** This study demonstrated that the expression of CGRP and SP is significantly higher in pulps with irreversible pulpitis compared with healthy pulps.

Keywords: CGRP, Human dental pulp, Substance P

2099P

Investigation of green tea extract effect in the treatment of *Candida*-associated denture stomatitis in comparison with nistatin

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Background: Denture stomatitis is a common inflammatory lesion in the palatal mucosa of denture wearers.**Candida** species have been identified in almost all patients. widespread emergence of microbial resistance to present drugs and their side effects, represents an increased interest to use natural antimicrobial compounds. Green tea extract including catechins and caffeine, have strong anti-microbial activity. The aim of this study is to evaluate the efficacy of green tea extract in compared with nistatin in the treatment of denture stomatitis. **Methods:** We studied 30 patients in two parallel groups.15 patients received nistatin drop and the other ones green tea extract. They used mouthwash in two weeks, 4 times a day each time 15-20 drops for 5 min. On days 0, 7, 14 the erythema of the palatal mucosa measured and recorded and mycological samples taked from the palatal mucosa and surface of the denture with sterile cotton swabs. Samples were cultured on Yeast extract Glucose Chloramphenicol Agar for colony counting, and CHROM agar for identification of *Candida* species. **Results:** *Candida* species have been identified in all patients and *Candida albicans* was isolated from 29 patients.The erythema surface of the palatal surface and density of yeasts was significantly reduced in both groups compared with the pre-treatment condition. No significant difference was seen between the two groups. **Conclusion:** This study indicated that the effect of Green tea extract in reducing both the number of *Candida* colonies and the erythematic area was comparable to nistatin drop in the treatment of denture stomatitis.

Keywords: Denture stomatitis, Green tea, Nistatin, *Candida*

2163P

Effect of chewing gum on oral mucositis in patients undergoing chemotherapy

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Background: Oral mucositis as an adverse effect of chemotherapy refers to inflammation and ulceration that occurs in the mouth. The transient decrease in saliva production exacerbates oral inflammation.Our goal was to study whether stimulation of salivary flow can protect the oral mucosa against chemotherapy. **Methods:** This study was done in Amir Kabir hospital, Arak, Iran. Control group was composed of 65 patients who received mucotoxic drugs. Test group was made up of 65 patients received similar drugs in addition to sugar free gums simultaneously. These patients consumed 6 pieces of gums per day for 15 days. Daily evaluation of the severity of mucositis was done according to WHO grading guideline during 15 days. **Results:** Severe oral mucositis occurred in 30 (46%) of 65 patients in the test group and in 26

of 65 (40%) patients in the control group. Difference was not significant. A small reduction in the incidence of grade 1-4 oral mucositis was seen in the test group compared with the control group (69% vs. 79%), but the difference was not significant in the overall population. The reduction was chiefly due to a decrease in cases of grade 1-2 oral mucositis (15% vs. 35%). **Conclusion:** stimulation in saliva flow can decrease inflammatory injuries of the oral mucosa during chemotherapy. However, it is not effective to decrease severe mucositis. The type of chemotherapy regimen is the main determinant of severe oral mucositis.

Keywords: Mucosistis, Gum, Chemotherapy

2036P

Comparison of osteopontin expression in human pulpal tissues with different degrees of dental caries and normal pulps

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Background: Pulpitis is an inflammatory condition of dental pulp which in turns is due to microbial invasion during dental caries, tooth filing or trauma. Of course, dental caries is more important than the others. During dental caries, bacterial by-products pass through dentinal tubules and reach the dental pulp and lead to stimulation of inflammatory cells, which in turn causes the inflammatory changes in dental pulp. Since the precise roles of immune factors in pathogenesis of pulpitis are not well so the aim of this study was to compare the expression of pulpal osteopontin. **Methods:** Sample collection was done from individuals with healthy teeth, teeth with superficial, moderate and deep carious lesions. After RNA extraction and synthesis of cDNA from each sample,the expression of different variants of osteopontin was measured by Real-Time PCR technique. **Results:** It is shown that the expression of variant 2 of osteopontin in healthy teeth group was more than different kinds of dental caries groups ($p<0.05$). There is no difference between different kinds of dental caries regarding to the expression of osteopontin.($p<0.05$). **Conclusion:** Based on the results of this study and the previous studies, which indicated that osteopontin has some role in protection of the periapical tissues or in regeneration of different tooth structures, it is concludes that osteopontin, specially variant 2 of osteopontin, has some protective role for dental pulp against bacterial invasion. Probably by exposing the dental pulp which is due to passing the carious lesion through dentine, other variants of osteopontin may be overwhelmed.

Keywords: Osteopontin, Dental caries, Pulps

2203P

Correlation between gingival expression of isoforms of MYD88 and TRIF genes in aggressive periodontitis and chronic periodontitis

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Background: Periodontal disease is a bacterial infection of periodontal tissue which can lead to bone resorption and in severe cases, causes tooth loss. Both innate and adaptive immune responses develop during the disease process. Most of the periodontopathic bacteria are gram negative, which through TLR4 can activate both adaptive and innate immune cells through two different signaling pathways, MyD88 and TRIF. Signalling through MyD88 leads to the production of inflammatory cytokines such as IL-1 and IL-18, while signaling through TRIF leads to the synthesis of type I interferons. Since, there is not sufficient data about signaling pathways on various kinds of periodontal diseases, the aim of this study was to determine the correlation between gingival expression of "MyD88 and TRIF" and "periodontal diseases". **Methods:** *G*gingival tissue samples were collected from 20 patients with moderate to severe chronic periodontitis and 20 patients with aggressive periodontitis. After homogenizing the gingival tissues, RNA was extracted by RNA isolation kit. The expression of MyD88 and TRIF were evaluated by Real-time PCR. **Results:** In aggressive periodontitis, higher expression of MyD88 total and TRIF were found ($P<0.05$). We also found higher expression of MyD88 in cases with severe tissue destruction chronic and aggressive periodontitis ($P<0.05$). **Conclusion:** TLR4 signaling through My88 pathways has an important role in tissue destruction during periodontal destruction.

Keywords: Chronic periodontitis, Aggressive periodontitis, MyD88, TRIF

2228P

The effect of menstrual cycle on inflammatory cytokines in periodontium

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Background: The effects of different levels of the steroid hormones in puberty, pregnancy and menopause in periodontium are demonstrated but changes in sex hormone levels during menstrual cycle and its influence on periodontium remained controversial. The aim of this study was to investigate the effect of menstrual cycle on Interleukin-1 beta (IL-1β) and Tumor Necrosis Factor Alpha (TNF-α) levels on gingival crevicular fluid (GCF) and periodontal clinical parameters including Gingival Bleeding Index (GBI) and Modified Gingival Index (MGI) in periodontally healthy women. **Methods:** Twenty-seven girls with regular menstrual cycle and periodontally healthy, entered into the study. Clinical parameters include GBI, MGI, and simplified oral health index (OHI-S) recorded in menstruation day (MD), ovulation day (OD), and pre-menstruation day (PmD). GCF and unstimulated saliva were collected for

assessing of IL-1β and TNF-α, estrogen and progesterone levels, respectively, in each phase of the study. **Results:** Both the GBI and MGI, increased significantly during menstrual cycle, and OD was significantly higher among the other phases (P<0.001), Whereas, OHI-S did not significantly change during menstrual cycle (P=0.18). IL-1β and TNF-α levels increased during menstrual cycle, but only TNF-α concentration change was significant (P<0.05). **Conclusion:** This study indicated that menstrual cycle influences on periodontium and induces inflammatory condition during menstrual cycle.

Keywords: Menstrual Cycle, Periodontium, IL-1β, TNF-α, GCF

2499P

Changes of interleukin-6 level in gingival cervical fluid (GCF) during orthodontic movements

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Background: Different substances have been considered in GCF as diagnostic markers. The aim of this study was to investigate the levels of IL-6 in GCF during orthodontic movements. **Methods:** Fourteen orthodontic patients (9 females and 5 males, mean age 15.1±2.5 years) with Cl I malocclusion needing first bicuspid extraction. In each patient one maxillary canine was distalized (DC) with a NiTi push coil spring. The contra-lateral canine (CC) was included in the orthodontic appliance but was not subjected to the orthodontic force and one of the mandibular canines was used as control with no orthodontic appliance (Antagonist canine: AC). The concentration of IL-6 was evaluated at the baseline and 14th and 28th days after intervention. Concentration of IL-6 detected by ELISA reader was compared by repeated measure ANOVA and LSD multiple comparison. **Results:** The amount of IL-6 in GCF increased on day 14th in DC teeth in comparison with AC and CC teeth. The concentration of IL-6 in DC teeth was significantly greater than the 1st and 28th days. The maximum concentration of IL-6 was detected in both pressure and tension sides of DCs at T14. At T28, although the IL-6 levels were significantly higher than baseline levels but, it was significantly less than T14. **Conclusion:** The results of this study support the hypothesis that mechanical stimuli cause an inflammatory reaction within the periodontal tissues.

Keywords: Orthodontic tooth movement, Gingival Cervical Fluid (GCF), Interleukin-6 (IL-6)

2027P

Leptin and IL-6 in aggressive periodontitis

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Background: One important function of leptin is the regulation of immune or inflammatory responses. Moreover, in a recent study no correlation between leptin and periodontal diseases was found. Therefore the aim of this study was to determine the correlation between leptin concentration and chronic periodontitis. **Methods:** In this analytical study, 20 healthy gingival

tissue samples (control group) and 20 gingival tissues with aggressive periodontitis (case group) were taken from 20 patients. Patients were consisted of 65% females and 35% males with average age of 25.55 yrs. Tissue samples were cultured for 72 hours. Then ELISA was used for detecting of leptin and IL-6 in supernatant fluids of explant cultures and leptin in serum samples. Statistical analysis was made by t-test and wilcoxon signed Ranks test. **Results:** There was higher leptin in gingival samples of aggressive periodontitis ($P<0.05$), the mean concentration of leptin was 105.55±45.98 (pg/ml). The mean concentration of IL-6 in control and case groups was 71.08±25.95 and 82.33±26.25 pg/ml, respectively. Statistical analysis has shown no difference between case and control groups regarding IL-6 concentration. **Conclusion:** It is concluded that probably leptin does have some pathogenic role as an inflammatory protein in aggressive periodontitis but we can not assumed any important role for IL-6 in periodontal disease.

Keywords: Leptin, Aggressive periodontitis

2031P

Leptin-an adipocytokine and chronic periapical lesion

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Background: It was shown that leptin regulates bone formation and its expression in adipose tissue and its level in circulation are increased after administration of inflammatory stimuli such as lipopolysaccharide (LPS). There is not many data about the role of leptin in the immunopathogenesis of periodontal disease. So the aim of this study was to evaluate the presence and concentration of leptin in moderate to severe chronic periodontitis. **Methods:** For this purpose, chronic periapical lesions were collected from twenty patients and were cultured for 72 hrs. ELISA method was used in order to determine the concentration of leptin in supernatant fluids of explants cultures. Statistical analysis was undertaken using non-parametric tests (Mann-Whitney U, Chi Square and Spearman's Correlation Coefficient. **Results:** Leptin was found in all samples with the average concentration of 405.55±102.98 (pg/ml). There was no significant correlation between the concentration of leptin and BMI or the diameters of lesions. **Conclusion:** It has been concluded that leptin could be considered as an inflammatory mediator during the early phases of dental periapical lesions.

Keywords: Leptin, Periapical lesion

2032P

Substance P and CGRP levels in human dental pulp in relation to caries progression

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Background: This study sought to explore the relationships between the concentration of

neuropeptides in the dental pulp with development of carious lesions. **Methods:** Extracted teeth (n=81) were split with an air turbine and their pulpal tissue were removed and transferred to sterile tubes and immediately frozen at -20'C then different groups of our study were selected by radiographic views and tooth sections. We prepared hemogenized tissue extracts by tissue lysis buffer (containing tris and sucrose) and centrifuged several times. The amount of SP and CGRP was measured by using commercially available high-sensitivity enzyme-linked immunosorbent assay kit. **Results:** Analysis revealed significant increases in CGRP and SP expression with caries progression**.** Significant differences were found between concentration of SP and CGRP in different groups of caries with the highest concentration in exposed pulp followed by groups that caries extended in internal half of dentin thickness, caries extended in external half of dentin thickness, enamel caries and non-carious teeth respectively. Also there was a significant statistical correlation between SP and CGRP concentration. **Conclusion:** It can be concluded that with propagation of caries lesion, SP and CGRP concentration is increased. Also the CGRP concentration is more than SP which is related to regulator effects of this neuropeptide in order to inhibit additional destructive changes in dental pulp.
Keywords: Substance P, CGRP concentration

2035P

Effect of surgical flap on concentration of IL-1beta and TGF-beta1 in the gingival crevicular fluid of patients with chronic periodontitis

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Background: TGF-beta1 is one of the most important anti-inflammatory cytokine and growth factorthat presents in the GCF. IL-1beta is a proinflammatory cytokines that presents in gingival inflammation and the GCF. Such factors might be of value as a prognostic marker of wound healing activity and therapeutic progress following flap surgery. The aim of this study was the assessment of the effect of surgical flap on concentration of IL-1beta and TGF-beta in the GCF of patient chronic periodontitis. **Methods:** The GCF sample were collected, using the Perio-Paper strip at phase 1 (the pre-surgery), phase 2 (the 4th week post surgery) and phase 3 (the 12th week post surgery) from 110 sites of 28 patients under going flap surgery. IL-1beta and TGF-beta concentration were measured my ELISA. **Results:** The mean of TGF-beta and IL-1beta concentration decreased from phase 1 to phase 3 ($P<0.05$). There is no significant statistical correlation between IL-1beta and TGF-beta1 concentration in the 3 assessment phases. **Conclusion:** The flap surgery has significant effect on decrease of IL-1beta concentration. In the case of TGF-beta1, probably its concentration increases in short time after treatment due to its role in initial stages of healing.
Keywords: IL-1beta, TGF-beta1

2227P

C - reactive protein levels in patients with periodontal disease and normal subjects

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Background: Although periodontitis is a chronic inflammatory disease but some factors of acute inflammation phase are involved in this disease among which is the C-Reactive protein (CRP). To minimize its effects, anti-inflammatory drugs or non-pharmacological approaches such as oral hygiene is recommended. CRP can also be used for the prediction and early detection of periodontal disease. The aim of the present study was the comparison of the amount of salivary C-Reactive protein (CRP) in healthy subjects and patients with periodontal disease. **Methods:** This case-control study was done on 90 patients referred to the Department of Periodontology of Babol Dentistry School. These subjects were divided into three groups of healthy (n = 30), gingivitis (n = 30), and chronic periodontitis (n = 30), based on Gingival Index (GI) and Clinical Attachment Loss (CAL) indices. 2ml saliva samples were collected from these people and clinical indicators including GI, CAL, Periodontal Pocket Depth (PPD), and Bleeding Index (BI) were assessed. ELISA method was used to evaluate the salivary CRP levels. Collected data were analyzed using SPSS statistical software by non-Parametric Kruskal-Wallis and Mann-Whitney test and Spearman correlation coefficient and $P<0.05$ was considered significant. **Results:** The mean salivary CRP levels were 5332.62±5051.63pg/ml in periodontitis patients, 3545.41±3061.38pg/ml in gingivitis group and 3108.51±3574.47pg/ml in healthy subjects. The statistic analysis showed a significant difference in salivary CRP concentrations between the periodontitis patients and healthy subjects ($P=0.045$). **Conclusion:** The results indicate that there is a significant association between periodontitis and salivary CRP concentrations.

Keywords: CRP, Periodontitis, Gingivitis, Saliva

2313P

TNF-α and TGF-β1 level in radicular cyst and odontogenic keratocyst fluid

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Faculty of Dentistry, Babol University of Medical Sciences and Health Services, Babol, Iran.
Background: TNF-α is a multifunctional proinflammatory cytokine and TGF-β1 is a secretory protein controlling epithelial proliferation and differentiation. Keratocyst presents an aggressive behavior and a growth mechanism different from that of radicular cyst. Aim: In this line, the present study aimed at evaluating TNF-α and TGF-β1 level and its association with histopathological findings in the two odontogenic lesions of different origins. **Methods:** In this case-control study, aspirated fluid of 15 cases of radicular cyst and 15 cases of keratocyst were investigated using ELISA method. The grade of inflammation and the mean number of blood vessels in three microscopic fields were provided with a magnification of 40 times on microscope slides. T-test, x2, Mann Whitney, and Pearson correlation tests were used for the comparison of TNF-α and TGF-β1 levels in the mentioned lesions and the association between cytokine levels and grade of inflammation and angiogenesis. **Results:** TNF-α and TGF-β1 were observed in aspirated fluid of all radicular cysts and keratocysts. Levels of TNF-α and TGF-β1 were found to be 6.72 ± 2.985 and 5.882 ± 2.985 respectively in radicular cyst fluid and 24.759 ± 94.849 and 63.38 ± 30.069 in keratocyst fluid; however, no statistically significant difference was observed in terms of TNF-α (P=0.450); increasing trend in TNF-α level in radicular cyst and keratocyst was accompanied by increased inflammation and angiogenesis (P<0.001andP=0.001). **Conclusion:** TNF-α and TGF-β1 are involved in the pathogenesis of radicular cyst and keratocyst. TGF-β1 level was higher in radicular cyst when compared with keratocyst; however, TNF-α level was similar in the two lesions. A positive correlation was found between TNF-α level and grade of inflammation and angiogenesis.
Keywords: Radicular cysts, Odontogenickeratocyst, TNF-α, TGF-β1, ELISA

2564P

Correlation between salivary sCD14 and TNF-alpha in chronic periodontitis

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Background: Oral epithelial cells do not express membrane CD14 and may be that high levels of the soluble form of CD14 (sCD14) can be assumed as a way for evading from the induction of inflammatory response in gingival epithelium by periodontopathis bacteria which most of them are gram negative and can elicit innate immune cells by attaching to CD14 and TLR4. So the aim of this study was to evaluate the relationship between salivary levels of CD14 and TNF-alpha as one of most important proinflammatory cytokine in patients with moderate to severe chronic periodontitis. **Methods:** For this purpose, salivary samples were collected from 50 patients with moderate to severe chronic periodontal diseases. The levels of soluble CD14 and TNF-alpha in saliva after diluting saliva by PBS, were measured by ELISA. Statistical analysis was made by SPSS 18.00. **Results:** We found negative significant correlation between salivary sCD14 and TNF-alpha, and also there were significant positive correlations between TNF-alpha concentration and CAL (Clinical attachment loss) and PD (Pocket depth) but there was a negative correlation between sCD level and CAL. **Conclusion:** It is concluded that sCD14 has a possible protective role against periodontopathic bacteria and disease process, probably by dampening the production of proinflammatory cytokines such as TNF-alpha.
Keywords: sCD14, TNF-alpha, Chronic periodontitis

1660P

The assessment of correlation between salivary toll like receptor 2 (TLR 2) concentration with early childhood caries (ECC)

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Background: early childhood caries are prevalent in developing countries. Knowledge about etiology and pathogenesis of this lesion plays an important role in prevention. Evaluation of immune responses followed by a decay, is one of new ways of prevention. Toll Like Receptor 2 has a vital function in innate immune system versus gram positive bacteria, so the evaluation of correlation between this factor and caries could have some role in prevention methods. **Materials:** 28 children with age of 36 to 71 months (15 ECC group and 13 caries free group) were chosen based on predetermined factors. 1-2 mililiter of their saliva was taken with disposable syringe. Moreover index plaque and dmft (Decayed, Missed, Filled Teeth) were evaluated. 3 months after dental restorations, 8 children in patient group were re-sampled. After that, the optical density of samples obtained by ELISA reader. Spss software and Kolmogorov Smirnov and parametric tests (T-test and Pearson correlation index) were used for analysis. for Second phase, paired sample-t was applied. **Results:** the mean concentration of TLR 2 in ECC and caries free groups, were 2.12 and 1.42 ng/ml, respectively. The difference between concentrations, were significant (3 months after treatment in 8 ECC children, the mean concentration was 0.925 ng/ml, which compared to ECC group and caries free group, was significant. **Conclusion:** The concentration of TLR 2 in ECC group was more than caries free group. 3 months after dental restorations, the concentration of TLR 2 in treated children decreased significantly, so that, the concentration of TLR 2 in this group was reduced in comparison with that, in ECC and caries free groups.

Keywords: TLR 2, Saliva, Early childhood caries.

Psychoneuroimmunoendocrinology

Oral Presentations:

1531O

Linkage Study of Primary Microcephaly in Pakistani Kindred

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Background: Microcephaly is heterogeneous, autosomal recessive trait with reduced head circumference of at least 4 SD below age and sex means due to reduction in neuron production. The brain of microcephalic patient is architecturally normal but severe to mild mental retardation. It is rare disease affecting 2-2.5% of total population specifically in Asia and Arab where the incidence of cousin marriages is relatively high. From seven known currently mapped loci *ASPM* is found to be the frequent causative agent. **Methods**: In the current investigations exclusion mapping of a microcephalic family was done. DNA from all blood samples was extracted using standard procedure and after gene specific PCR amplifications, 8% non-denaturing PAGE was done. **Results**: Linkage was observed at MCPH5 locus where *ASPM* is a candidate gene on chromosome 1q31. The results of DNA sequencing showed G to A transition and Leucine (CTG) to Leucine (CTA) was noted. There are six triplet codons which differ by single nucleotide encoding for Leucine. Hence, no overall change in the effect of protein expression was observed due to the degeneracy of codons. **Conclusion**: Therefore, the sequencing of the entire *ASPM* gene with intervening sequences was suggested in order to find the actual cause of microcephaly.

Keywords: Exclusion mapping, Microcephaly, ASPM

3298O

ASC provides a potential link between depression and inflammatory disorders: A clinical study of depressed Iranian medical students

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Background: AIM2 is a member of the inflammasome which can activate caspase-1 via an adaptor protein (ASC) after PAMPs and DAMPs recognition. Activation of caspase-1 is trigger for the activation of IL-1 and IL-18 which are important pro-inflammatory cytokines. IL-1b has previously been associated with depression. Previous studies revealed that depressed patients suffered from altered immune responses, but the mechanisms underlying this correlation are unclear. Thus, the aim of this study was to determine the mRNA levels of AIM2 and ASC in the PBMCs isolated from depressed Iranian medical students. **Methods:** Subjects of the study were 38 Iranian depressed medical students and 43 healthy students as a control group. The mRNA levels of AIM2 and ASC were evaluated using β-actin as a housekeeping gene using Real-Time PCR technique. **Results:** Our results showed that mRNA levels of AIM2 were similar in both groups. However, ASC levels were significantly increased in PBMCs isolated from depressed patients when compared to healthy subjects. **Conclusions:** Based on the current results, it appears that ASC transcript expression may represent a potential link between depression and the altered immune responses seen in these patients.

Keywords: Depression, AIM2 and ASC.

1992O

Physical activity, fatigue, depressed mood and immune function in breast cancer patients

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Background: Physical activity has benefits above and beyond weight control. Physical activity has been documented to help maintain healthy bones, muscles, and joints, to reduce the risk of high blood pressure and diabetes, to promote psychological well-being, to reduce the risk of premature death and death from heart disease. In addition to these health benefits, researchers are learning that physical activity can also affect the risk of cancer. **Methods:** A sample of 54 breast cancer survivors was recruited through a convenient sampling procedure. Demographic and clinically relevant information, cancer-related fatigue, physical activity level, and depressed mood were assessed in all participants. Immune function was estimated using NK and T cell function. **Results:** Significant positive correlations between depressed mood and fatigue ($r = .52$ $p < .01$) and between physical activity and immune function ($r = .38$, $p < .05$) were found. In addition, significant negative correlations between depressed mood and physical activity, ($r = -.44$ $p < .05$) and depressed mood and immune function ($r = -.36$, $p < .05$) were found. Regression analyses revealed that cancer-related fatigue and physical activity level were significant predictors of depressed mood, and when combined, they explained 32.8% of the variance in depressed mood. Similarly, fatigue, physical activity and depressed mood significantly predicted immune function and together explained 19.4% of the variance in immune function. **Conclusion:** Cancer-related fatigue, physical activity level, and depressed mood partially explain the variability of immune function in breast cancer survivors.

Keywords: Physical activity, Fatigue, Mood, Immune function, Breast cancer

2289O

Down-regulation of IL-6 gene attenuates clinical course of active experimental autoimmune encephalomyelitis

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Background: T_{H17} cells play a pivotal role in the pathogenesis of experimental autoimmune encephalomyelitis (EAE). Meanwhile, IL-6 induces the development and differentiation of TH_{17} cells from naive T cells. This study aimed to investigate the therapeutic effect of IL-6 gene downregulation in mouse model of EAE. **Methods:** A plasmid encoding short hairpin RNA (shRNA) against IL-6 was designed and constructed (P240-shRNA6). Then, this plasmid was twice injected to EAE mice on days 15 and 22 after EAE induction. The clinical signs of the treated mice were evaluated daily and scored according to a standard method. At the end of treatment period, all the EAE mice were sacrificed for histopathological and immunological analysis.**Results:** EAE severity significantly decreased in the P240-shRNA6 treated mice. Although, P240-shRNA6 had no considerable inhibitory effect on the infiltration of inflammatory cells into the CNS tissues of the EAE mice, but it could significantly decrease pro-inflammatory cytokines levels IL-6, IL-17 and IFN-γ; and increase anti-inflammatory cytokine level IL-4. Moreover, a significant increase was detected in the $CD4^+CD25^+$ regulatory T cells population of both CNS and spleen of the treated mice.**Conclusion:** Dowregulation of IL-6 gene by means of shRNA may be considered as a therapeutic tool for EAE, and perhaps multiple sclerosis.

Keywords: IL-6, shRNA, EAE, Regulatory T cells, T_{H17} cells

2326O

Alternation of Immune cells in patients with schizophrenia: response quality more important than quantity

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Background: A variety of immunologic alterations have been observed in patients with schizophrenia and progressive loss of brain volume has been discussed as possible consequences of an active immune process. We therefore assessed cellular changes of both innate and adaptive immunity (humoral and cell mediated) in schizophrenia patients. **Methods:** In this study, we used immunofluorescent flowcytometry to measure CD19 + (B) - and CD3 + (T)-lymphocytes and also CD14 monocytes of peripheral blood (PB) samples in patients who met DSM-IV-TR criteria for schizophrenia and in normal control subjects. In addition, total WBCs and neutrophils were analyzed using automated hematology systems. **Results:** The schizophrenic patients showed a higher levels of B and T lymphocytes and

also inflammatory monocytes and neutrophils when compared to healthy controls. While, the increase observed for absolute WBC count (ranges 4600–11000 cells/cu mm, mean 7960 cells/cu mm) was not significant compare to healthy groups(ranges 4100–13600 cells/cu mm, mean 7650 cells/cu mm) and also normal ranges (ranges 4000–11000 cells/cu mm, mean 7500 cells/cu mm).**Conclusion:** The present data confirm the presence of immunological alternations of both innate and adaptive immunity in schizophrenic patients. However, small shift in quantity of all subsets of WBC without significant alternation of total WBC compare to normal ranges indicated that adaptive immune cells differences and subsequent changes of responses and function of immune cells seem to be more important than alternation in total count of WBCs caused by increased number of inflammatory cells.

Keywords: Schizophrenia, Immune cells, Flowcytometry, Lymphocyte,WBC

2705O

α-Galactosylceramide enhanced TNF-α in post-weaning social isolated rats

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Background: Psychological stress is associated with an increased expression of markers of peripheral inflammation, and there is growing evidences describing a link between periodontal pathogens and systemic inflammation. In present study, we investigate the immune-response status of post-weaning social isolation rats and evaluated the effect of α-galactosylceramide on lymphocyte proliferation and tumor necrotic factor-α in splenocytes. **Methods:** Wistar rats after weaning (21–23 days old) were either housed individually or grouped for 6-8 weeks. The animals were treated twice in social isolation period with either α-galactosylceramide or saline. For immunoassay, spleens were removed and cultured RPMI 1640 medium in 96 well plates for 72 h and exposed with PHA (10 mg/well) and incubated. All assays were performed in triplicate. **Results:** Results were expressed as stimulation index defined as the ratio of the mean absorbance of cells stimulated with antigen to the mean of unstimulated cells. Data indicated significantly enhancement of stimulation index of α-galactosylceramide in isolated rats compared to control group ($p<0.05$). Social isolation decreased ($p<0.001$) stimulation index in all rats. **Conclusion:** Our results indicate that α-galactosylceramide may stimulate the immune-response and improve the immunity in the social isolation rats.

Keywords: α-galactosylceramide, TNF-α

2325O

New cytokines network involved in Schizophrenia, targeting by antipsychotic treatment

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Background: The involvement of immunological mechanisms in the etiopathogenesis of schizophrenia has been a matter of research, with recently increasing effort. Cytokines are crucial mediators of the cross-talk between the brain and the immune system. Furthermore, some studies reported that treatment with antipsychotic drugs affects the cytokine network. **Methods:** 23 patients, who met DSM-IV-TR criteria for schizophrenia and 42 patients at stable phase showed clinical improvement by treatment (treatment group), were compared with 33 healthy controls. Serum levels of TNF-α, IL-1 β, IL-2, IL-6, INF-γ and IgG were measured by sandwich enzyme-linked immunosorbent assay (ELISA). Also, we attempted to determine potential correlation between cytokine levels and disease control by comparing results in patients and treatment groups. **Results:** The serum levels of TNF-α, IL-1 β, IL-6 and INF-γ were significantly lower in the treatment group($p<0.001$) when compared with both healthy and patient groups; Besides, IgG and Il-2 decreased significantly compared with patient and healthy groups, respectively. IL-6 and IgG were increased in patients in comparison with controls ($p<0.001$). Interestingly, there was a significant correlation between IL-1 β and IL-6, TNF-α and IL-6 and also between IL-1 β and TNF-α only in patient group ($p <0.001$); While, this significant correlation were not demonstrated between IL-6 and IgG. **Conclusion:** These findings provide the first evidence of establishment of [TNF-α, IL-1 β, IL-6] cytokines network in schizophrenia, which can be paralleled with antipsychotic treatment results. Our data revealed a notable immune disturbance after schizophrenia treatment, which may cause clinical improvement, but some alternations, especially INF-γ reduction, need to be considered for Th1/Th2 imbalanced shift.

Keywords: Schizophrenia, Immune system, Cytokine, Antipsychotic treatment, IgG

2950O

Behavioral, cardiovascular and endocrine responses effects of acupuncture in mice under stress of captivity

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Background: Acupuncture is an ancient healing technique that dates back about 2000 years in this study, effects of acupuncture on some physiological responses were evaluated in mice under captivity stress. **Methods:** 40 mature male mice balb / c with an average weight of 30-25 g, were kept in individual cages and divided into two equal groups of 20. During adaptation period was the two-week and in the experiment, the mice were stored in the same conditions. The first group contains The mice endured daily for 60 minutes a week in captivity stress within the glass cylinder individually. The second group: This group, like the previous group were affected by stress but after the period of captivity were treated with acupuncturel needles number 82 in area, REN17 S36, SP6, P6. **Results:** Considerations come into operation on the natural behavior of induction like jumping, gasping, roaring and …, In term of imprisonment and afterwards in various categories, in the first group was compared with the control group indicate the motion. Heart rate and blood pressure did not show significant differences between

different groups. The serum levels of plasma corticosteroids, adrenaline and noradrenaline in group two was lower than the level of this hormone in control. **Conclusion:** Study results confirmed that the acupuncture points have a significant impact on reducing levels of stress hormones is caused the movements induced by captivity in mice. However, Does not have a significant effect on the cardio-vascular system the mice.

Keywords: mice, endocrine responses, behavioral effects, cardio – vascular, acupuncture

1871O

Islet amyloid polypeptide is not a target antigen for CD8$^+$ T-Cells in type 2 diabetes

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Background: Type 2diabetes (T2D) is a chronic metabolic disorder in which pancreatic β cells are destroyed. The islet amyloid polypeptide (IAPP) produced by β cells has previously been reported to play role in the destruction of b cells. We hypothesized that IAPP might act as an autoantigen and hence CD8$^+$ T cells specific for this protein might be present in T2D patients. **Method:** Peripheral blood mononuclear cells (PBMC) were obtained from HLA-A2+ T2D patients and non-diabetic healthy subjects. Cells were then screened for peptide recognition using ELISPOT assay to investigate the presence of IFN-γ producing CD8+ T cells against two HLA class-I restricted epitopes derived from IAPP ($IAPP_{5-13}$ and $IAPP_{9-17}$) and some common viral peptides (as control). **Results and conclusion:** A total of 36.4% of patients and 56.2% of healthy subjects elicited a specific response against $IAPP_{5-13}$ peptide, however the difference between two groups was not statistically significant. For $IAPP_{9-17}$ peptide, yet again no significant difference was observed between T2D and healthy subjects, 36.4% of patients vs. 37.5 % of controls. Finally, control group showed stronger response toward common viral peptides compared to T2D patients; however the difference was not statistically significant. In conclusion, it is unlikely that IAPP would be a suitable target for CD8$^+$ T cells in diabetic patients. However a trend toward a lower response of T2D patients against IAPP and common viral peptides was observed that raises the possibility of decreased immune response of these patients.

Keywords: Islet amyloid polypeptide, CD8$^+$ T-cells, Type 2 diabetes

3218O

Decreased peripheral blood mononuclear cells viability in women with gestational diabetes Mellitus

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Background: Gestational diabetes mellitus (GDM) is defined as glucose intolerance of variable severity with onset or first recognition during pregnancy. It is associated with, metabolic disorders and inflammatory responses in the maternal circulation. Many previous studies have shown differences in cytokines and Adipokines level in GDM women compared with healthy pregnant women. And the role of T lymphocytes in this disease has been reported. The aim of this study was to investigate viability of Peripheral Blood mononuclear cell (PBMC) in patients with GDM in comparison with healthy pregnant women. **Methods:** During a case -control study37 pregnant women with GDM and 45 healthy pregnant women in gestational age28-36 week were recruited. Fasting venous blood was collected from all subjects .PBMC was separated from ficoll density-gradient centrifugation. Then Cells were suspended in culture medium RPMI 1640, 10% FBS and cultured in the absence and presence of PHA in 96-well plate (1* 10^6 cells/ml) at 37^{0C} 5% CO2 for 48h. cell viability was assessed with MTT assay. **Results:** There was no significant difference in age, body mass index, and gestational week in the two groups. MTT assay show a significant decrease in cell viability of PBMC in GDM in compared to the control group in the absence and presence of mitogen. **Conclusion:** These results show that GDM is associated with Decreased peripheral blood mononuclear cells viability.
Key word: cell viability, GDM, PBMC

Poster Presentations:

1582P

Serum prolactin level and retinopathy in type 2 diabetes

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Background: Retinopathy is a common complication of type 2 diabetes. Although there are several factores including hyperglycemia and hypertension that seem to play important role in retinopathy, the actual mechanistic pathways are not yet discovered. Prolactin may have protective effects on retin due to its antiangiogenic characteristics. We performed a case control study in order to investigate the potential role of 23 kd prolactin in retinopathy. **Methods**: A total nummber of 212 type 2 diabetics who were visited at out pateint endocrine clinic were recruited in this study. According to retinopathy they were divided into two groups.Group one (70 pateintes)suffered diabetic retinopathy and group two (142 pateints)did not have retinopathy. **Results:** There was significant difference in diabetes duration and hyperglycemia and systholic hypertention in two groups .However we did not found any significant difference in prolactin level between two groups. **Conclusion:** Serum 23 kd prolactin does not seem to have preventive role in diabetes retinopathy.
Keywords: Retinopathy, Prolactin, Diabetes, Anti-angiogenesis

1849P

Impact of thyroid dysfunction on airway responsiveness in the Guinea-pig *in vitro*

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Background: Bronchial asthma worsens after the development of hyperthyroidism. However, the mechanism of this phenomenon, which is induced by thyroxine (T4), remains obscure. To investigate the possible relationship between thyroid dysfunction and asthma we studied airway responsiveness in guinea pig. **Methods:** Twenty four male Dunkin- Hartley guinea pigs were randomly placed in to 3 groups: 1. Control (CTL), 2. Hyperthroid (HPR), 3. Hypothyroid (HPO). HPR and HPO were induced by administration of ip L-thyroxin and po methimazole, respectively. After treatments animals were anesthetized by CO2 gas and blood samples were taken via heart puncture. Thereafter, they were euthanized by an excess dose of the gas, thyroid glands and tracheas were removed for further experiments. Tracheas were divided in to segments of 3 rings each and were placed in the organ bath. Airway responsiveness was evaluated after administration of increasing concentrations of histamine, acetylcholine and isoprenaline.**Results:** L-thyroxin and methimazole administration caused HPR and HPO situation as shown in biochemical, clinical and histopathological examinations. In the HPR guinea pigs an increased airway hyperrespnsivness was demonstrated ($p<0.05$).**Conclusion:** Our data showed that hyperthyroidism, not hypothyroidism, is exacerbated asthma. It is suggested to study this interaction in more details, e.g., epidemiologic trials to screen asthmatic patients for their blood thyroid hormones levels, is highly recommended.

Keywords: Hyperthyroid, Hypothyroid, Airway responsiveness, Guinea pig

1708P

Differential Expression level of CXCL10 as anti-angiogenesis CXC chemokine in gestational diabetes mellitus mothers and their neonates

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Background: Gestational diabetes mellitus (GDM) is the most frequent metabolic disorder in pregnancy, affecting 1–10% of all pregnancies. Several types of regulators including cytokine and chemokine network is considered to play a crucial role in pregnancy by local modulation of the immune system at the level of peripheral leukocytes. Therefore, current study aimed to determine systemic level CXCL10 in GDM mothers and their neonates. **Methods:** The study group consisted of 54 pregnant women suffering GDM in the third trimester of pregnancy and 54 healthy normal pregnant women matched for gestational age served as a normal control group. The serum and cord blood level of CXCL10 was measured by ELISA in studied groups. **Results:** Our results showed decreased antistatic chemokine CXCL10 neonates of delivered from mothers with GDM. Our results also showed that the level of studied CXC chemokine was not changed in mothers with normal or GDM-associated pregnant women. **Conclusion:** According to the results of this work it could probably be concluded that GDM the expression of CXCL10 Chemokine is related with the balance between angiogenesis / angiostatsis phenomenon associated with pregnancy and follows a pattern of inflammatory in pregnant women.

Keywords: CXCL10, Gestational diabetes mellitus.

1707P

Elevated level of CXC chemokine CXCL12 as angiogenesis in gestational diabetes mellitus mothers and their neonates

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Background: The GDM usually manifests itself in the latter half of pregnancy and is described by carbohydrate intolerance of variable severity. It has been reported that, the prevalence of GDM is proportional to the frequency of Type 2 Diabetes Mellitus within a community. Several types of regulators including cytokine and chemokine network is considered to play a crucial role in pregnancy by local modulation of the immune system at the level of peripheral leukocytes. Therefore, current study aimed to determine role levels of CXCL12 as angiogenesis in gestational diabetes mellitus mothers and their neonates. **Methods:** The study group consisted of 54 pregnant women suffering GDM in the third trimester of pregnancy and 54 healthy normal pregnant women matched for gestational age served as a normal control group. The serum and cord blood level of CXCL12 was measured by ELISA in studied groups. **Results:** Our results showed increased levels of angiogenesis chemokine CXCL12 neonates of delivered from mothers with GDM. Our results also showed that the level of studied CXCL12 chemokine was not changed in mothers with normal or GDM-associated pregnant women. **Conclusion:** According to the results of this work it could probably be concluded that GDM the expression of CXCL12 chemokine is related with the balance between angiogenesis/ angiostatsis phenomenon associated with pregnancy and follows a pattern of inflammatory in pregnant women.

Keywords: CXCL12, Gestational diabetes mellitus

2112P

The relationship between Seasonal variation and the incidence of congenital hypothyroidism in Markazi Province, Iran

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Background: Congenital hypothyroidism is the most common cause of preventable mental retardation in newborns. Retardation occurs when treatment is useless, but the CHT can be detected easily with a simple test in the third to fifth day after the birth so this problem can be prevented rapidly. Any delay in treatment would reduce IQ and infant health. In this study the relationship between seasonal variations and the incidence of congenital hypothyroidism (CHT)was evaluated with the screening program of CHT in Markazi Province, Iran. **Methods**: data were collected with colorimetric Method (Guthrie paper) in the Markazi province screening center.CHT screening results of 85112 neonates from 14 health center in Markazi province since 2009 to 2012 were collected and surveyed. The data's were evaluated and analyzed and the relationship between seasonal variation and incidence of CHT has been proved. **Results**: from 85112 neonates that were screened, 290 neonates were diagnosed with CHT. overall incidence from April to September (Spring & summer) was 2.9 out of 1000 live birth(2.9/1000), but the results of October to March (autumn & winter) are increased and the

rate changes to 3.9/1000 live birth. **Conclusion**: our identifications about high incidence of CHT in cold seasons were similar to the results of west midlands, England. Therefore, it can be concluded that there are some seasonal factors such as temperature, vegetables and fruits diet and air pollutions that have effect on incidence of CHT. So recognition of these factors can aid development strategies of prevention.

Keywords: Congenital hypothyroidism, colorimetric Method, seasonal variations, mental retardation.

2666P

Impact of growth hormone (GH) deficiency and GH replacement upon thymus function in adult patients

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Background: Despite age-related adipose involution, T cell generation in the thymus (thymopoiesis) is maintained beyond puberty in adults. In rodents, growth hormone (GH), insulin-like growth factor-1 (IGF-1), and GH secretagogues reverse age-related changes in thymus cytoarchitecture and increase thymopoiesis. GH administration also enhances thymic mass and function in HIV-infected patients. Until now, thymic function has not been investigated in adult GH deficiency (AGHD). The objective of this clinical study was to evaluate thymic function in AGHD, as well as the repercussion upon thymopoiesis of GH treatment for restoration of GH/IGF-1 physiological levels. **Methods and results:** Twenty-two patients with documented AGHD were enrolled in this study. The following parameters were measured: plasma IGF-1 concentrations, signal-joint T-cell receptor excision circle (sjTREC) frequency, and sj/b TREC ratio. Analyses were performed at three time points: firstly on GH treatment at maintenance dose, secondly one month after GH withdrawal, and thirdly one month after GH resumption. After 1-month interruption of GH treatment, both plasma IGF-1 concentrations and sjTREC frequency were decreased ($p<0.001$). Decreases in IGF-1 and sjTREC levels were correlated ($r=0.61$, $p<0.01$). There was also a decrease in intrathymic T cell proliferation as indicated by the reduced sj/b TREC ratio ($p<0.01$). One month after reintroduction of GH treatment, IGF-1 concentration and sjTREC frequency regained a level equivalent to the one before GH withdrawal. The sj/b TREC ratio also increased with GH resumption, but did not return to the level measured before GH withdrawal. **Conclusions:** In patients with AGHD under GH treatment, GH withdrawal decreases thymic T cell output, as well as intrathymic T cell proliferation. These parameters of thymus function are completely or partially restored one month after GH resumption. These data indicate that the functional integrity of the somatotrope GH/IGF-1 axis is important for the maintenance of a normal thymus function in human adults.

2667P

Expression of the growth hormone (GH)/insulin-like growth factor (IGF) axis during Balb/c thymus ontogeny and effects of GH upon *ex-vivo* T-cell differentiation

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Background: Human growth hormone (GH) is a polypeptide with a molecular weight of 22kDa, containing 191 amino acids and is secreted by the anterior pituitary. Growth hormone has a wide range of biological activities including growth, metabolism of proteins, carbohydrates, fats and minerals. Growth hormone deficiency in human occurs both in children and adults. The routine treatment for this condition is administration of recombinant human growth hormone (rhGH) made by prokaryotes. *Lactococcuslactis* is a suitable microorganism for production of recombinant proteins as it is regarded safe. It also has the potential of localization in human and animal body for in-suite production. The aim of this study was to clone and optimize the production of rhGH in *Lactococcuslactis.* **Methods:** The sequence related to human GH was amplified and cloned into *Lactococcus lactis* using NICE expression system. Different media and culture conditions including 0, 0.5% and 0.7% of lactose and temperatures (25°C, 30°C, 37°C) were tested. Production of rhGH was assessed using, dot blotting, western blotting and ELISA. **Results:** The rhGH sequence was cloned successfully as confirmed by PCR and sequencing. The rhGH was produced and released into the medium by bacteria at high levels. Protein expression levelwas higher at 0.7% of lactose and at 37°C compared to other conditions. Conclusion: The rhGH could be produced at high levels in as safe and probiotic bacteria such as *Lactococcuslactis* successfully.

Keywords: Thymus - Growth hormone (GH) – Insulin-like growth factors (IGFs) – GH receptor (GHR) – GHR antagonist – Fetal thymic organ cultures (FTOC)

2601P

CXCL5 gene polymorphism association with diabetes in Ardebil province

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Background: CXCL5-known as epithelial cell-derived neutrophil-activating peptide (ENA-78), is a chemokine that has a role in some diseases. CXCL5 blocks insulin signaling by activating the Jak2/STAT5/SOCS2 pathway. It is reported the association between 156G>C (rs352046) polymorphism in the promoter region and diabetes. The aim of this study was to examine whether there was an association between this polymorphism and diabetes mellitus in Ardebil province population. **Methods:** A total of 100 patients affected diabetes were recruited from Ardebil province population; 100 healthy control subjects were recruited from the same area. The region containing the CXCL5 - 156G>C polymorphism was genotyped by PCR amplification and restriction fragment length polymorphism analysis, and allele frequency data were analyzed using Fisher test. **Results:** The results show a higher frequency of carrying both the G/G and G/C genotype in patients with diabetes compared with healthy controls (P-value=0.01 and 0.006, respectively). In addition, the frequency of allele C was significantly increased (P-value = 0.028) in patients with diabetes (25.5%) compared with controls (12%). **Conclusions:** Our findings suggest a role of CXCL5 in the pathogenesis of diabetes. Also, replications in other populations with larger sample sizes are required to confirm these findings.

Keywords: Diabetes, CXCL5, Polymorphism

2635p

Cell therapy and gene therapy as new approaches for the cure of diabetes mellitus

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Background: Diabetes mellitus is a clinical condition caused by insulin deficiency or a resistance to insulin, resulting in elevated blood glucose levels. The consequences of long-term hyperglycemia can cause to end stage micro- and macrovascular damage leading to organ failure such as neuropathy, nephropathy, retinopathy, peripheral vascular disease, morbidity and mortality. 371 million people are living with diabetes and it is estimated that this number will reach 600 million by 2030.Curative therapy for diabetes mellitus mainly implies replacement of functional insulin-producing pancreatic cells, with pancreas or islet-cell transplants, but new methods gene therapy may be helpful. **Methods:** The query was conducted by using standard keywords (MeSH Term) in the main database of clinical trial Studies as GeMCRIS, ClinicalTrials.gov, American Society of gene & Cell therapy, European Society of gene & cell therapy. All studies were enrolled completed or on the verge the end and Timeframe considered the beginning of the studies so far have been recorded. **Results:** In entire queries, 7101 clinical trial studies on diabetes is being done that 1074 cases related to Type I diabetes, 3691 cases related to Type II diabetes and the remaining cases involving be shared between the both. Less than 10% of the studies have focused directly on the treatment of diabetes and other studies have relied on the treatment of Diabetes complications. **Conclusion:** Cell therapy with emphasis on the differentiation of stem cells into beta cells and transplanting it to specific location or an Ectopic site as liver, Success is associated with approximately insignificant. Bone Marrow derived Mesenchymal cell, Epidermal Keratinocytes and Fibroblast are of other options. Since 1997, different types of gene therapy studies have been undertaken were often mediated by viral vectors, However recently, non-viral gene transfer methods as Calcium Phosphate Co-precipitation, Lipofection (Liposome), Nanoparticle and Particularly Electroporation and Biolistic have been used. However, all the above has not led to a cure for diabetes, but this has opened new windows.

Keyword: Diabetes, gene therapy, cell therapy, clinical trial

2235P

Effect of different culture condition on production of recombinant human growth hormone in Lactococcus lactis

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Background: Human growth hormone (GH) is a polypeptide with a molecular weight of 22kDa, containing 191 amino acids and is secreted by the anterior pituitary. Growth hormone has a wide range of biological activities including growth, metabolism of proteins, carbohydrates, fats and minerals. Growth hormone deficiency in human occurs both in children and adults. The routine treatment for this condition is administration of recombinant human growth hormone (rhGH) made by prokaryotes. *Lactococcuslactis*is a suitable microorganism for production of recombinant proteins as it is regarded safe. It also has the potential of localization in human and animal body for in-suite production. The aim of this study was to clone and optimize the

production of rhGH in *Lactococcuslactis*. **Methods:** The sequence related to human GH was amplified and cloned into*Lactococcuslactis*using NICE expression system. Different media and culture conditions including 0, 0.5% and 0.7% of lactose and temperatures (25°C, 30°C, 37°C) were tested. Production of rhGH was assessed using, dot blotting, western blotting and ELISA. **Results:** The rhGH sequence was cloned successfully as confirmed by PCR and sequencing. The rhGH was produced and released into the medium by bacteria at high levels. Protein expression levelwas higher at 0.7% of lactose and at 37°C compared to other conditions. **Conclusion:** The rhGH could be produced at high levels in as safe and probiotic bacteria such as *Lactococcuslactis*successfully.

1878P

Changes in serum levels of the TNF-α, IL-10 and IL-2 in schizophrenic patients before and after treatment

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Background: Schizophrenia is a disorder of the executive function of both sensory and central nervous system (CNS).Recent studies suggest the existence of effective immunological changes in the pathophysiology of this disease. The variations in cytokine levels have been associated with schizophrenia, psychopathology and treatment. In this study we investigated the changes in serum levels of TNF-α, IL-10 and IL-2in schizophrenic patients before and 40 days after treatment. **Methods:** In a case-control study26 schizophrenic patients and 26 healthy individuals as a control group were enrolled. PANSS scale questionnaire used for diagnosis and severity of disease. All patients were treated with risperidoneor clozapine for 40 days. Serum levels of TNF-α, IL-10 and IL-2 were measured by ELISA before and after treatment and also in control group. Paired t-test and Independent t-test used for comparison of data. **Results:** In comparison with healthy Controls, serum levels of TNF-α in schizophrenic patients was increased but IL-10 levels was decreased before treatment. A significance decrease of TNF-α and IL-10 levels were observed in the sera of patients after treatment (P = 0.002 , 0.008 respectively).Serum levels of IL-2 was less than the lower limit of detection of assay and was not detectable. There was no correlation between cytokines level and the positive and negative scale (PANSS), also no significant difference with admission, relaps and duration of illness before and after treatment. **Conclusion:** Increase of TNF-α and decrease of IL-10 may have an important role in Psychopathology of Schizophrenia.

Keywords: Schizophrenia, TNF-α, IL-10, IL-2, Psychopathology Symptoms

2713P

Differential expression of miRNA-150, miRNA-155 and miRNA-18a correlate with PU.1 and AMWAP levels in an animal model of multiple sclerosis

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Background: MicroRNAs are regulatory molecules which are involved in many physiological and pathological processes, including diseases of the nervous system. Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS), which is characterized by leukocyte infiltration and microglial activation, followed by demyelination. PU.1 is a transcription factor that is upregulated in macrophages of the CNS during MS. In this study we investigate miR-155, miR-150 and miR-18a expression levels and their relation with PU.1 transcription factor and downstream AMWAP gene in the CNS of EAE mice, an animal model of MS. **Methods:** RNA was extracted from the lumbar spinal cord tissue of mice with EAE at different stages of disease. Gene and microRNA expression were analyzed in tissue samples from EAE and control mice using real- time RT-PCR with SYBR Green method. **Results:** A comparison between the control and study groups showed that miRNA-150, miRNA-155, miRNA-18a and also their target genes, PU.1 and AMWAP, were significantly increased in the nervous system acute and chronic stages of disease. In addition, we observed a statistical relationship between these three microRNAs and their target genes expression. **Conclusions:** These findings show that miRNA-155, miRNA-150, miRNA-18 might play a in inflammatory responses in CNS, suggesting that they might be a promising target for therapeutic intervention in autoimmune disorders such as multiple sclerosis.

Keyword: MicroRNA, Multiple sclerosis, Experimental autoimmune encephalomyelitis, PU.1

1699P

Purification of brain antigens and its reaction with the patient sera with Alzheimer disease

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Background: Alzheimer's disease is one cause of progressive perception failure in the elderly. This disease is a disorder of the CNS that affects approximately 1-6% of people over 65 years. **Methods**: The study included two categories of response. The one is Precipitation reactions between a brain antigen in animal models and the antibody in the serum, the procedure was performed in 2 methods, immunoelectrophoresis and counter-immunoelectrophoresis. Because of the absence of a clear precipitation lines, result was not acceptable. Another class of reaction is flocculation, in which the procedure is similar to the VDRL test. Serial dilutions of sera (1/40,1/20,1/10,1/5)were performed, with using this method the semi-quantitative autoantibodies titration was performed. **Results:** Undiluted serum of all patients showed the significant positive response to this test, while this figure is indicated in the control groupwas29.51% (P=0.00011) , control group showed a positive response only to a dilution of1/10, while 3.44% of the patients showed a positive response to a dilution of1/40. Nosignificant differences between male and female patients were seen in the incidence of positive or negative responses. As well, titration in patients was not significantly related to

age. **Conclusion:** These data indicated that increased titers of anti-lipid antibodies against antigens of brain in Alzheimer's patients compared to healthy controls and Autoimmunity may be said that at least one of the factors involved in the genesis and progression of the disease.
Keywords: CNS: central nervous system, VDRL Vernal Disease Research Laboratory

1466P

The lost ring of asthma control assessment; psychosocial functions of asthmatic patients and their families

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Background: Guidelines for the management of asthma recommended to asses all aspects of asthma control by multidisciplinary approach. However, the determinants for well-controlled asthmatic patients do not inform about these patients' quality of life. We aimed to compare physical and psychosocial functions in asthmatic patients with different level of asthma control and healthy children aged 8-12 year-old. **Methods**: A total of 160 asthmatic children and an age-sex matched control group were participated. Asthma control levels in children were correlated to their Pediatric Quality of Life Inventory™ (PedsQL™) and Family Information Form scores. **Results**: Those in the asthma' group scored significantly lower on Emotional Function scale of PedsQL, as compared to the control group ($p<0.05$). Moreover, the asthma had significantly negative effect on the parent's daily works and activities. According to these results, the PedsQL scales of the asthmatic children in three levels of asthma i.e. controlled, partly controlled and uncontrolled had no significant differences except Physical Function scale ($p<0.05$).**Conclusion**: In conclusion, the asthma not only impairs the Physical and Emotional functions of asthmatic children, but also affects parent's daily works. The clinical controls of asthma just improve the asthmatic physical functional and not their psychosocial functions. Therefore, asthma specialist should pay attention to the importance of psychosocial factors in asthmatic children and their families, additionally; it is suggested to consider the psychosocial assessment for assigning the level of asthma control.

Keywords: Asthma, PedsQL, Children

2962P

The role of inflammation in the severity of major depressive disorder

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Background: It has been shown by several studies that inflammation can lead to alterations in the brain function which resemble those found in patients with depressive disorder. Changes in the inflammatory profile may induce depression by influencing the 5-HT, noradrenergic and HPA systems .The main aim of the current study was to investigate whether immune dysregulation is correlated with the severity of depressive disorder. **Methods:** Our study included 58 unipolar untreated outpatients with major depressive disorder (MDD) .Psychiatric diagnosis were established using the structured clinical interview for DSM-IV .The severity of psychopathology was quantified by using Beck Depression Inventory (BDI) questionnaire. Serum levels of tumor necrosis factor-alpha (TNF-alpha), Interlukin-5 (IL-5) and Interferon gamma (IFN-gamma) were measured by Enzyme-Linked Immunosobent Assay (ELISA). Serum levels of CRP and ESR were also measured. **Results:** Analysis of our data showed significant correlation between BDI scores of the patients and serum TNF-alpha (r = 0.33), IL-5beta (r = 0.35), IFN-gamma (r = 0.40), BMI was implicated as a moderating factor. No significant correlation was observed for ESR or CRP. **Conclusion:** Our data were consistent with the possibility that there may be a dose-response relationship between some circulating inflammatory markers level and the severity of depression disorder. These findings could be leading to novel therapeutic approaches for treatment of MDD.
Keywords: Inflammation, Cytokine, Depressive disorder

3198P

Allergic contact dermatitis is strongly associated with psychological distress: a case-control study

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Background: Recent studies have demonstrated that a high proportion of Allergic contact dermatitis (ACD) patients show an association with psychological factors. While a few study was conducted on the investigation of psychological features of ACD patients in Iran, so, we aimed to evaluate the relationship of psychological distress with ACD in outpatient subjects. **Methods:**153 consecutive outpatients were diagnosed ACD based on physical exam, medical history and patch test. 163 controls were interred to study and invited to complete the Symptom Checklist 90 Revised (SCL-90-R) instrument in order to assessment of psychological distress. Univariate (t-test and Chi-square) and multivariate (logistic regression) methods was used for data analysis. **Results:** A significant association of ACD with all nine subscale and three global indices including Global Severity Index (GSI), Positive Symptom Distress Index (PSDI) and Positive Symptom Total (PST) of the SCL-90-R were detected. Patients with ACD reported significantly higher levels of poor appetite, trouble falling asleep, thoughts of death or dying, early morning awakening, disturbed sleep and feelings of guilt compared to the controls. Multivariate analysis indicated that interpersonal sensitivity, somatization, paranoid ideation, depression and phobic anxiety subscales and PST, PSDI and GSI global indices were significantly associated with ACD (age, gender, educational level, marital status, employment status, smoking, alcohol use, and BMI).**Conclusion:** Psychological features are strongly associated with ACD; notably, interpersonal sensitivity, somatization, paranoid

ideation, depression, phobic anxiety and all global indices including PST, PSDI and GSI is significantly associated with. So, the appropriate psychological assessment in these patients is critically important.

Keywords: Allergic contact dermatitis, Psychological distress, Symptom Checklist-90-Revised, Global Severity Index

3275P

TIM-3 gene polymorphisms in multiple sclerosis

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Background: The family of T-cell immunoglobulin and mucin domain (TIM) proteins is known to be expressed on T cells. The TIM gene families are located on chromosome 5q33.2 of human. Multiple sclerosis is an autoimmune disease of the CNS that involved brain and spinal cord.TIM-3 might have a key role in the autoimmune diseases. It seems that some gene polymorphisms can contribute to the pathogenesis of MS. Therefore, this study was undertaken to analyze the TIM-3. **Methods:** In this study, 102 definite MS patients and 102 healthy controls selected after consent confirm. The polymorphism of the TIM- 3 genes was identified by RFLP-PCR method. **Results:** In current work, of the two polymorphisms genotyped, the allele - C in genotypes (CC) of -574A>C polymorphism was more frequent in MS patients than in controls [P =0.015, odds ratio (OR) =0.165, 95% confidence interval (CI): 0.039 - 0.703].These findings suggest that -574A>C polymorphism in TIM3 gene may affect the disease susceptibility. There was no significant difference in SNP -1516 C>A genotype and allele distributions between MS patients and control (P =0.86, Confidence Interval 95% = 0.421 – 2.058).**Conclusion:** Our findings indicate that SNP -574 A>C in the TIM3promoter region is important. These findings suggest that -574A>C polymorphism in TIM3 gene may affect the disease susceptibility. It is concluded that SNP -574 A>C may be important role in innate and adaptive immunity and would be having a key factor in contributing of the pathogenesis in MS patients.

Keywords: MS, TIM- 3, polymorphism, RFLP- PCR

2317P

The status of interleukin (IL)-6 serum levels in nephropathic type 2 diabetic patients

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Background: Nephropathy complication of type2 diabetes is a complex disorder which is most likely depends on several environmental and genetic factors and currently the interplay

between these factors yet to be clearly understood. The present study was aimed to examine the serum levels of the IL-6 (an inflammatory cytokine) in nephropathic and non-nephropathic type 2 diabetic patients. **Methods:** In this study, serum samples were obtained from 100 non-nephropathic type 2 diabetic patients, 100 nephropathic type 2 diabetic patients and 100 non diabetic controls. Serum levels of IL-6 were detected by ELISA. **Results:** Our results showed that the serum levels of IL-6 level were not differ significantly in three evaluated groups (nephropathic and non- nephropathic type-2 diabetic patients and healthy controls). **Discussion:** According to these findings, it can be concluded that the serum levels of IL-6 are not associated with T2D with and without nephropathy patients.

Keywords: IL-6, T2D, Nephropathy.

3241P

Evaluation of Prostaglandin Inhibitors on IL-6, IL-10,IFN-ɣ Levels and Clinical Symptoms in Schizophrenia

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Background: Schizophrenia is a range of chronic psychological disorders with unknown ethiology. Genetic and environmental factors have a role play in pathophysiology of schizophrenia. Prostaglandin inhibitors such as celecoxib are effective on cytokines, IL-6, IL-10 and IFN-γ and clinical symptoms of schizophrenia. Some evidence suggests abnormalities in the immune system and Th1/Th2 imbalance in schizophrenia. The aim of this study was to determine the efficacy of adjuvant therapy with celecoxib on cytokines level and clinical symptom in schizophrenia. **Method:** In this prospective study 32 patient with a DSM-IV-diagnosed schizophrenia were randomly assigned to regular antipsychotic plus celecoxib or regular antipsychotic plus placebo. 16 patients received regular antipsychotics plus 400 mg/day celecoxib and 16 received regular antipsychotics plus placebo for 5 weeks. Before and after treatment an assessment of the psychopathology was performed using the Brief Psychiatry Rating Scale (BPRS) and IL-10 and IL-6. And IFN-γ was evaluated. **Results:** Before starting treatment, no significant differences found on IL-6 in both groups but after treatment a significant decrease was observed in celecoxib group ($p < 0.01$). No significant difference was found in IL-10 and IFN-γ in both groups before and after treatment. Over 5 weeks, both groups of patients showed significant improvement in BPRS. However celecoxib group showed significantly greater improvement in the BPRS ($p < 0.03$).**Conclusion:** The result of this study suggest that celecoxib given as adjuvant therapy to regular antipsychotic treatment is effective on chronic schizophrenia and its effectiveness on cytokines level needs to further investigation.

Keywords: schizopherenia, celecoxib, cytokines

2167P

Involvement of Serotonergic system (5-HT3 receptors) of accumbens shell area on ACPA-induced amnesia in male wistar rats

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Background*:* Cannabinoids are thought to affect mood, memory, cognition, and pain perception by activation of the presynaptic cannabinoid CB1 receptor, which is expressed at high levels in many brain regions. Previous investigations on serotonin (5-HT) and cannabinoid systems have suggested that a direct interaction exists between cannabinoid and serotonergic systems. **Methods:** Adult male wistar rats, weighing 250-*280 g* at the time of surgery ,were used. Bilateral microinjections of drugs into the nucleus accumbens (Nac) shell were in a volume of 0.6µl/rat (0.3µl/each side). Based on the fact that neural serotonergic system is related to learning and memory, the Elevated Plus Maze (EPM) test has been employed to investigate the involvement of the system in these processes. **Results:** *Post*-training intra-NAc-shell administration of cannabinoid CB1 receptor agonist (ACPA) (2ng/rat) and 5-HT3 receptor agonist (0.04mg/rat) induced amnesia in EPM test [$p < 0.01$]. However, ACPA and 5-HT3 receptor agonist at equal doses did not significantly affect locomotor activity in the EPM test. The results also reveal that 5-HT3 receptor antagonist (0.1mg/rat intra-NAc-shell) significantly improved learning in EPM test [$p < 0.01$]. But 5-HT3 receptor antagonist at equal doses did not significantly influence locomotor activity in the EPM test. **Conclusion**: The results of this study showed that post-training intra-NAc-shell administration of ACPA impaired memory consolidation. The findings also support those of previous enquiries by indicating that cannabinoid CB1 receptors play a specific role in memory extinction. Further research can be conducted on the probable interaction between cannabinoid and serotonergic system

Keywords: 5-HT3 receptors, NAc shell, ACPA, amnesia

Research, Development & Manufacturing

Oral Presentations:

2959O

Establishment and preservation of immortal lymphoblastoid cell lines as a promising *in vitro* model system

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Background: lymphoblastoid B cell lines (LCLs) immortalized by Epstein-Barr virus can be established and are banked as cellular reference material for immunological or genetic analysis of study populations and can produce and secrete immunoglobulins.**Methods:** One hundred and twenty four LCLs were established from healthy ethnic groups of Iranian in cell bank of Iranian Biological Resource Center. Characterization was done with respect to morphology, cytogenetic analysis. Also Inter species identificationconfirmed by multiplex PCRandPattern of short tandem Repeats (STR) of each Blood sample was determined with DNA of blood samples are authenticated. **Results:** LCLs showed rosette morphology with doubling time of approximately 24 h. Ploidy analysis showed diploid DNA content. When compared with parent lymphocytes there appeared no change at genetic and gene expression level.**Conclusions:** Our results show that EBV allows cell immortalization with minimal genetic and phenotypic aberrations, with ease of establishment and maintenance making LCLs provide an unlimited source of biomolecules like DNA, RNA or proteins and are a promising in vitro model system for genetic screening studies, genotype-phenotype correlation studies, a variety of molecular and functional assays along with immunology and cellular biology studies.

Keywords: EBV, Lymphoblastoid, Immortal

2573O

Recombinant expression of vascular endothelial cell growth factor (VEGF) in baculovirus expression system

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Background: Vascular endothelial growth factor is one of the essential factors in angiogenesis. VEGF applies its biological effect on target cells through interaction with membrane tyrosine kinase receptors. Difficulties in the way of producing recombinant proteins in*E.coli* have led to using other expression systems. One of these systems due to the high similarity with mammalian expression systems is Baculovirus expression system. In this study, human VEGF protein was expressed in this expression system. **Methods:** The gene constructs containing the VEGF was cloned in pFastBac-HTvector, and transformed in DH10BAC. The recombinant bacmid was extracted and used for Sf-9insectcellstransfection using the cell fect in method. Transfected cells were harvested and VEGF expression was confirmed by Western blotting using specific antibody. **Result and Conclusion:** In this study we've shown that human VEGF could be expressed in Baculovirus expression system.

Keywords: Baculovirus, Vascular endothelial cell growth factor

2115O

Cloning, expression and purification of recombinant soluble mouse endostatin as an anti-angiogenic protein in *Escherichia coli*

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Background: Inhibition of angiogenesis has become a particular interest for treatment of solid tumors. Endostatin, a C-terminal fragment of collagen XVIII, has been reported to exhibit potent inhibitory effect on endothelial cells proliferation, migration and tube formation. **Methods:** In this research, the cDNA library of endostatin was synthesized from mouse liver and inserted into the *Sac*I and *Sal*I enzyme-cutting sites of pUC18 cloning vector. The recombinant vector was transferred into *E. coli* DH5a and the recombinant clone was selected on LB agar plate plus ampicillin. PCR analysis and DNA sequencing proved the presence of intact endostatin gene in pUC18. The endostatin gene subcloned into pET32a expression vector and the competent bacterial cells of *E. coli* BL21 were transformed by the vector harboring endostatin gene. In the optimum conditions, expression plasmid was induced with IPTG and recombinant soluble endostatin as a fusion with thioredoxin was purified with Ni-NTA (Ni^{2+}-nitrilotriacetate) resin. **Results and conclusion:** The results showed that soluble recombinant endostatin as a fusion protein with thioredoxin is a homogenous polypeptide that inhibits angiogenesis (capillary tube formation) in human umbilical vein endothelial cells (HUVECs) by 200 ng/ml.

Keywords: Molecular cloning, Endostatin, *Escherichia coli*, Angiogenesis

1604O

Agrobacterium-mediated transient expression of Hepatitis B surface antigen in Lettuce leaves (Lactuca sativa L.)

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Background: The recombinant vaccine is generally regarded as the most economical and safe type of vaccine. In addition, oral vaccines are considered the easiest vaccine administration method. Plants expression system has provided an optimal system for the expression of recombinant vaccines free of contamination by bacterial toxins and animal pathogens. Hepatitis B surface antigen (HBs-Ag) was the first viral antigen chosen to be produced in transgenic plants. The capacity of plant transient expression systems to produce vaccines in large amounts has been well established therefore in this research transient expression system has been investigated for expressing of hepatitis B surface antigen gene in lettuce leaf by agro-infiltration. **Methods:** Hepatitis B surface antigen (HBsAg) gene was cloned in plant binary vector pBI121 under control of pCaMV 35S promoter and NOS terminator. pBI121vector containing the (HBsAg) gene has been transformed to *Agrobacterium tumefacienes* strain GV3850 using freeze-thaw procedure and then introduced into *Lettuce leaves* by infiltration. Transient expression assays have been performed within 3-7 DPI (days post infiltration). RNA and protein were extracted from agro infected lettuceleaves and used for reverse transcription polymerase chain reaction (RT-PCR), Northern dot-blot, and enzyme assays. Results: The amplified sensitivity immunoassay (EASIA) was performed on extracted protein samples using HBS AG BIOLISA kit, highest expression yield in vacuum agro-infiltration experiments reaching 0.9 % of total soluble protein. Conclusion: Our results had shown the expression of recombinant HBsAg in tissue of lettuce by agro-infiltration may lead to production of a cheaper vaccine locally where it is needed.

Keywords: Hepatitis B virus, Recombinant vaccine, Transient, Lettuce leaves

1890P

Production of humanized anti-IL-2α receptor antibody in *Leishmaniatarentolae*

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Background: Therapeutic mAbs are used extensively in the treatment of diseases. Anti-IL-2α receptor antibodies are one of these therapeutic antibodies used for organ-allograft rejection and T-cell-mediated autoimmune diseases. Mammalian cells are the first choice for the production of humanized antibodies because of posttranslational modifications needed for biological activity. However, the expression efficiency of mammalian cells is relatively low compared to other expression systems, such as Escherichia coli or yeast. A novel protein expression system based on *Leishmaniatarentolae*, a non human pathogen was developed. This system allows not only easy handling like E. coli and yeast, but also full eukaryotic protein folding and the mammalian-type posttranslational modifications of target proteins. Here, we attempt to produce recombinant humanized anti-IL-2α receptor antibody in the *Leishmaniatarentolae* in a secretory form. **Methods:** The expression cassette constructed into a pLEXSY vector. The leishmania cells were transfected by electroporation. After selection of transfectants, the protein expression was evaluated at protein levels **Results:** Expression of recombinant anti-IL-2α receptor antibody was proved by western blotting. **Conclusion:** The adventages of this system are Easy handling and culture, and human cell like posttranslational

modifications, but the expression yield of this host is relatively low and needed to improve for large scale production.

Keywords: Anti-IL-2α receptor antibody, *Leishmaniatarentolae*, mAb

1607O

Development of enzyme-linked immunosorbent assays using 2 truncated ORF2 proteins for detection of IgG antibodies against hepatitis E virus

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Background: Without appropriate culture systems for hepatitis E virus (HEV), sufficient natural viral proteins are difficult to generate for use in serological tests. Therefore, it is important to produce large amounts of HEV recombinant proteins in an economical way. The present study developed ELISAs using 2 truncated forms of the HEV open reading frame (ORF) 2 protein in order to detect anti-HEV IgG in serum samples. **Methods:** Two truncated forms of the ORF2 protein were expressed in *Escherichia coli* and were purified by Ni^{2+}-chelate-affinity chromatography (Qiagen, Germany). Two ELISAs were developed using these proteins and were compared with DIA.PRO HEV IgG ELISA kit (DIA.PRO. Italy) in 220 serum samples. **Results:** High yields of the target proteins were obtained through codon optimization. The concentration and purity of the proteins were improved with Amicon filters (EMD Millipore, USA). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting analysis of the resultant proteins showed a protein band of approximately 60 kDa corresponding to ORF2.1 (amino acids 112 – 660) and a protein band of approximately 55 kDa corresponding to ORF2.2 (amino acids 112 – 607). Positive agreement, negative agreement, and concordance of the 2 in-house ELISAs compared with DIA.PRO HEV IgG ELISA kit were 87%, 99.5%, and 98.1%, respectively (kappa = 0.899, P = 0.625). **Conclusion:** The newly developed ELISAs are useful for detecting anti-HEV IgG in serum samples and are highly concordant with DIA.PRO HEV IgG ELISA kit.

Keywords: Hepatitis E virus, ORF2, Optimization, Expression, *Escherichia coli*

1543O

Anti transforming growth factor-beta receptor type II (TGF-βR2) monoclonal antibody

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Background: Transforming growth factor-beta receptors (TGF-βR) play a key role in TGF-β signaling pathway and pathological states. In this Study, monoclonal (mab) and polyclonal antibodies against two peptides of human TGF-β receptor type II (TGF-βR2/A, and TGF-βR2/B) were produced and characterized. **Methods:** BALB/c mice were immunized with

TGF-βR2/A-KLH and TGF-βR2/B-KLH. After five times immunization, splenocytes of hyper immunized mice were fused with murine myeloma Sp2/0 cells. Three positive hybridomas were selected by ELISA using TGF-βR2/A and TGF-βR2/B peptides as coating antigen. The mAbs from asciticfluides were purified using Hi-Trap protein G HP column. The reaction of purified mAbs with TGFβR2 in breast cancer tissue was assessed by immunohistochemistry (IHC) method. Polyclonal antibodies were also produced by immunization of rabbits with the above mentioned peptides. The rabbit sera were affinity purified using a column of CNBr-activated sepharose 4B. **Results:** The results showed one specific mab against TGF-βR2/A (clone: 1D3-H1, IgG3/K) and two against TGF-βR2/B (clones: 1D4-G2, 3F6-G1, IgG1/ K). It is also shown that 1D4-G2 mAb recognized expression of TGFβR2 in breast cancer tissue. The ELISA and SDS-PAGE results showed that produced monoclonal and polyclonal antibodies have a high reactivity with their synthetic peptides and monospecific band respectively. **Conclusion:** According to appropriate reactivity of produced antibodies to their peptides, it seems that humanization of these antibodies might be a good candidate for therapeutic strategy.
Keywords: Monoclonal antibody, Polyclonal antibody, TGF-β, TGF-β receptor II

Poster Presentations:

2361P

Cloning of human CD52 gene from Raji B cell line

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Background: The CD52 antigen is a glycoprotein anchored on the cell membrane of mature B, T lymphocytes, monocytes, eosinophils and also expressed on cells from most B and T cell malignancies and a minority of myeloid leukemias. Anti CD52 is currently approved for treatment of patients with refractory chronic lymphocytic leukemia (CLL) and is undergoing phase III clinical trials for the treatment of multiple sclerosis. The aim of this study was to clone and express Human CD52 gene in order to produce recombinant CD52 protein in CHO cell line. **Methods:** Total RNA was extracted from Raji cell line and cDNA synthesized then specific primers of CD52 was used for amplification. Amplified fragment was digested with pstI/ xbaI enzymes and subsequently cloned in pBudCE4.1 vector. Positive Colonies were selected and confirmed using PCR and restriction pattern. **Results:** Amplification of CD52 gene using specific primers on RajicDNA showed a 220 bp band. Restriction pattern confirmed the fragment and cloning of CD52 gene was achieved using pstI/xbaI sites. The new construct was designated as pBudKT1. **Conclusion:** Change in serum free circulating soluble CD52 as a biomarker has been reported in chronic lymphocytic Leukemia. Cloning of CD52 gene provide standard recombinant CD52 protein for production of mAb and to set up an immune assay system to evaluate changes of this protein in various disease conditions.
Keywords: CD52, Gene clonining

2094P

Preparation of morphine-HRP conjugate using mixed anhydride method

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Background: Most widely used immunoassay methods require “tracer” molecules. A tracer molecule is one of the immunoreagents which is labeled with a marker or tag to monitor the formation of the specific antibody-antigen complex. Enzymes are used as ELISA labels. One of the most common enzymes used in immunoassays is horseradish peroxidase (HRP). In this study we prepared morphine-HRP conjugate using mixed anhydride method to develop a competitive ELISA for measurement of morphine. **Methods:** At first the morphine was converted to 6- morphine hemisuccinate (6-MHS) derivative, solved in DMSO, and kept on ice-ethanol. Isobutylchloroformate and triethylamine were added to cold 6-MHS solution respectively. Mentioned solution was added to HRP drop wise (during 20 min) while stirring and incubated overnight at 4◦C. The conjugate was finally purified using G-25 gel filtration column. At the end, the success of conjugation was assessed by checkerboard titration. **Results:** The data obtained from checkerboard titration showed morphine successfully has been conjugated to HRP. **Conclusion:** Horseradish peroxidase (HRP) is a 44kDa glycoprotein with six lysine residues. The presence of few lysine residues in this enzyme leads to lower desired hapten -HRP conjugate yields while applying active ester method using carbodiimide (EDC). Our data showed that the mixed anhydride is an efficient method for conjugation of morphine to HRP.

Keywords: Morphine, Conjugation, Mixed Anhydride, HRP

2378P

Development of a qualitative homogeneous immuno-FRET assay method for tetanus toxin detection

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Background: Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule without emission of a photon. The efficiency of FRET is dependent on the inverse sixth power of the intermolecular separation, making it useful over distances comparable to the dimensions of biological macromolecules. The distance over which FRET can occur is limited to between 1-10 nm. In this case of FRET immunoassay, a quencher (QSY-7) conjugated toxin compete with unlabeled toxin for binding to fluorochrome (OG514) conjugated antibody. It is clear that quenched OG514-antibody in proximity to its quencher QSY-7 can increase its fluorescence emission only by introducing unlabeled toxin. **Methods:** First of all, Anti tetanus toxin mAb and tetanus toxin were

conjugated to OG514flurochrome and QSY7 quencher, respectively. Finally for evaluating the practicability of our designed FRET method, we run a competitive method of FRET in soluble phase; various amounts of unlabeled toxin was exposed to constant concentration of labeled antibody and antigen. After incubation, the decrease in fluorescent intensity was measured. **Results:** Our result shows: in absence of quencher, and in a concentration-dependent manner, the fluorochrome-labeled Ab that binds to toxin could show emission spectrum that it was detectable by spectrofluorometer. The sensitivity for this method was 1µg/ml. **Conclusion:** Here we design an immune-FRET that could be an important technique for investigating a variety of biological phenomena that produce changes in molecular proximity as like as antibody-antigen interaction.

Keywords: Fluorescence, immuno-FRET assay

2548P

Optimization of transient gene expression (TGE) in suspension-adaptedExpi293F for the production of recombinant proteins

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Background: Nowadays, making recombinant proteins mainly monoclonal antibodies (mAbs) is an essential and challenging step of the drug discovery process that developed for the treatment of various diseases such as asthma and allergy. Transient gene expression (TGE) is a rapid method for the production of milligram to gram quantities of recombinant proteins in suspension-adapted mammalian cells, especially human embryo kidney-293 (HEK-293) and Chinese Hamster Ovary (CHO) cell lines. Optimization of TGEis continued to achieve significant amounts of r-proteins and produce in pilot scale for pre-clinical studies. Tissue plasminogen activator (t-PA) drug has been used for treatment of thromboembolic diseases. The aim of this experiment was to optimize the transiently gene expression in Expi293F cells in order to increase the expression of r-protein t-PA for preclinical experiments. **Methods:** On the day of transfection, cell densities were 2.5×10^6 cells/ml, and for each 1 ml of culture, 1 µg of vector DNA and 2.7 µg of ExpiFectamine 293 reagent were required. After 24h, 48h and 72h rate of transfection was investigated by flowcytometry. The cell culture supernatant was analyzed for protein production by an ELISA based immunoassay. **Results:** After 24h, 48h and 72h, transfection efficiencies were 42%, 61% and 86%, respectively. Analysis of protein production on day 3, 5, 6, 7 and 9 showed maximum protein production on the fifth day (approximately 2 mg/L).**Conclusion:** TGE has become an important tool in the biopharmaceutical production pipeline development and is a widely used method for the rapid production of r-proteins in mammalian cells. By these cells, enhanced level of productivity of r-proteins for pre-clinical and clinical investigations is achieved.

Keywords: Transient gene expression (TGE), Expi293F, Tissue plasminogen activator (t-PA), produc

2147P

Cloning and expression of extracellular domain of TGF-β1 type II receptor in PichiaPink

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Background: TGF-β, a transforming growth factor, has been implicated in the cell differentiation, tissue regeneration, embryonic development, regulation of the immune system, induction of apoptosis in a number of cells and carcinogenesis in human. All TGF-B isoforms are key mediators for fibrosis. TGF-β receptor types I& II are serine/threonine kinase receptors both of them have a characteristic structure including an extracellular domain, transmembrane domain and an intracellular domain. **Methods:** The TGF-β type II receptor ectodomain gene sequences were prepared from the NCBI gene bank, and then were optimized for expression in yeast. Synthesis was carried out by ShineGene Molecular Biotech, Inc in the pUC57 cloning vector.pUC57 and PichiaPink-HC vector was digested by double digest enzyme and ectodomain gene 579bp was subcloned into PichiaPink-HC expression vector. Recombinant plasmid was linearized with AflII restriction enzyme and was expressed in methylotrophic yeast PichiaPink. The recombinant protein was produced and purified. **Results:** Subcloning and expression was correctly confirmed by the result of molecular methods such as PCR, enzyme digestion and sequencing process. Recombinant protein was purified withnickel affinity column chromatography. The band of 38KDa was observed in gel electrophoresis and approved by immunoblotting. **Conclusion:** The presence of 6 cysteine amino acids and 3 position of N-glycosylation in the glycoprotein structure was led to select in the PichiaPink yeast system. The expected 24KDa molecular weight of this glycoprotein was observed 38KDa in polyacrylamide gel electrophoresis because of hyperglycolisation and reduction of electrophoresis mobility for recombinant glycoprotein.

Keyword: TGFβ, PichiaPink, pPink-HC, Type II receptor, Recombinant glycoprotein

2332P

Production of specific egg yolk immunoglobulin (IgY) against *Helicobacter pylori*OipA recombinant protein

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Background: Passive immunization with oral antibodies could be effective against a variety of intestinal pathogens in both prophylactic and therapeutic studies. Recently, egg yolk immunoglobulin (IgY) has been considerable as a means to prevent and control disease because

of a large number of advantages compared with treatment with mammalian IgG including cost-effectiveness, convenience and high yield**.** **Helicobacter pylori** is the most common cause of gastritis and gastric ulcers and plays a pivotal role in the development of gastric carcinomas. OipA is one of *H. pylori* outer membrane protein that has function as a adhesin and is an important virulence factor. In this study, Igy against *H. pylori* recombinant OipA (rOipA) was produced. **Methods:** The gene encoding OipA protein was cloned in *Escherichia coli* BL21.Expression of recombinant protein was induced with IPTG. Purification of rOipA was performed by Ni–NTA affinity chromatography. The hens were immunized by rOipA three times. After 28 days egg yolks from immunized hens were collected. Purification of IgY were performed with different percentages of poly ethylene glycol (PEG 6000). The purified IgY was analyzed by ELISA, SDS- PAGE and Western-blot methods. **Results:** SDS- PAGE was confirmed production of rOipA (30KD). Hens Immunization was evaluated by ELISA Method. The Purified Igy from egg yolks were also verified by ELISA assay to titrate the amount of active specific IgY and was assessed with SDS-PAGE and confirmed by western-blot. **Conclusion:** IgY against rOipA*H. pylori* was successfully produced and can be considered for passive immunization against *H. pylori* infection.

Keywords: IgY, recombinant OipA, *H. pylori*

2457P

Camel IgG preparation as a snake anti-venom

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Background: Snake envenoming is a significant cause of global mortality and mortality and a particular burden on the rural poor communities of Asia and Africa. Intravenous administration of anti-venom, prepared from hyperimmunized horses or sheep, is the only effective treatment of systemic envenoming. Conventional anti-venom, formulated as intact IgG, papain cleaved (Fab) or pepsin-cleaved $F(ab)_2$ fragments. Camel IgG is less immunogenic than horse and sheep IgGs. Comparison of different IgG preparations has been shown the lowest propensity to induce adverse reactions for camel IgG. **Methods:** In this study effectiveness of immunized camel with snake venom was investigated for production of potent IgGs, Camel IgGswere prepared by ammonium sulfate precipitation and ion exchange chromatography. In the ammonium sulfate precipitation, the best state for fractionation of IgG was 55% precipitate. **Results and Conclusion:** It can be included that, the methodology of IgG purification which proposed this study, can prepare an effective anti-venom.

Keywords: Snake, Ammonium sulfate, Camel, IgG, Anti-venom

2701P

Cloning, expression and analysis of outer membrane protein D of *Haemophilusinfluenzae* in *E. coli*

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Background: Non-typeable *Haemophilus influenzae* is a significant pathogen in children, causing. otitis media, sinusitis, conjunctivitis, pneumonia, and occasionally invasive infections. protein D.belong to the minor OMPs of *H.influenza.* This protein is highly conserved surface lipoproteinand The main function of this protein is damaging the ciliary function in human nosopharyngealtissue. This protein has shown to be one of the most capable vaccine candidates against non-typeable*Haemophilusinfluenzae strain.* **Methods:** A 1095 bp fragment of *hpd*gene was amplified by PCR from *H.influenzae* and then cloned into prokaryotic expression vector pBAD-gIIIA. For expression of recombinant protein, pBAD-pdplasmid was transformed into competent Top10 cells. Recombinant protein was expressed with Arabinose. In this study we aligned *hpd* gene with *hpd* gene which were published in GenBank and our study we used I-TASSER server which is an internet service for protein structure and function predictions. It allows predictions of 3D structure and biological function of protein molecules from their amino acid sequences. **Results:** Cloning of PDwas confirmed by colony-PCR and enzymatic digestion. In comparison with the corresponding sequences in GeneBank, the nucleotide sequence homology of the cloned *hpd*gene was 98%. Arabinose 2% could efficiently induce protein expression. SDS-PAGE analysis showed that our constructed pBAD-PD-Top10 efficiently produces a target recombinant protein with a molecular weight of 42 kDa.The recombinant PD was reacted with Peroxidase Conjugated rabbit anti mouse immunoglobulins . The result shows the sequences of *hpd* gene have high homology with our *hpd* amino acids sequence . I-TASSER server predictions of 3D structure. This protein consist of 33.6 percent of alpha helix and 66.6 percent of beta sheet and this proteins have hydrophobic properties. **Conclusion:** the pBAD-PD-Top10 system successfully expressed recombinant protein, that could be used for further immunological studies.

Keywords: *Haemophilusinfluenzae*, PD, Ni-NTA agarose, pBAD-gIIIA

1971P

Using recombinant *Chlamydia trachomatis* OMP2 as antigen in diagnostic ELISA test

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Background: Obligate intracellular bacterium *Chlamydia trachomatis* is a factor of sexually transmitted diseases. Timely and sensitive detection of this pathogen is very important. Due to the difficulties in cross-reactions observed during bacteriological and serological detection methods, to achieve a specific antigen for serological tests was the aim of this study. **Methods:** Blood samples were takenfrom192womenwith suspected chlamydial infection and sera were isolated. ELISA plate wells were coated by recombinant *Chlamydia trachomatis* OMP2 as antigen. Cut-off ratio was determined with40negativesera.The results of recombinant antigen coated plate were compared with Euroimmun commercial kit. **Results:** Cut-off of ELISA system was calculated at 0.27 using negative sera samples. OD of positive samples was higher than 0.27 and of negative samples was lower than of cu-off. A total of 30samples (15.62%) were positive and162cases (84.37%) were negative. Sensitivity and specificity of the recombinant antigen

were 90%and86%, respectively. This antigen showed no cross-reactivity with sera of patients infected by Hydatid cyst, HCV, *Epstein Barr* virus, HBV, *Helicobacter pylori*, *Toxoplasma gondii*, *Cytomegalovirus*, *Mycoplasma*, *Measles* and Varicella *zoster* virus. Conclusion: The sensitivity and specificity ofrOMP2 in ELISA for detection of *Chlamydia trachomatis* are 90%and 86%, respectively, which is more sensitive than Euroimmuncommercial kit although its specificity is lower than Euroimmuncommercial kit.

Keywords: *Chlamydia trachomatis*, ELISA, OMP2

2085P

Construction of recombinant ScFv library of human antibodies by phage display method against tetanus toxin

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Background: Monoclonal antibodies represent the vital category of biotechnology products for treatment of human diseases. Progress in the antibody engineering and the appearance of selection technology such as phage display permitted to produce the human antibodies against specific antigen with high affinities. In this technique, the library of antibody genes is constructed and biopanning of library is performed. The purpose of this study was to construct an Immune antibody library from a vaccinated donor against tetanus toxin. **Methods**: A blood sample was drawn from a person vaccinated with tetanus toxoid. PBMC were isolated by using ficoll. After RNA extraction and cDNA synthesis, two uniplex PCR were performed to amplify the VH and VL genes and cloning of these genes was accomplished. Recombinant phagemids were extracted from *E. coli* XL1Blue and sequenced. For screening of the library, panning was carried out by phage display method. The accuracy of recombinant phage screening was confirmed by phage ELISA. Final clones with high affinity and specificity were sequenced. **Results**: In this study, the recombinant human antibody library was constructed and confirmed using DNA sequencing. The results indicated, after panning and screening cycles, the specific antibody against tetanus toxoid with high affinity was obtained successfully. **Conclusion:** Our aim was the construction and panning of immune library to obtain the specific human antibody. These immune libraries could be valuable sources for selection of specific antibodies for developing the effective therapeutic agents.

Keywords: *E. coli* XL1Blue, Tetanus toxin, ELISA, Biopanning, Phage.

3118P

Real-time PCR: An appropriate approach to confirm ssDNA generation from PCR product in SELEX process

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Background: Aptamers are low molecular weight RNA or ssDNA, selected from a random

library of nucleic acids through the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process. ssDNAaptamers are usually used for therapeutic goals due to the greater stability of DNA and low cost of production compared to RNA aptamers. As the success of DNA selection process through SELEX experiments is dependent on the conversion of the dsDNA to ssDNA, we employed a simple and convenient approach for verifying the production of ssDNA from PCR product in SELEX process. **Methods:** Quantitative real-time PCR was performed to evaluate the amplification of template. The synthetic ssDNA library used as template in different concentrations of 1/4, 1/20 and 1/50.The amplification process, followed by enzymatic digestion with lambda exonuclease which was facilitated through using a phosphorylated 5'-end reverse primer. Subsequently, melting curve analysis carried out to evaluate the ssDNA generation from PCR product. **Results:** The real-time PCR results showed the best amplification of library molecules for 1/20 of concentration, moreover the melt curve after exonuclease lambda digestion, revealed the dsDNA conversion to ssDNA through Tm reduction from 75.6 to 29.9° c. Tm of undigested PCR products remain unchanged. **Conclusion:** To the best of our knowledge this is the first report to use real-time PCR for verifying the ssDNA generation from PCR product after enzymatic digestion rather than conventional Polyacrylamide Gel Electrophoresis (PAGE). Thus real-time PCR could be a reliable and time saving method for aptamer SELEX.
Keywords: Aptamer, lambda exonuclease, real-time PCR, SELEX

3061P
Production and Characterization of Murine Monoclonal Antibodies against Synthetic Peptide of CD34

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Background: The treatment of hematologic malignancies and immunodeficiency diseases are offered by hematopoietic stem cells (HSCs) as a unique self-renewal and differentiation source which most commonly is selected by CD34 surface marker for HSC. The purpose of this study was to develop and characterize monoclonal antibody against CD34 antigen for detection of hematopoietic stem cells.**Methods:** Balb/c mice were immunized with two synthetic peptides of CD34 and Spleen cells were fused with SP2/0. Fused cells were grown in hypoxanthine, aminopterine and thymidine (HAT) selective medium and cloned by limiting dilution. Large scale of monoclonal antibodies was produced by mouse ascites production of mAb (in vivo) method. Monoclonal antibody was purified by chromatography. Then reactivity of these antibodies was evaluated in different immunological assays including ELISA, immunofluorescence (IF), western blot (WB) and flowcytometry. **Results**: In this study, between five positive clone wells, two clones were chosen for limiting dilution. Limiting dilution product was one monoclone (3-D5 monoclone) with absorbance about 2. Isotype of this mAb was identified as IgG1 class with Kappa (κ) light chain. **Conclusion:** This antibody is highly specific and functional in biomedical applications such as ELISA, flowcytometry, immunofluorescence, and western blot assays.
Keywords: Monoclonal antibody, CD34, Synthetic Peptide

1536P

Improvement of protein A production from the staphylococcus aureus

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Background: Protein A is produced intracellulary or extracellulary by *Staphylococcus aureus*. It is covalently linked to the cell wall and bind specifically to the FC potion of IgG. Protein A is used in different immunological tests and also in the affinity purification of monoclonal antibodies. its major therapeutic application is the removal of IgG plasma in the treatment of certain types of cancer. **Methods:** protein A could be Isolated from strain Cowan 1 of *Staphylococcus aureus*by lysozyme, hotacid, freeze and thaw, sodium meta priodate and toluene - triton X100-EDTA to lysis of cell wall bacteria were performed. the purified proteins by precipitation with ammonium sulfate , 80 % were done and the results of Gram staining and microscopic observation methods , SDS-PAGE, protein measurement methods are compared and evaluated in terms of cell wall lysis and release the greatest amount of protein A .**Results:** The process has been successfully used to purify protein A of cell wall Staphylococcus aureus. **Conclusion:** The process is simple, rapid, and inexpensive when compared to other reported processes**.**

Keywords: Protein A, Cowan 1, *Staphylococcus aureus*, affinity purification, IgG.

1511P

Extraction and purification and molecular evaluation protein A of *Staphylococcus aureus*

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Background: Protein A is well known for its interaction with the Fc-region of IgG from various mammals and also in the affinity purification of monoclonal antibodies that is economically valuable. IgG could be purified from serum by affinity chromatogeraphy on protein A-DEAE or sephrose. **Methods:** Staphylococcus aureus strain NCTC8325 was used to extraction and purification of protein A. extraction processes of protein A consists two steps, cell wall lysis of bacteria and purification protein A. Five Ways 1) hot acid , 2) freez and thaw, 3) toluene - triton X100-EDTA, 4) lysozyme and 5) sodium meta priodait wall to lysis of cell wall bacteria were performed. The purified proteins by precipitation with ammonium sulfate, 80 % were done and the results of Gram staining and microscopic observation methods, SDS-PAGE, protein measurement methods are compared and evaluated in terms of cell wall lysis and release the greatest amount of protein A. **Results:** Our results show that any of the protocols mentioned above have ability for protein extraction, but with the lysozyme protocols we have high volume of protein. **Conclusion:** In many researches lysostaphin was used to lysis staphylococcus cell wall. In this research in order to extract and purify of A protein, we used lysosome and affinity chromatography. Our results indicate that this procedure is an alternate procedure and have economically advantage for purification of this valuable protein.

Keywords: Staphylococcus aureus, Protein A, Monoclonal antibodies, Affinity chromatogeraphy, Lysozyme.

3120P

New method for production and standardization of *clostridium perfringens*type C beta antitoxin

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Background: *Clostridium perfringens*type Cis a gram positive, anaerobic, spore-forming pathogen of most mammalian species. This microorganism causes necrotic enteritis in human and animals including sheep, cattle, pigs, goats and chickens. This disease most frequently occurs in the young animals of these species. Beta toxin is secreted by *C. perfringens* type C strains and plays a key role in the lethal outcome of type C strains infections. Beta toxin is a pore-forming toxin. In the present study, we established the new procedure for production *clostridium perfringens*type C beta antitoxin for treatment of poisoning related to beta toxin. **Methods:** Beta toxin was purified from the culture filtrate of *Clostridium perfringens* type C. Then, five New Zealand white rabbits were injected four times subcutaneously with 40 µg of beta toxin in 300 µl of complete Freund's adjuvant. At fourteenth day, the rabbits were boosted with 40 µg of beta toxin in 300 µl of incomplete Freund's adjuvant. Twenty-eight days following the initial immunization, blood sampling was done. The blood was allowed to clot at room temperature for 1 h and the serum was collected following centrifugation. Titration of produced antibodies was done using the enzyme-linked immunosorbent assay or ELISA method. Finally, **Human umbilical vein endothelial cells** (**HUVEC**) were exposed with several dilutions of beta toxin-antitoxin mixture. **Results:** Bata antitoxin (dilution 1/1000) neutralized lethal dose of beta toxin in mice. The titer of *Clostridium perfringens* type C beta antitoxin in the serum of animals increased to more than 1/800. HUVEC cells exposed to beta toxin showed cell rounding and cell shrinkage. However, HUVEC cells exposing with toxin-antitoxin mixture did not show any changes up to dilution1/400. **Conclusion:** The results show produced beta antitoxin can neutralize lethal dose of beta toxin in mice and counteract with beta toxin poisoning.

Keywords: *Clostridium perfringens* type C, beta-toxin, Antitoxin, ELISA, HUVEC cells.

2943P

Purification of mouse IgG2a subclass

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Background: ProA, ProG are two bacterial proteins that produced recombinantly and used routinely for affinity purification of key antibody types from a variety of species. These antibodys are important reagents utilized in a variety of experimental techniques in many fields of biomedical research and play a key role in medicine as well as in analytical biotechnology. **Methods:** Pro G chromatography was carried out for purification of mouse IgG and ProA chromatography used for IgG2a purification .The low-PH condition dissociated the antibody frome the immobilized Protein A and Pro G. We purified IgG from Pro G in PH 2.8 with

0.1M glycine and IgG2a from Pro A in PH4.5 with citrate buffer. The method of choice for determining purity was SDS-PAGE. ELISA was used for determining isotype. **Results:** The results of SDS-PAGE for determining the purity of IgG showed distinct band with molecular weight about 50-KDa at IgG heavy chains 20-30 KDa at light chain. **Conclusion:** We used ProG, ProA and ion-exchange chromatography for purification of mouse IgG2a subclass and conclude that the purified mouse IgG2a subclass with purity higher than 95% is a suitable and economical product towards self –sufficiency of country at the boycott era. The SDS-PAGE analysis showed that purification of IgG by Pro G, Pro A and ion-exchange chromatography resulted in a highly pure product.

Key word: Polyclonal antibody , Purification,IgG2a, Ion-exchange chromatography, ProG chromatography, Horse radish peroxidase conjugation.

2063P

Enzyme-linked Immunosorbent Assay design for staphylococcal superantigens detection in Rheumatoid arthritis patient′s Blood

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Background: Rheumatoid arthritis (RA) is the most common chronic inflammatory disease of unknown etiology. The roles of staphylococcal super- antigens as effective agents were considered in this study. Therefore, the aim of this study was to design the Elisa plate for assay on common staphylococcal super- antigens of rheumatoid arthritis patient′s Blood samples. **Methods:** During the 18 months 60 blood samples from rheumatoid arthritis patients were collected. Blood culture was carried out. Using the Monospecefic polyclonal antibodies against Staphylococcal enterotoxins A and B (Super- antigens A and B), the Elisa plates were designed. Then, the blood samples were subjected to assess for above super- antigens. The data descriptively were analyzed. **Results:** While, the results of blood cultures were negative for bacterial growth but the designed Elisa plate were able to detect the staphylococcal super-antigens in blood samples. The results of Elisa revealed that23% and 29% of blood samples were positive for staphylococcal enterotoxins A and B respectively. The significant level of results was $P \leq 0.05$. **Conclusion:** However, the role of Bacterial super antigens was considered in the pathogenesis of rheumatoid arthritis, but its origin is unknown. The results of this study have been shown some evidence of being endogenous origin for involved super antigens. The probability relationship between disease and these super- antigens need to further study. However, this finding may alter diagnosis and treatment methods of RA disease.

Keywords: Rheumatoid arthritis, Super- antigens, Elisa

2278P

Production of cytokine antagonist using site directed mutagenesis

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Background: Human growth hormone (hGH) is a typical hormone/cytokine and its receptor belongs to class I cytokine receptor family and signals mainly through JAK2-STAT5 pathway. It is a single-chain polypeptide with 191 amino acids and a molecular mass of 22 kDa. hGH not only stimulates linear growth but also is responsible for a variety of metabolic processes, such as metabolism of proteins, carbohydrates and lipids as well as in growth, development and immunity. hGHhas two distinct sites (sites 1 and 2) that bind to the two identical GHRs at the cell surface. In this study, we used hGH as a model for producing a cytokine antagonist using site directed mutagenesis. **Method:** Appropriate primers were designed, and plasmid containing human growth hormone gene was used as a template. Amplified gene fragment were gel recovered and ligated in to expression vector. This expression construct were then transform in to E coli. The resulting protein was purified and its antagonist properties were investigated using a reporter gene assy. **Results:** hGH analogue was created with a single amino acid substitution (glycine[G] to arginine[R]) in the third R-helix of the hGH molecule. The hGH mutant was obtained after 2 rounds of PCR. This hGH analogue was found to be an hGH antagonist. **Conclusion:** There is an ever increasing demand for cytokine antagonists that may have potentials in treatment of different immune system related conditions. We used site directed mutagenesis to generate a cytokine antagonist which may be used to antagonize the effects of cytokine (growth hormone).

Keywords: Human growth hormone, Cytokine, Site directed mutagenesis, Glycine

2764P

Cost analysis of childhood asthma in Iran: A cost evaluation based on referral center data for asthma and allergies

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Background: Asthma as the most common chronic disease in childhood reduces the quality of life of children and their families. We aimed to estimate the cost of managing childhood asthma in Iran and to examine its variability depending on asthma severity. **Methods:** The cost of asthma was estimated by building a cost assessment model regarding the factors that influence the cost of asthma in children including age and sex distribution, prevalence of disease severity, level of resource utilization depending on disease severity (3 groups of controlled, partly controlled and uncontrolled were defined). The model was comprised of both medical (cost of medication, physician visit and respiratory tests) and non-medical (transportation and hoteling) costs. Furthermore, the average family income in each category was figured and the share of asthma managing costs from the average income was calculated in different groups. **Results:** According to model, the total cost of childhood asthma in Iran was around 516.5 million dollars. Moreover, direct medical cost represented 49% of the total costs, among which 66% accounting for medication cost. Direct non-medical costs were

estimated 51% with the majority (93%) expended on transportation. In addition, the mean annual cost per child was approximately 466 dollars. In addition, the results indicate the vast majority of patients (46%) are categorized in the uncontrolled group. **Conclusion:** The cost of childhood asthma in Iran is extremely high comparing to the average income of Iranian families in all categories of asthma severity. Considering the high amount of transportation cost, the accessibility of asthma treatment does not appear to be acceptable. The major source of costs is found to be related to medicine expenditure. Since it has been proven that using medicine does not necessarily result in a well-controlled disease status, alternative approaches should be considered in asthma management.

Keywords: Childhood asthma, cost analysis, pharmacoeconomics, Iran

2845P

Evaluation of a simple and rapid protocol for purification of mouse IgG1

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Background: Affinity purification of immunoglobulins has been largely confined to the use of Protein A and Protein G chromatography. Protein A has the ability to specifically bind to the Fc region of IgG subclasses molecules. In the mouse, initial studies showed that IgG2a, IgG2b and IgG3 but not IgGl could bind protein A. However, more recently, a weak interaction between IgGl and protein A Sepharose has been found.**Methods:** An outbred strain of Swiss White mice (either sex, 20-24 g) was used as a source of serum. Mouse serums were filtered and precipitated by dropwise addition of an equal volume of a saturated solution of ammonium sulphate at 4°C. Then the samples were dialyzed overnight at 4°C against 0.15 M, pH 7.2 PBS buffer and applied to a Protein A affinity chromatography column. Elution was performed at 25 ml/h using a discontinuous pH gradient with steps at pH 6, 4.5, 3.5 and 2.7.finally, purity of the product was confirmed by SDS-PAGE. **Results:** In this study, we applied samples that containing 30 mg of mouse serum protein to protein A-sepharose and 5mg of IgG1 were collected with high purity. **Conclusion:** A simple and rapid method for isolating pure mouse IgG1 subclasses with nearly 100% yield was described. The method should prove useful to all researchers working with mouse Igs and will, for the first time, enable antibodies of the IgG1subclasse to be simply and rapidly isolated in good yield and purity from whole serum. Importantly, production of purified mouse IgG1 could be considered another step toward Iran self-sufficiency.

Keywords: Affinity chromatography, Mouse IgG1, Purification, SDS-PAGE

2992P

Tetra-Primer ARMS-PCR designing for single nucleotide polymorphisms genotyping

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Background: Single nucleotide polymorphisms are one of the most common types of genetic diversity. SNPs have a significant role in the etiology of many human diseases and are becoming of major interest in pharmacogenetics. The upswing in interest in SNPs has been reflected by the rapid and massive development of a wide range of SNP genotyping methods. Many genotyping methods depend on the use of advanced, automated equipment coupled with costly chemistry and detection systems. Subsequently, designing of effective techniques, plain and cost-effective to polymorphism identification, help to gather data that identifies gene function and treatment. A simple and economical method involving in single PCR reaction is reported here using the Tetra-Primer ARMS-PCR technique. **Methods:** In the present study, 135419018 C/T polymorphism genotyping that is located in the HBS1L-MYB intergenic region, is going to be detected by designing of two pairs of primers using Primer3 and NCBI databases and OLIGO software. The reverse primers were designing in such a way that they pair to different alleles of SNP specially. The outer primers could generate a large size product as a positive control in allele PCR reactions. **Conclusion:** After assessment of the characteristics of the designed primers by the appropriate softwares, and also its position in genome by BLAST analysis, primers were verified. The primers were tested in the laboratory and expected bands were observed. **Results:** Tetra-Primer ARMS-PCR is a quick, simple, low-cost and easy method to use for SNP genotyping. It can be used even in low-tech laboratories.

Keywords: Tetra-Primer ARMS-PCR, SNP, Genotyping

3330P

Determination and comparative evaluation of *in vitro* cell based proliferation assay of human recombinant erythropoietin with classical *in vivo* assay

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Background: Erythropoietin (Epo) is a 35-39 kDa glycoprotein that acts on immature erythrocytes to stimulate their proliferation and differentiation into mature red blood cells. Recombinant Epo is used clinically to treat anemia resulting from chronic kidney disease, chemotherapy and complications from AIDS therapies. In this study we evaluate the correlation between *in vitro* and *in vivo* bioassay of EPO. **Methods:** *In vivo* bioassay for EPO is based on the stimulation and counting of reticulocytes in several groups of normocytaemic B6D2F1 female mice according to standard British Pharmacopeia monograph. For *in vitro* bioassay of EPO, the UT-7 cells were cultured in αMEM medium supplemented with 10% FBS, 40 mg/mL Gentamycin, 2 mM glutamine and 5 ng/ml GM-CSF to a final concentration of $1 \times 10^5 ml^{-1}$. Serial 2-fold dilutions of EPO samples were prepared in the assay medium. Diluted protein samples were added in triplicate to the test wells and the plates incubated for 3 days at 37° C in a humidified 5% CO_2 atmosphere. The positive control contained EPO and the negative control didn't contain any forms of EPO. Proliferative effect of different concentration of EPO was evaluated on the basis of the colorimetric MTT assay .The potency of EPO quantified by comparing the responses of samples to that of the reference material using the standard

statistical methods for a parallel line assay. **Results**: The correlation of two above methods for determination of biological activity was 0.7 for ten baches of active pharmaceutical ingredient of human recombinant erythropoietin. **Conclusion**: *In vivo* bioassays are laborious, require many animals and are generally not very sensitive. Their accuracy is also influenced by other compounds that can modify erythroid responses. *In vitro* EPO bioassays are generally less laborious and more sensitive than *in vivo* assays and could be used in most investigation on EPO structure and related function researches as a more rapid and less expensive reliable method.

Keywords: Erythropoietin**,** *In vivo* bioassay, *In vitro* bioassay

Tolerance & Autoimmunity

Oral Presentations:

2497O

Autoantibodies with hydrolytic activity against hCG in infertile womenand spontaneous abortion

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Background: Infertility is one of the common problems seen in couples at reproductive age. Certain autoantibodies which are found in auto immune diseases such as lupus anticoagulant, anti-cardiolipin or anti-beta2 glycoprotein Ican impair fertility. Antibodies against hCG have been reported in some investigations, for example vaccination against hCG has been shown to block fertility in women when titers of the antibodyremained above 50 ng/ml. On the other hand, anti-hCG antibodies have been detected in young males who had been treated with exogenous hCG. Also the presence of naturally occurring antibodies to hCG/LH has also been reported in young women. This study investigates the possible influence of anti-hCG auto antibodies with hydrolytic activity on reproduction. **Methods**:Fiftyinfertile women or women with recurrent miscarriage were included as the test group and 20 healthy men and women were included as normal controls. Serum IgG samples were found to be >95% pure as determined by SDS-PAGE. Presence of anti hCG antibodies in sera of patients was assessed by ELISA, immunoblot and immunoprecipitation.Hydrolytic and protease activity of anti hCG antibodies were determined by enzyme assay and zymogram, respectively.**Result**: Presence of anti hCG antibodies was confirmed by immunoblot in 8 patients.Theresults of zymogramshowed that 7 patientshadprotease activityand two patientsandonecontrolhad catalytic activity that hydrolyzedβ-chain of hCG.**Conclusion:** This study suggests that anti hCG antibodies with catalytic activity may be associated with pregnancy loss and may be considered as a possible mechanism for IVF Failure.

Keywords: Anti hCG antibody, Catalytic activity, Infertility, Spontaneous abortion

2849 O

In silico designof GABA agonists, as immunomodulatingagents for thetreatment of autoimmune diseases

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Background: It has become clear that there is an extensive cross-talk between the nervous and the immune system. In addition to its role in the brain as the main inhibitory neurotransmitter, gamma-aminobutyricacid (GABA) is an effective immunomodulatory molecule and has a number of effects on immune cells such as activation or suppression of cytokine secretion, modification of cell proliferation and cell migration. GABA appears to have a modulatory role in autoimmune diseases like multiple sclerosis, type I diabetes, and rheumatoid arthritis.GABA agonists, especially those recently designed to act primarily on peripheral receptors,have great potential in the treatment of autoimmune diseases.**Methods:** Using the 3D structure of $GABA_BR$, fetched from the Protein Data Bank, a set of chemical compounds were docked with the receptor to find GABA agonists. For this to be done, 7 agonist molecules which had been shown to be GABA agonists before, were undergone a similarity search. 21 final compounds were docked with $GABA_BR$.**Results:**Among the seven known agonists, baclofen showed the best docking score and among the compounds from the similarity search, 5 compounds showed high potency in binding to $GABA_BR$. From the five best compounds, two of them showed better results than the baclofen itself according to their binding energy scores.**Conclusions:** It is clear that*insilico* techniques can diminish the heavy workload of experimental tests. Here the computationally designed compounds were shown to have the potential to be substituted with GABA, hence exertgreaterimmunomodulating effect.

Keywords: $GABA_B$ receptor, agonist, immunomodulating effect

2967 O

Alpha1-Antitrypsin phenotypes in Behcet's syndrome

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Background: Behcet's syndrome is a rare disorder that causes inflammation in blood vessels throughout body that leads to numerous symptoms. The symptoms of Behcet's syndrome include skin rashes, mouth sores, eye inflammation, and lesions. This condition is an autoimmune disorder, which means the body's immune system mistakenly attacks some of its own healthy cells. Both genetic and environmental factors may be responsible for Behcet's syndrome. The cause of this disease is not known. But these disorders may be developed due to some factors such AAT (alpha-1- antitrypsin) is a type of protein called a protease inhibitor. AAT has an important role in the inflammation. In this study we determine prevalence of AAT PhenotypesinBehcet's syndrome. The prevalence of PI*M, PI*S, and PI*Z allele was determined in 18 patients with Behcet's syndrome and compared with 40 healthy control group.**Methods:**Phenotyping was performed by isoelectric focusing (IEF) with ampholin 4.2-

4.9. **Results & Conclusion:** The phenotype among patients was: M in 90%, S in 7% and Z in 3%. There was not any significant difference in distribution of phenotypes between patients and control subjects and we did not find evidence to association between AAT phenotypes and Behcet's syndrome in this study.
Keywords:Behcet's syndrome, Alpha-1- antitrypsin, Isoelectric focusing

2882O
Chymotrypsin ameliorates experimental autoimmune encephalomyelitis in a site-specific and dose-dependent manner

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Background: Multiple sclerosis is an inflammatory autoimmune disease in which inflammatory cells including migratory macrophages and resident microglia release pro-inflammatory cytokines, proteases, and other toxic mediators. Proteases are involved in many aspects of inflammatory process. There are many reports regarding effect of proteases on inflammation. Chymotrypsin, a serine protease, has been demonstrated to possess anti-inflammatory activity. We investigated chymotrypsin effect on experimental autoimmune encephalomyelitis (EAE). **Methods:** EAE was induced in female Lewis rats using guinea pig spinal cord and complete Freund's adjuvant. Intra-CSF injection with 0.1mg/ml, 0.2mg/ml chymotrypsin, or saline was done on day 7 after EAEinduction.The animals were evaluated for weight loss and clinical signs from day 0 to day 14 after the disease induction. Expression of mRNA for IFN-γ, IL-4, IL-17, and FoxP3 was determined in brain and spinal cord using real-time PCR in which β-actin was used as reference gene.**Results:** Administration of 0.2mg/ml chymotrypsin led to decreased clinical sign, IL-17 and IFN-γ with increased FoxP3 in brain and increased IL-4 in S.C. Increased FoxP3 expression in the brain of 0.1mg/ml chymotrypsin-treated animals did not decrease clinical signs. In addition, chymotrypsin effect on IL-4 level was notable as it was increased in the inflammatory foci, i.e. spinal cord, of 0.2mg/ml chymotrypsin-treated animals, but not in 0.1mg/ml chymotrypsin-treated animals. **Conclusion:** Our study demonstrated that 0.2mg/ml chymotrypsin manipulate immune response in both brain and spinal cord. Presumably, chymotrypsin acts site-specific in a dose-dependent manner and concentrations of chymotrypsin more than 0.2mg/ml may have more beneficial effects.
Key word:EAE, Chymotrypsin, Immunomodulation, Dose-dependent, Site-Specific

3164 O

The protective effect of Thymoquinone against experimental autoimmune encephalomyelitis

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Background: Multiple sclerosis is the most abundant central nervous system inflammatory disease and Experimental autoimmune encephalomyelitis (EAE) is used as an animal model of this disease. Thymoquinone (TQ) a component derived from Nigella sativa, has been investigated for its anti-oxidant and anti-inflammatory activities. **Methods**: 40 C57BL/6 female mice were divided into 5 groups: normal, control andtreatmentgroups with 3 different doses of TQ. All Groups except normal group were given Myelin Oligodendrocyte Glycoprotein subcutaneously to induce EAE. Mice in treatment groups received intraperitoneal injection of low(1 mg/kg),intermediate(10 mg/kg) and high(100 mg/kg)dose of TQ for 10 days. Clinical and weight assessments were performed daily. On day 25 animals were sacrificed. Brain was stained for histological studies. Spleen cells were analyzed using flowcytometer and Real-time PCR. Brdu assay was used for splenocyte proliferation and Griess reaction performed for detection of NO.**Results**: Our results showed significant mean weight increase and Clinical score decrease in treatment groups. Histological studies revealed lower lymphocytic infiltration and demyelination in treatment groups. Serum level of NO showed significant reduction in treatment groups. The percentages of spleen Foxp3$^+$Treg increase in treatment groups. Expression of transcription factor and cytokines related to Treg and Th2 showed significant increase and related to Th1 and Th17 showed significant decrease in treatment groups. We demonstrated that TQ has an optimum therapeutic effect at dose 10 mg/kg.**Conclusion**: It seems that TQ alleviate disease condition in EAE mice through reducing inflammatory immune responses andshift the immune responses from Th1 and Th17 to Th2 and Treg.

Keywords: Multiple sclerosis, Experimental autoimmune encephalomyelitis, Thymoquinone, Immune response

1919O

Study of *FOXP3* gene Polymorphism in multiple sclerosis patients

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Background: Multiple sclerosis (MS) is a demyelinating disease of central nervous system which has different clinical manifestations. It is the most common neurological disorder among young adults in which women are affected twice as frequently as men.Thedysregulation of inflammatory responses is considered to be a key element in the autoreactive immune response in MS. Loss of peripheral tolerance mechanisms has been suggested as a prerequisite to allow activation and migration of self-destructive inflammatory cells to the target organ. Active suppression by natural FOXP3$^+$ regulatory T cells (Treg) maintains peripheral tolerance and controls autoreactive T cells.The *FOXP3* transcription factor is predominantly expressed by the Treg cell lineage and appears to act as a master regulator for cytokine production and cell–cell contact dependent inhibition of T effector cell activation. Associations have been reported between *FOXP3* gene variants and some autoimmune diseases. The aim of this study was to investigate the possible association between single nucleotide polymorphisms (SNP) in the *FOXP3* gene and predisposition to MS.**Methods:** This study comprised 115 MS patients and

115 healthy controls, which were genotyped for the SNP rs 3761549. DNA extraction from peripheral blood mononuclear cells was performed using DNA extraction kit and also by a salting outprocedure.After primer designing with PRIMER3 software for the rs 3761549 SNP, the related restriction enzyme was selected using restriction mapping software. The desired fragment was amplified by thermo cycler and the amplicons were verified by agarose gel electrophoresis. RFLP analysis was performed using *AluI* restriction enzyme and the digestion results were assessed using agarose gel electrophoresis.**Results:** The frequency of A allele was 15.7% in patients and 11.3% iv normal controls (p=0.33), allele G was 99.1% in MS case and 98.3 of controls. The rs 3761549(GG) was found in 84.3% of MS patients and in 88.7% of controls (p=0.33), rs 3761549 (AA) was found in 0.9% of MS cases and in 1.7% of controls (p=0.5), rs 3761549 (AG) was found in 14.8% of MS cases and in 9.6% of controls (p=0.27). No allele and genotype was significant in, rs 3761549 in patients and controls. **Results:** Based on our knowledge, this is the first study to investigate the association of FOXP3 SNP of rs 3761549 with MS. Results of the present study suggest that the mentioned functional polymorphism is not likely to cause susceptibility to MS.
Keywords:Multiple sclerosis, FOXp3, Polymorphism

2704O
Differential expression of CD22 and CD40 on regulatory B cells in RRMS patients

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Background: B cells are generally considered as positive regulators of immune response by producing regulatory cytokines such as IL-10. Inhibitory function of regulatory B cells has been investigated in many murine and human studies. Regulatory B cells with different surface markers have been considered in Multiple sclerosis (MS) and Experimental autoimmune encephalomyelitis (EAE). It is well established that CD22 on B cells is a suppressive molecule and CD40 under special stimulation could play the same role as well. We studied thefrequency of CD19+CD22+CD40+ B cells that produce IL-10 as regulatory B cells and expression levels of CD22 and CD40 as inhibitory marker on the B cells in MS patients in comparison to controls.**Methods:** We cultured peripheral blood mononuclear cells from relapsing remitting MS(n=27, Mean age: 31.68±9.91)and controls (n=10, Mean age: 32.1±7.37) and stimulated with CPG, CD154 and Anti human Ig. After 66h, PMA/ionomycin, and brefeldin A were added and 6h later, Cells were harvested and stained using CD19-PE-cy7, CD22-percp and CD40-APC. Cells were also stained for intracellular IL-10- PE and analyzed with flow cytometry. **Results:** we found that the frequency of $CD19^{+}CD22^{+}CD40^{+}$ IL-10 $^{+}$B cells and expression level of CD22 and CD40 on the surface of $CD19^{+}$ IL-10 $^{+}$B cells were reduced in MS patients in comparison to healthy individuals. **Conclusion:** $CD19^{+}CD22^{+}CD40^{+}$ IL-10 $^{+}$B cells could be a subset of regulatory B cells which reduced in MS patients and CD22 and CD40 on the surface of these cells could have inhibitory function.
Keywords: Regulatory B cell, Multiple Sclerosis, CD22, CD40, IL-10

3318O

Identification of Antigenic Epitopes of Chromogranin A Autoantigen in Nonobese Diabetic (NOD) Mouse Model of Type 1 Diabetes

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We have identified a peptide from the vasostatin-1 fragment of chromogranin A autoantigen, ChgA 29-42, as the antigenic epitope of ChgA for BDC2.5 T cells in NOD mice(Nikoopour et al. J Immunol.2011, 186(7):3831-5).The sequence KCVLEVISD derived from truncation of the ChgA 29-42 epitope is the minimal nine amino acid peptide required for binding to the MHC class II I-A^{g7} molecule and various peptides centered around this core segment are able to stimulate BDC2.5 T cells (Nikoopour et al. Eur. J. Immunol. 2014 *In press*). Anchor residues for binding to the I-A^{g7} molecule are $P1^{K}$, $P4^{L}$, $P6^{V}$ and $P9^{D}$. We have also found a peptide nonamer (VLEVISDSL) corresponding to residues 36-44 of vasostatin-1 that binds to the MHC class I K^{d} molecule and elicits an immune response in $CD8^{+}$T cells of NOD mice. This nonamer peptide overlaps with the $CD4^{+}$T cell epitope of vasostatin-1. We have used tetramers for the $CD4^{+}$ T cell epitope, ChgA 29-42/I-A^{g7}, and $CD8^{+}$ T cell epitope, ChgA 36-44/K^{d}, to monitor ChgA-specific T cells in lymphoid tissues and pancreatic islets.ELISA-based measurement of ChgA specific autoantibodies in NOD mice at different ages demonstrate that there is a significant rise in the level of ChgA 29-42 peptide-specific antibodies in diabetic mice compared to young NOD mice

2678O

Analysis of NFATc1 isoforms and their regulation by miRNAsinan animal model of multiple sclerosis

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Background: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), which is characterized by T cell infiltration and demyelination of CNS. MicroRNA are small non-coding RNA molecules which regulate gene expression by binding to complementary mRNA sequences. Herein, using EAE animal model, we investigated the role of miRNAs in regulating the levels of long and short isoforms of NFATc1, a transcription factor which is crucial for T cell activation.**Methods:**EAE was induced in C57/BL6 mice, and CNS tissue was extracted at different time-points after the induction of disease. The levels of short and long isoforms of NFATc1 mRNA were measured using real-time RT-PCR with SybrGreen method. The levels of miR-124, miR-137, miR-329 and miR-669 were also measured with real-time RT-PCR.**Results:** Expression of both isoforms of NFATc1 was significantly increased in the spinal cord in acute and chronic phases of disease. Expression of miR-124 and miR-137 which target the long NFATc1 isoform was significantly diminished in acute and chronic phases of disease. However, the expression of miR-669 was induced at the acute phase, while the levels of miR-329 did not show significant changes during disease process.**Conclusions:**

Our findings indicate that altered expression of miRNAs might be involved in regulating the expression of NFATc1 isoforms in the context of autoimmune neuroinflammation.
Key word: MS, MiRNA, NFATC1

2475O

Concentration-dependent effect of estrogen on human Regulatory T cells induction

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Background: It is now well accepted that sex hormones have immune regulatory activity. The potential of estrogen in driving suppressive activity of Foxp3+ regulatory T cells (Tregs) involves programmed death-1(PD-1) molecule. In this study, we investigated the co-expression of Foxp3 and PD-1 in 17-β estradiol (E2) conditioned T cells. **Methods:**The magnetic bead separated peripheral blood naive T cells were treated with different concentrations of E2 at pregnancy (1ng/ml, 4 ng/ml,7 ng/ml) andhigher than pregnancy (36 ng/ml) levels in the presence of anti-CD28 antibody in anti-CD3 coated plates. Induction of regulatory T cells was surveyed by flow cytometric analysis by staining cells with antibodies against human Foxp3 and PD-1. **Results:** Naive T cells differentiated into Tregs by all applied concentrations of E2 *in vitro*. The frequency of cells expressing Foxp3 was 47.14 % , 23.2% , 43.6% and 19.3% at 1, 4, 7 and 36ng/ml, respectively. PD-1 expression was higher than 80% in all concentrations. Co-expression of FOXP3 and PD-1 was determined to 46.5%, 23.1%, 43.5% and 19.2%at1, 4, 7 and 36ng/ml, respectively. **Conclusion:** These data demonstrate that E2 at pregnancy levels can affect multiple regulatory elements that influence Treg suppression. In addition, our data suggest that E2 at even 1 ng/ml (E2 concentration at first trimester) affects on tolerance induction and can be considered as a therapeutic agent in immune dysregulated conditions.

Keywords: 17β-oestradiol, FOXP3, PD-1, Regulatory T cells.

1416O

Immunomodulation of auto-reactive cells by mesenchymal stem cell-derived exosomes

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Background: Auto-reactive cells-mediated immune responses are responsible for the current tissue damages during autoimmunity. Accordingly, functional modulation of auto-reactive cells has been a pivotal aim in many of recent studies. Recently, MSC-derived exosomes have found to harbor many of MSC associated regulatory molecules. In the current study, we investigated the possibility for insertion of regulatory molecules onto auto-reactive cells

through exosomalnano-shuttles as a novel approach for phenotype modification of auto-reactive cells.**Methods:** The exosomes were isolated from supernatant of mesenchymal stem cell (MSC) culture at second passage. Resultant exosomes co-cultured with lymphocytes were harvested from established experimental autoimmune encephalomyelitis (EAE) mice in the presence of antigenic MOG_{35-55} peptide. After 24hr, insertion of exosomaltolerogenic molecules (PD-L1, TGF-β, galectin-1) onto auto-reactive cells were explored through flow cytometry. The potency of exosomal inserted membrane molecules to modulate phenotype of auto-reactive lymphocytes was assessed upon ELISA test for their-derived cytokines IFN-γ and IL-17. **Results:**Incorporation of exosomal molecules into lymohocytes membrane was confirmed by flow cytometric analyses for surface levels of mentioned molecules. Additionally, the decreased secretion of IFN-γ and IL-17 were detected in exosome pre-treated lymphocytes upon stimulation with MOG peptide. **Conclusion:** MSC-derived exosomes showed to be efficient organelles for insertion of bioactive tolerogenic molecules onto auto-reactive cells and modulation of their phenotypes.

Keywords: Auto-reactive cell, EAE, MSC, Exosome, Tolerogenic molecule

Poster Presentations:

2046P

Trichuris suis: new weapon against inflammatory bowel disease

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Background: Gastrointestinal nematodes modulate the immune system to establish infections. Potent immunomodulatory effects of nematodes lead to the induction of regulatory pathways. They may be suppressing unrelated inflammation such as inflammatory bowel disease (IBD), probably results from failure to downregulate chronic Th1 intestinal inflammatory process. Induction of Th2 immune response by intestinal helminths diminishes Th1 responsiveness. **Methods:** In a trial performed at University of Iowa Health Care to evaluate safety and effectiveness of helminthic antigens in the treatment of IBD, four patients with active Crohn's disease (CD) and three with ulcerative colitis (UC) were studied. A single dose of 2500 live *Trichuris suis* ova (TSO) was given orally, and patients were followed every 2 wk for 12 wk. Safety was monitored by clinical and laboratory studies. Patients also were monitored using the Crohn's Disease Activity Index. Two patients with CD and two with UC were given 2500 ova at 3-wk intervals as maintenance treatment. **Results:** During the treatment period, all patients improved clinically without any adverse effects. In the maintenance period, multiple doses caused no adverse effects and sustained clinical improvement in all patients treated every 3 wk for >28 wk. **Conclusions:** This trial demonstrates improvement in the common clinical indices also it is safe to administer TSO to patients with CD and UC. The administration of

parasitic antigens is discussed as a possible treatment of IBD due to their regulatory properties. Future IBD therapies will develop from excretory/secretory proteins or nematode components.
Keywords: IBD, *Trichuris suis*, helminth therapy

2139P

Cytokine pattern of islet-stimulated C57BL/6 diabetic mouse splenocytes shifts to regulatory cytokines in the presence of adipose-derived mesenchymal stem cells

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Background: Type 1 diabetes mellitus (T1D) is characterized by autoimmune destruction of pancreatic beta-cells and consequent metabolic complications. Transplantation of human cadaveric pancreas or isolated islet cells could be considered therapeutic in this condition. However, the shortage of cadaveric pancreas donors and overcoming the autoimmunity are still challenging issues. Mesenchymal stem cells (MSCs) due to immunomodulatory properties as well as plasticity in differentiation might be attractive in T1D therapy. The aim of this study was to evaluate immunomodulatory effects of adipose-derived mesenchymal stem cells on cytokines produced by C57BL/6 diabetic mouse splenocytes against syngeneic islet cell lysate. **Methods:** MSCs were extracted from abdominal fat tissue of healthy C57BL/6 mouse and cultured to proliferate. Then, they were immunophynotyped and their trans-differentiation to osteocyte, adipocyte, and chondrocyte was approved. Diabetic C57BL/6 mouse model was prepared by administration of consecutive low-doses of sterptozotocin and diabetic condition was confirmed. Pancreatic islets were isolated from healthy mouse and splenocytes prepared from healthy and diabetic mice. Splenocytes were co-cultured with MSCs in the presence of islet lysate for 72 hours. Inflammatory (IFN-γ, TNF-α, and IL-17) and regulatory (IL-4, IL-10, and TGF-β) cytokines were assayed in culture media by ELISA technique. **Results:** Our results demonstrated that MSCs significantly increased the levels of regulatory cytokines IL-4, IL-10, and TGF-β in cell culture supernatants ($P<0.05$). In contrast, inflammatory cytokines IFN-γ, TNF-α, and IL-17 were decreased in co-culture media ($P<0.05$). **Conclusion:** Immunomodulatory effects of MSCs may provide a new horizon for treatment of T1D in the near future.
Keywords: Islet of pancreas, Immunomodulation, Mesenchymal stem cells, Type one autoimmune diabetes

2726 P

Optimization of in-vitro human Th17 cell differentiation by evaluating of different polarizing cytokines

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Background: T helper-17 (Th17) cells are a CD4+ Th subset that plays a critical role in pathophysiology of several inflammatory disorders, autoimmune diseases and organ transplant rejection. Th17 cells are characterized by production of IL-17, IL-22 and expression of transcription factor RORC. The differentiation of Naive human CD4+ T cells into Th17 cells requires combination of inflammatory cytokines. Regarding discrepancies that exist among different studies which have tried to clarify critical factors in human Th17cell differentiation, the aim of this study was to identify the best condition for Th17differentiation.**Methods**: Naive CD4+ T cells were isolated from peripheral blood samples and cultured either in X-VIVO 15 serum-free medium or RPMI 1640 containing 10% FBS. Purified cells were treated with different combinations of polarizing cytokines (TGF-β1, IL-1β, IL-6 and IL-23) and neutralizing anti-IFN-γ and anti-IL-4 antibodies followed by analysis of the expression of RORC, FOXP3, GATA-3 and TBX-21 genes and their relevant cytokines by real-time quantitative RT-PCR and flowcytometry, respectively.**Results**: We found that combination of IL-1β, IL-6 and IL-23 in X-VIVO 15 serum-free medium could be applied as the best condition for developing human Th17 cells in compare with other cytokine treatments. It is shown that TGF-β1 is a negative regulator for human Th17cell differentiation.**Conclusion**: We concluded that human Th17 cells could be differentiated in the presence of inflammatory cytokines together with IL-23 as a growth and stabilization factor and TGF-β is considered as a negative regulator of human Th17 cell differentiation.

Keywords: Th17, CD4+ T, Cytokine, Differentiation, TGF-β

1525 P

Immunomodulatory effect of mesenchymal stem cells on allergic airway inflammation via modulation of regulatory T cells

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Background: Mesenchymal stem cells (MSC) in addition to potential for tissue repair possess potent anti-proliferative and anti-inflammatory effects which support their therapeutic use for immune mediated diseases. Regulatory T cells (Treg cells) play a role in maintaining immune homeostasis, preventing autoimmunity, moderating inflammation, and minimizing collateral damage to tissue. A primary function of Treg cells is to inhibit the function of antigen-presenting cells and effector T cells. The aim of present study was to evaluate the effect of MSC on Treg activity in allergic airway inflammation in mouse model.**Methods**: Following induction of allergic airway inflammation in mice, MSC administered via intravenous route. Treg mediated cytokine include TGF-β and pathologic allergic airway inflammation indexes were evaluated.**Results**: Results show that administration of MSC increased the production of TGF-β, an anti-inflammatory cytokine secreted by Tregs. According to histopathologic study, MSC administration reduced the allergic airway inflammation pathologic indexes. **Conclusion**:Present study suggesting that the major therapeutic targets of MSC could be Tregs. These results indicate that MSC efficiently diminishes bronchial inflammation in an allergic

airway inflammation in murine model, andthis effect might correlate with Tregs, which play an important role in maintaining immune homeostasis and suppressing the function of other T cells to limit the immune response.

Keywords: Mesenchymal stem cell, Regulatory T cells, TGF- β, Mice.

1495 P

Expression patterns of Th1/Th2 transcription factors in patients with guttate psoriasis

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Background: Many lines of evidence propose that psoriasis is a T cell-mediated diseasewhere T cell activation is followed by secretion of inflammatory cytokines.**Methods:**To elucidatethe functional state of T cells in guttate psoriasis, we analyzed mRNA expressionlevels of T-bet and GATA3 for Th1 and Th2 differentiation, respectively together withTh1 (IFN-γ) and Th2 (IL-4) cytokine mRNA expression. Relative quantification ofT-bet, GATA3, IFN-γ and IL-4 transcripts in peripheral blood leukocytes(PBL) was conducted by real-time reverse transcriptase PCR (RT-PCR). Serum levels of IFN-γ and IL-4 were also determinedby ELISA. **Results:** GATA-3 and IL-4 mRNAexpression levels were lower in psoriatic patients as compared to normal healthy controls. The expression levels of T-bet andIFN-γ genes were relatively similarin the patients and controls. In addition, a marked decrease in plasma IL-4 levels wasobserved in the psoriasis group, while no differences were observed with regard to levelsof IFN-γ between patients and normal subjects. Furthermore, a clear correlationbetween decreased IL-4 mRNA expression and IL-4 ($P < 0.05$) was revealed.**Conclusion:**These results suggested that altered balance between Th1 and Th2 cellstranscriptionfactor genes and their products may be implicated in the pathogenesis of psoriasis.

Keywords: Psoriasis, T cell, Autoimmunity.

1800 P

Association of SNP rs2104286 on IL2Ra gene in Multiple Sclerosis patients in IRAN

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Background: The prevalence of MS 20-60/100000 in Iran and 73/100000 in Isfahan. Isfahan is considered as an area with high risk of MS. The patients affected MS interface with physical, psychological, family, occupation, societal, and family problems.This disease has extended anegative impact on the health system due to high drug expenses and long term hospital bed occupation.MS is one of the so-called complex genetic diseases, which are common disorders that are characterized by modest disease risk heritability and multifaceted gene–environment interactions.Up 2010, some new susceptibility genes have been identified and replicated using this approach.Estimations revealed that the HLA region accounts for 20-60% of genetic susceptibility in MS. IL2Rαgene is other genetic susceptibility loci in MS.Multiple

sclerosis (MS) is an inflammatory, demyelinating, neurodegenerative disorder of the central nervous system (CNS) of unknown etiology and is known as autoimmune disease. MS is the most common neurologic disability disease among young adults. Women are affected approximately fourth as often as men in our area. It is characterized clinically with recurrent attacks; the clinical presentation of individuals with MS is extremely variable. Recent genome-wide association studies have identified a number of single nucleotide polymorphisms (SNPs) associated with susceptibility to MS. Since the genetical model of the disease would be varied in different ethnic and geographic areas, we decided to study the polymorphism of the IL2Ra (CD25) geneinRelapsingRemittingMultiple Sclerosis (RRMS) patients in order toidentify some diseasesusceptibilitygenevariants**. Methods:** After written consent, blood samples from 200 patients with RRMS (180 females and 20 male; mean age=31.65±8.3) with clinically and MRI definitedRRMS according to the classification of Mcdonald et al (2005) who had recuited to MS research center in Alzahra Hospital (Isfahan, IRAN), and 200 age and sex matched healthy subjects of blood donors (160 females and 40 male; mean age=31.74±7.75) with no history of neurological disorders were included in the study.DNA was extracted from whole blood using a commercially available kit (Qiagene) and stored at -20°C until used for genotyping. SNP analysis was performed using HRM Real Time PCR (Corbett).Differences in allele and genotype frequencies among the respective groups were evaluated by chi square calculation.**Result:** According to our findings frequency of the minor allele for rs2104286 did not differ significantly in patients with RRMS when compared with healthy subjects (P=0.058) but it was trended toward significant when note in 0.1 level.**Conclusion:** This result suggests that rs2104286 SNP is not correlated to RRMS susceptibility in studied population.

Keywords: RRMS, CD25 (IL7Ra) gene, rs2104286

1561 P

Anti-PhosphatidylserineAntibodies in Acute Myocardial Infarction

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Background: Acute myocardial infarction (AMI) is the combined result of environmental factors and personal predispositions. Many factors play a role in AMI. One those anti-Phospholipid (aPL) antibodies, that may act in the induction of immunological response leading to the development of AMI. The study of anti-Phosphatidylserine (anti-PS) antibody in AMI might shed light on etiologic mechanisms in the pathogenesis of acute coronary syndromes. This study was conducted to evaluate whether prevalence of anti-PS antibodies, in patients who had AMI and to analyze their relationship with traditional cardiovascular risk factors. **Methods:** The prevalence of anti-PSIgG and IgM in a well characterized group of patients with AMI as a case group and in age and sex matched healthy subjects as control group. Sera from two groups were tested to evaluate the presence of IgG and IgMisotypes to anti-PSby ELISA method.**Results:** The frequencies of positive test for anti-PS IgG were26.70% and 8.90%among patients and controls respectively with significant difference (P=0.003). The anti-PS IgM frequencies were12.20%and 1.10% in patients and the controls, with significant difference (P=0.005).**Conclusion:** The findings of this study suggest that anti-PS antibodies seemed to play a role in AMI, independent risk factors for AMI, which may represent a link between autoimmunity and atherosclerosis inpatients with AMI. Further studies with bigger

sample size including patients with AMI and healthy people are recommended to explore the exact role of anti-PSantibodiesinAMI.

Keywords: Anti-Phosphatidylserine(PS) antibodies, Acute Myocardial Infarction (AMI), Cardiovascular Ischemia

1789 P

IFN-α stimulates CD26 gene expression in Systemic Lupus Erythematosus patients.

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Background: Systemic lupus erythematosus (SLE) is an involute autoimmune disease that involves several organs. Increasing evidences infer that in lupus patients, alpha interferons (IFN-α) have an essential role in both disease onset and pathogenesis. CD26 is a T cell activation marker with dipeptidyl peptidase activity. Our previous study revealed that soluble CD26 level increased in SLE patients. Additionally, previous studies proposed that IFN-α stimulate expression of genes which have GAS element instead of TATA box in their promoter. In silico analysis revealed that CD26 has GAS element in promoter and could be target of IFN-α signaling. To analysis this hypothesis we investigate the level of IFN-α in serum, along with CD26 gene expression in peripheral blood mononuclear cells (PBMCs) of SLE patients. **Methods**: Forty three SLE patients and 39 age and sex-matched healthy control have been included in this study. IFN-α level in serum were quantified by sandwich ELISA. Additionally CD26 mRNA in PBMCs was analyzed by Real-time PCR.**Results:** CD26 mRNA level was significantly higher (4 times) in SLE patients in comparison with controls ($p<0.001$). Moreover significant correlation between IFN-α concentration and CD26 gene expression was observed ($p<0.05$).**Conclusion:** For the first time, we showed that IFN-α up-regulates CD26 gene expression in lupus patients. Therefore CD26 could be considered as a potential disease marker. As well CD26 beside IFN-α could be a target for therapeutic approach.

Keywords: CD26, SLE, Interferon

1790 P

Soluble CD26 is associated with nephritis in SLE

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Background:CD26 is a dipeptidyl peptidase expressed on activated T cells. To elucidate the immunopathological role of CD26 in the pathogenesis of SLE, we investigated the level of soluble CD26 (sCD26) in the serum of SLE patients.**Methods:**sCD26 level in serum were quantified by sandwich ELISA in 47 patients with SLE and 44 sex-matched healthy control. **Results:**Although the level of sCD26 in SLE patients (579.66ng/ml)were higher than controls (438.96ng/ml), but this difference was not significant. Nevertheless, analysis of sCD26 levels in

association with different clinical variables indicated that patients with nephritis had significantly higher levels than patients without this disorder ($p<0.05$). **Conclusion:**Collectively, the results presented hereassociate sCD26 level with nephritis outcomes in lupus patients. Moreover, increased sCD26 could be also of diagnostic or therapeutic significance.

Keywords: Systemic lupus erythematosus,Dipeptidyl peptidase IV, Nephritis

2527 P

Influence of Vitamin D on the cell cycle and apoptosis in patientswith systemic lupus erythematosus

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Background: Systemic lupus erythematosus is an autoimmune disease characterized by production of antibodies against nuclear antigens. Etiology is unknown but genetic and environmental factors activate the disease. In normal individuals autoreactive T and B lymphocytes are cleared through apoptosis which is a tightly controlled procedure. Any increase and decrease in apoptosis may disturb tolerance. In tumor cells vit D has anti-proliferative effects and controls cell cycle progression. In present study we investigated the molecular effects of vit D on cell cycle progression and apoptosis induction in lupus erythematosus patients.**Methods:**Study group comprised of 30 patients with systemic lupus erythematosus, and 30 age and sex matched controls. Lymphocytes were isolated and cultured in presence of vit D for 48 hours, then one part of cells stained with FITC labeled Annexin V and PI and analyzed on a flow cytometer. Another part of cultured cells were treated with PI and cell cycle was analysed using Flow Cytometer.For detecting gene expression, RNA was isolated from cells, cDNA was synthesized. Using specific primers and Real Time- PCR method, the expression levels of Fas and FasL, Caspase8, Caspase9, Bcl2, and Bax were determined. **Results:**Vit D decreased apoptosis rate in lupus patients in comparison to healthy controls. Expression level of Bcl2 increased, while FasL decreased significantly .Cell cycle analysis showed an increase in G1.**Conclusion:** Vit D has regulatory effects on cell cycle, and apoptosis induction in lupus patients.

Keywords: Vit D, Cell cycle, Apoptosis, Systemic lupus erythematosus, Human

2483 P

Effects of vitamin D on Th17 cells and related cytokines in systemic lupus erythematosus patients

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ital,MashhadUniversity of Medical Sciences, Mashhad, Iran.

Background: Systemic lupus erythematosus is a multisystem inflammatory autoimmune disease.Genetic and Environmental factors are involved in the pathogenesis of SLE disease and vitamin D deficiency has been proposed as a potential environmental factor triggering SLE.Furthermore, Emerging data suggests that Th17 cells are the main pathogenic cells involved in SLEthat secrete proinflammatory cytokines and increased frequency of these cells in peripheral blood is correlated with disease activity. In present study we investigated the effects of vitamin D on of Th17 cells and related cytokines.**Methods:**Blood samples were obtained from 30patients with SLE.PBMCs isolated using Ficoll-Hypaque density gradient centrifugation. IsolatedPBMCs were cultured in the presence and in the absence of vitamin D and were incubated overnight.After incubation, for the detect of Th17 cells,cell suspension was harvested and stimulated for 4-5 hours with PMA and ionomycin in the presence of brefeldin A and IL-17 secreting cells were analyzed by flowcytometry.Also RNA extracted, cDNA synthesized, and the expression levels of IL-6, IL-17and IL-23 genes was assessed by Real-time PCR**. Results:**The percentage of Th17 cells among CD3 +CD8- T cells was significantly decreased in 1,25(OH) 2D3-treated cells (mean ± SD 3.67± 2.43%) compared with untreated cells (4.65±2.75p<0.003). Theexpression of IL6,IL17 and IL23 genes in 70,63.3 and 80 percentage of patients was decreased by1,25(OH)2D3 treatment,respectively. **Conclusion:**Our data indicate that 1,25dihydroxyvitamin D3 modulateTh17 -related immune responses.Thus,1,25dihydroxyvitamin D3-mediated immunomodulation might be a beneficial strategy to control tissue inflammation and to treat autoimmune diseases including SLEin therapeutic strategies.

Keywords: Systemic lupus erythematosus, Vitamin D, Th17 cells

3200 P

IL-13 rs20541 (2044A>G) Single Nucleotide Polymorphism in Iranian Patients with Multiple Sclerosis

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Background: Multiple Sclerosis (MS) is a chronic autoimmune and inflammatory disease of central nerves system characterized by demyelination and axonal damage with genetic predisposition. Interleukin 13 (IL-13) is an anti-inflammatory mediator of immune responses. The aim of the present study was to investigate association of IL-13 rs20541gene polymorphism with MS**. Methods:** Total of 76 patients with clinically definite MS and 110 healthy controls were enrolled in this study. Blood samples were collected and DNAs were isolated. Afterward, genotyping was performed by PCR-RFLP using NlaIV restriction enzyme. Comparison of genotypes and allele frequencies were made by logistic regression and genotype counting statistic tests, respectively.**Results:** Our results showed significant higher frequency of allele

A (p=0.004) and AA genotype (p= 0.019) in MS group in comparison with control group by applying Chi-squared test. Also, results from regression logistic reported that genotype AA has higher possibility to being in MS group. (Odds ratio [OR] =3.01, P=0.02). **Conclusion:** We foundsignificant associations between allele A of IL-13 rs20541 SNP and MS which has never been reported before in Iran. The studied SNP is located in exon 4 and may be affect the conformation of IL-13 protein and subsequently the binding affinity to its receptor. The allele A would give rise to lower affinity and decreased anti-inflammatory effect of IL-13 which leads to predisposition to MS. However, more studies are needed to evaluate exact mechanism(s) by which SNPs predispose a population to MS.

Keyword: IL-13, Multiple sclerosis, Polymorphism, rs20541, PCR-RFLP

1401 P

Suppressive Role of IL-27 against Pro-inflammatory Role of IL-17A in Patients with Multiple Sclerosis

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Background: Effector $CD4^+$ T cell subsets have important role in Multiple Sclerosis (MS). Interleukin-27 (IL-27) suppresses Th (Th1, Th2 and Th17) cells and dampens autoimmunity and tissue inflammation via promoting the generation of Tr1 cells. IL-27 significantly inhibits both non-polarized and IL-23 driven IL-17 production by myelin-reactive T cells. A strong suppressive effect of IL-27 has been demonstrated in active experimental autoimmune encephalomyelitis (EAE). IL-27 can potently suppress the IL-17A effects and effective phase of EAE in vivo and may have potential therapeuticeffect in autoimmune diseases such as MS. The aim of this study was identifying the role of IL-27 and whether this cytokine suppresses the inflammatory cytokine IL-17 in MS patients. **Methods:** Venous blood was collected from forty MS patients and forty-three healthy subjects as control group. Serum levels of IL-27 and IL-17A were measured by ELISA method. **Results:** Our study has shown significant difference between patients serum IL-17A concentration (120.68 ± 209.85 pg/ml) and control group (67.26 ± 117.76 pg/ml) ($P < 0.05$). Serum IL-27 levels of the MS patients (159.7 ± 581.4 pg/ml) were significantly lower than control subjects (180.35 ± 507.84 pg/ml) ($P < 0.05$). **Conclusion:** Our findings suggest the inhibitory role of IL-27 on inflammatory process of MS.

Keywords: Interleukin-27, Interleukin -17A, Multiple Sclerosis

1407 P

Do thyroid hormones associated with C-reactive protein?

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Background: Thyroid-stimulating hormone(TSH) is a hormone that stimulates the thyroid

gland to produce thyroxine(T_4), and then triiodothyronine(T_3) which stimulates the metabolism of almost every tissue in the body(1), which regulates the endocrine function of the thyroid gland.C-reactive protein (CRP) is a protein found in the blood,and it's acute-phase protein, the levels of which rise in response to inflammation. CRP is synthesized by the liverin response to factors released by macrophages. The aimof this study was checking the amountof TSH, T3 and T4 withamountofCRPin the serum.**Methods:**This Cross-sectionalstudyon531 patientsreferred to the Zanjan'sClinical Laboratory,TSH, T3 and T4weremeasuredby the chemiluminescence method(Diasorin, Italy) with Liaisonanalyzer,and CRP weremeasuredby LIA method(Bionik, Iran).Then thedatawere analyzedwith Chi square testby SPSS13Software. **Results:**The female: male ratio was1.84. The mean age ofthe Patientswas 39.42±15.13. The results oftestsrevealed that thedatanotshowedasignificant difference between groups in level of CRP, T3 and T4.Butwe found significant differences between groups in level of CRP and TSH ($p<0.05$).**Conclusions:**This studyshowed thattherelationship between CRP and TSHcanbe thebackgroundfor the development of Hashimoto's thyroiditis that the slow progress to Hypothyroidismwas showed.StudingCRP and TSH with antibody against thyroglobulin (anti-TG) and antibody against thyroid peroxidase (anti TPO) suggested.

Keywords:TSH, CRP, LIA,Chemiluminescence

1527 P

Cytokine profile in new cases patients with Multiple Sclerosis

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Background: Multiple sclerosis (MS) is characterized by multiple areas of inflammation, demyelination and neurodegeneration. Multiple molecular and cellular components mediate neurodegeneration in MS. They involve: adhesion molecules, chemokines, cytokines, meta-loproteases and some immune cells such as CD4 T cells, CD8 T cells, B cells, microglia and macrophages. Infiltrating Th1 CD4 T cells secrete proinflammatory cytokines.They stimulate the release of some cytokines, expression of adhesion molecules and can be factors that cause damage to the myelin sheath and axons. **Methods:**In this study, we analyzed amounts of 5 important cytokines in the new cases multiple sclerosis Patients by ELISA and Real time PCR. **Results:**Our result was shown significant differences in IL-10, IL-27 and TGF-β but there was no significant difference in theIL-17 and IL-23 between two groups.**Conclusions:** This study may help to clear the probable role of these cytokines in neurodegeneration mechanism and current/future use of them in managing and treatments of multiple sclerosis.

Keywords: Multiple sclerosis, Inflammation, Neurodegeneration, Cytokines, Autoimmunity

2703 P

IL-10 producingCD19+CD38+CD24+ Regulatory B cells in RRMS patients

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Background: B cells are recently considered as regulatory cells by producing cytokines such as IL-10. Inhibitory function of regulatory B cells has been investigated in many murine and human studies. Regulatory B cells with different markers has been considered in Multiple sclerosis (MS) and Experimental autoimmune encephalomyelitis (EAE). Frequency and inhibitory function of regulatory B cells have been shown defected in EAE and MS patients. In this study, we analyzed thefrequency of a subset of regulatory B cells, IL-10 producing CD19+CD38+CD24+ B cells, in MS patients in active phase in comprison to healthy controls. **Methods:** We cultured peripheral blood mononuclear cells (PBMCs) from relapsing remitting MS patients(n=27, Mean age:31.68±9.91) and healthy individuals(n=10, Mean age:32.1±7.37) stimulated with CPG, CD154 and Anti human Ig. After 66h, PMA ionomycin, and brefeldin A were added. After 6h, cells were harvested and stained with CD19-PE-cy7, CD38-Biotin, PE-Texas Red streptavidin and CD24-FITC. Cells were also stained for intracellular IL-10- PE and analyzed with flow cytometry. **Results:** We found that the frequency of $CD19^{+}CD38^{+}CD24^{+}$ IL-10^{+}B cells are reduced in MS patients in comparison to healthy individuals.**Conclusion:**Altered frequency of $CD19^{+}CD38^{+}CD24^{+}$ IL-10^{+}B cells may affect regulation of immune responses in RRMS patient.

Keywords: Regulatory B cell, Multiple Sclerosis, CD38, CD24, IL-10

1564 P

Aqueous humor and serum concentrations of soluble MICA and MICB in Glaucoma patients

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Background: The destruction of the retinal ganglion cells (RGCs) in glaucoma has been previously reported to be related with immune reactions. In this study, the concentration of MICA and MICB molecules has beenmeasured in patients with glaucoma and compared with those cataracts.**Methods:** The aqueoushumor and serum of 15 glaucoma patients and 45 cataract ones that needed to be surgery,were obtained. TheconcentrationofMICAandMICB molecules weremeasuredwith the use of sandwichELISA technique in the aqueous humorandserumof T test was used for the comparison of the utilized results.**Results:**TheconcentrationofMICAandM

ICB molecules in the aqueous humor of glaucoma patients was136.71 pg/ml and 257.41 pg/ml and the concentration of these molecules in the aqueous humor of cataract patients was51.16pg/ml and 45.49pg/ml, in addition, the concentration of MICA and MICB molecules was 146.28 pg/ml and 231.31 pg/ml in the serum of glaucoma patients and 214.13 pg/ml and 157.77 pg/ml in the serum of cataract patients.Both MICA and MICB concentrations were higher in aqueous humor of patients with glaucoma compared to those of cataract (p=0.013 and p=0.004, respectively); however, in serum samples, no significant difference was observed. MICA and MICB concentrations showed no significant difference among the four types of glaucoma, family history of glaucomaor the optic nerve condition.**Conclusion:** IncreasedIOP and other pathological conditionsare associated with increasing expression of the MICA and MICB molecules which may initiate the destruction of RGCs and development of glaucoma.
Keywords: Glaucoma, MICA,MICB,Aqueous humor

1930 P

High serum levels of chemokine CCL20 in patients with multiple sclerosis

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Background: Chemokines play an important role in the autoimmune disease. The aim of this study was to investigate the circulating levels of CCL20 in patients with multiple sclerosis (MS).

Methods: The blood samples were collected from 135 MS patients and 135 healthy control subjects. The serum levels of CCL20 were measured by ELISA. **Results:** The mean serum levels of chemokine CCL20 in MS patients (76.52 ± 10.54 Pg/mL) was significantly higher than that in healthy control group (33.18 ± 2.56 Pg/mL; P<0.001). In both MS and control groups, no significant differences was observed between men and women regarding the mean serum levels of chemokine CCL20. In male patients the mean serum levels of chemokine CCL20 was significantly higher as compared to healthy men (P<0.01). Similarly, in female patients the mean serum levels of chemokine CCL20 was significantly higher in comparison to healthy women (P<0.001). **Conclusion:** These results showed higher levels of CCL20 in patients that represents the chemokine may play an important role in pathogenesis of MS.

Keywords: Multiple sclerosis, CCL20, Serum, ELISA

2052 P

Study of the expression of FCRL molecules in peripheral blood lymphocytes of patients with Hashimoto's thyroiditis and Grave's disease

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Background:B cells play a crucial role in humoral immunity by production of immunoglobulins and also as antigen presenting cells. FCRLs (Fc Receptor Like molecules) are new class of molecules belonging to immunoglobulin superfamily. These moleculesare encoded by genes in the human chromosome 1q21–23 region,which may share a common ancestor with the Fcreceptor family of molecules. They are dominantly expressed by B lymphocytes and their action has not clearly been identified.Recent studies have focused on the role of FCRL molecules in the pathogenesis of various autoimmune and malignant diseases. Graves' disease (GD) and Hashimoto's thyroiditis (HT) are common organ-specific thyroid diseases. In the present study the gene expression of the above molecules in two groups of HT and GD patients has been investigated.**Methods:** Peripheral blood lymphocytes were obtained from HT and GD patients as well asa group of healthy subjects. FCRL1, FCRL2 and FCRL4 expression was determined by quantitative Real time PCR.**Results:**Results of this study showed a decreased level of FCRL1 mRNA in both groups of patients compared to the healthy subjects. FCRL2 gene expression showed an elevated level in HT and GD patients and FCRL4 a decreased level only in GD patients compared to the controls. Measurement of anti-thyroid peroxidase and thyroid globulin in the serum of patients showed no correlation with the gene expressions.
Conclusion: Changes in the level FCRL gene expression may indicate their contribution in thyroid autoimmunity.Further studies are needed to explore the function of these molecules in relation to the pathogenesis of these diseases.
Keywords: Grave's disease, FCRL molecules,Autoimmunity

2679 P

Investigating the expression of C-FOS transcription factor and its regulation by miRNAs in an animal model of MS

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Background: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system. MicroRNAs are small, noncoding RNA molecules which function via base-pairing with complementary sequences within mRNA molecules, usually resulting in gene silencing. Herein, we analyzed the expression of C-FOS T cell transcription factor and the miRNAs which are known to target it in the spinal cord of mice affected by EAE, an animal model of MS. **Methods:** After induction of EAE in C57/BL6 mice using MOG 35-55 peptide, the CNS tissue was isolated at three different stages of disease (pre-onset, peak and chronic phase). The expression of C-FOS gene was measured in the lumbar spinal cords of EAE and control mice by real time RT-PCR. In the next step, expression of miR-7b1, miR-101a and miR-101b which target C-FOS transcription factor were measured in the spinal cord by real time RT-PCR.**Results:** Expression of C-FOS was significantly reduced during the chronic phase of EAE compared with control mice. Expression of miR-7b was induced in the acute phase of

EAE compared with control mice. The expression of miR-101a and miR-101b showed a trend towards reduction during the chronic phase of EAE, but it did not reach statistical significance. **Conclusions:** While miR-7b might be involved in regulating C-FOS expression level during EAE, miR-101ab do not seem to be strong regulators of CFOS expression.
Keywords: Multiple sclerosis(MS),MiRNA,C-FOS

2233 P
Numerical Status of Regulatory T Cells in Multiple Sclerosis

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Background: Regulatory T cells, including CD4+CD25+Fox3+ and CD8+CD28- cells play an important role in regulating the balance between immunity and tolerance. Since multiple sclerosis is an inflammatory autoimmune disease, regulatory T cells are considered to be involved in its pathogenesis. In this study, we investigated the circulatory number of 2-mentioned types of regulatory T cells and also their association with different clinical characteristicsin one large population of multiple sclerosis patients.**Methods:** 84 patients with multiple sclerosis and 75 normal individuals were studied. The peripheral blood frequency of two different subgroups of regulatory T cells (CD4+ CD25+Foxp3+ and CD8+CD28- cells) were analyzed by Flow cytometry. The statistical indices of regulatory T cells were calculated using *t* and chi-square tests. Correlations between variables were tested by Pearson's univariatetest and multiple linear regression analysis was used to test the effects of various factors on Tregs.**Results:** The frequency of CD4+CD25+Foxp3+ cells in multiple sclerosis patients was significantly less than that in healthy controls (P=0.006) and in mild forms less than that in sever forms (P=0.003). There was not any correlation between the frequency of regulatory T cells and different clinical variables.**Conclusion:** Our results show that the number of CD4+CD25+Foxp3+ cells reduces significantly in multiple sclerosis patients, a concept which probably shows the regulatory role of these cells in multiple sclerosis.
Keywords: Multiple Sclerosis, CD4+CD25+Foxp3+ Regulatory T cells, CD8+CD28- Regulatory T cells

2287 P
Detection of protein A effects on immune system

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Background: Highly-purified form of the Staphylococcal protein A (SpA) has the capability,

at very low concentrations, to down regulate activation of human B-lymphocytes and macrophages which are the key cells interfering tumefaction in determined autoimmune diseases.**Methods:**In a PBMC in vitro model, we investigated the effect of different doses of three highly-purified forms of protein A,in comparison of commercial drug, on the synthesis of selected cytokines after stimulation with lipopolysaccharide (LPS) in adult patients with active rheumatoid arthritis (RA) and healthy donors.**Results:** Protein A in therapeutic serum condensations significantly prevented the expressionof a majorityof the cytokines examined.**Conclusions:** Immune-moderating effects of Protein A were shown in clinically relatedconcentrations, in low doses reflecting serum concentrations found generally in patients. Protein A potentiates the suppressive influences of commercial drug on cytokine synthesis.
Keywords: Protein A, Immune, Therapeutic, Cytokine

2315 P

The association of different polymorphisms of NF-KB signaling pathway genes with multiple sclerosis in Iranian population.

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Background: The aim of this study is to investigate theassociation of polymorphisms of NF-KB signaling pathway genes including NF-KB1 and IKBa promoter polymorphisms with thedevelopment of Multiple Sclerosis (MS) disease in Iranianpopulation. We selected some important andcommon polymorphismswhich were well-studied in NF-KB signaling pathways genes associated with several common autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematous.**Methods:** For this purpose, we analyzed nearly two hundredsex and age matched MS patients along with the same unrelated healthy controls were enrolled inthis study. The NFKB1 –94 ins/del ATTG promoter polymorphism and three regions in IKBapromoter including -881A/G (rs3138053), -826C/T(rs2233406) and -519C/T (rs2233408) polymorphismswere determined by PCR/RFLP methods. **Results:** The data revealed no significant differences in the frequency of the -94 ins/del ATTG polymorphism in MS patients compared with the control group. In contrast to NF-KB1, our studydemonstrated that the genotype frequencies of IKBa -881A/G and -826T/T, and allele frequencies of IKBa-881G were significantly higher in patients with MS withrespect to the controls. We also found thatthe estimated haplotype frequencies of IKBa promoter-881G–826T–519C were significantly increased in thepatients with MS in comparison to that of the healthyindividuals. **Conclusion:** This study reveals for the first time that polymorphisms in theIKBa promoter (-881 A/G, -826 C/T) are strongly associatedwith the susceptibility of Iranian MS patients.
Keywords: NF-KB, Polymorphism, Multiple Sclerosis

2425 P

Association analysis of TIM-3 -1541C>T polymorphism with multiple sclerosis in Isfahan population.

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Background: The family of T cell immunoglobulin domain and mucin (TIM) proteins are expressed on T cells. A member of TIM family, TIM-3,isan important regulator of Th1 immunity and tolerance induction. Previous studies have shown an association between polymorphism in the TIM-3 gene and autoimmune disease. We aimed to determine the association of TIM-3 -1541C>T polymorphism with multiple sclerosis in Isfahan population. **Methods:**Blood samples were collected from 150 patients with multiple sclerosis disease and 150 healthy controls. After DNA extraction, TIM-3 -1541 C>T polymorphism was detected with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques.**Results:**The results of this study showed that there is a significant relation between multiple sclerosis and TIM-3 -1541 C>T polymorphism in Isfahan population. **Conclusions:**T cell immunoglobulin and mucin gene family (TIM) is located on human chromosome 5q33.2 and play a critical role in regulating immune responses, including allergy, transplant tolerance and autoimmunity. Our results suggest that TIM-3 -1541 C>T polymorphism is associated with multiple sclerosis in Isfahan population.

Keywords: Polymorphism, Mucin,Multiple sclerosis

2565 P

Determinationof the plasma levels of IL-36inpatients with RRMS in Esfahan Province

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Background: Multiple sclerosis is a common disease of the central nervous system in which the interplay between inflammatory and neurodegenerative processes. It has been reported that cytokines play an important role in the pathogenesis of multiple sclerosis (MS). IL-36 cytokines act directly on naive T cells by enhancing both cell proliferation and IL-2 secretion. IL-36 acts in synergy with IL-12 to promote Th1 arousal and IL-36 signaling is also involved in mediating of Th1 immune responses. The aim of this study was to evaluate the serum levels of interleukin IL-36 in patients with RRMS. To measure the IL-36 levels in Plasma of multiple sclerosis (MS) patients compared to healthy subjects.**Methods:** In a case-control study, venous blood was collected from healthy subjects as control group (n=45) and MS patients (n=45). All selected patients were clinically diagnosed as having relapsing remitting multiple sclerosis (RRMS) .The plasma levels of the cytokine IL-36 were measured using

ELISA method.**Results:** Elevated of IL-36 plasma level has been observed in patient with RRMS in comparison with control group. **Conclusions:** We found a highly significant increase of serum levels in MS patients in comparison with the control group. The results indicating a pivotal role of IL-36 in immunopathogenesisof MS.
Keywords: Multiple sclerosis, IL-36, Immunopathogenesis, Isfahan, Iran

2575 P

Interleukin 18 genetic variants and susceptibility of multiple sclerosis in RRMS patients

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Background: IL-18 is produced by monocytes/macropharges, dendritic cells, B cells as well as by astrocytes and microglia. IL-18 is a unique cytokine that stimulates both Th1 and Th2 responses. IL-18 plays an important role in Th-1 response through its ability to induce IFN-γ production in T cells and NK cells. It has been reported that role of IL-18 has a key role in IFN-γ production in the different stages of multiple sclerosis.To measure the frequency of rs360719 and rs1946518 SNPs on IL-18 gene in RRMS patients compared to the healthy control.**Methods:** 105 Iranian patients that clinically definite as multiple sclerosis (RRM) and 113 healthy controls were enrolled. IL-18 gene SNPs were assessed by HRM-PCR method. The statistical analysis was performed in order to find the presence of association between this SNP in the patients compared to healthy subjects.**Results:** These findings show that carriers of the (rs360719) and (rs1946518) display a slight protection against MS development. Patients homozygous for both SNPs showed a significantly higher proportion of MS patients. **Conclusions:** These protective effects might be related to functional outcomes of these IL-18 variations. This investigate confirms that IL-18 and the gene may be contribute in MS development and its progression particularly.
Keywords: Multiple sclerosis**,** IL-18**,** Polymorphism, Isfahan.

1537 P

Laboratory Evaluation of the relationship between vitiligo and diabetes mellitus

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Background: Vitiligo is a common acquired disorder of skin. The disease is characterized by depigmented macules and patch on the skin. Autoimmunity has the crucial role in the pathogenesis of the vitiligo. Vitiligo is frequently associated with different autoimmune diseases such as thyroid abnormalities and diabetes. This study aimed to evaluate association between vitiligo and diabetes mellitus. **MethodS:** This case-control study conducted on 70 patients with established vitiligo disorder and 70 non vitiligo individuals as control. On case group we performed two tests FBS and OGGT while on control group only FBS wasperformed

and finally datawere analyzed with SPSS software. **Result:** The results of our study showed from total 70 people as case, 18/70(25.71%) had impaired FBS while only 4/70(5.7%) had impaired GTT3. From the statistical analysis we got p-value=0.015 which showed significant difference of impaired FBS between case and control groups. As we had half and 1 hour GTT data (GTT1 and GTT2) and all patients with impairedGTT1 &GTT2 were females that were statistically significance.**Conclusion:**According to the results of this study, periodically laboratory checkup for diabetes mellitus in vitiligo patients with a special consideration to female gender seem to be necessary .

Keywords: Vitiligo, diabetes mellitus, FBS, GTT

1595 P

Reduced IL-27 levels in peripheral blood of patients with diabetes type1

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Background: Type 1A diabetes (T1D) is an autoimmune disease resulting from the selective destruction of pancreatic beta cells by T cells most likely due to interaction of environmental and genetic factors. IL-27 is a cytokine that is involved in proinflammatory and anti-inflammatory responses. IL-27 expressed in macrophages and dendritic cells in type1 diabetes which causes development of TH17. It seems IL-27 regulates proinflammatory cytokine such as IFN-γ that have a role in the pathogenesis of type1 diabetes. We therefore examined IL-27 expression in peripheral blood of patients with diabetes type I. **Methods:** For this reason, peripheral blood was collected from patients with diabetic case (41 patients) and normal control volunteer (47 healthy people) as control from hospitals of Jahrom University of Medical Sciences. Then serums were isolated and assessed for IL-27 using by ELISA (ebiosciences kit). **Results:** As a result,analysis of cytokine production profiles revealed that IL-27 serum level in patients was significant lower than control group (*P*=0.0001).**Conclusion:** Low level of IL-27 is because of opposite role of TH17 (IL-17 and IL-21) and TH1 (IL-12 and IFN-γ) which causes an increase level of TH17 cytokine and maintain TH17 phenotype and reduces TH1 cytokines. Disease process by increasing the activity of TH17 cytokines and decreasing cytokine of IL-12 group (IL-23 and IL-27) may causes destruction of beta cells by inflammatory cells (TH17). Majority of patients were passed early stage of disease probably TH1 cytokines were decreased and neutrophilic inflammation was induced by TH17. So, it seems Th17-related cytokinescan be used as an imminent target in autoimmune diseases such as diabetes.

Keywords: Type1 diabetes, TH17, IL-27

3287 P

The Role Of miR-181 Family inAutoimmuneNeuroinflammation

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Background: Inflammation is a key component of neurodegenerative disorders such as multiple sclerosis (MS).MicroRNAs are small non-coding RNA molecules which control geneexpression by translational repression ormRNA cleavage.Recent studies have revealed that MS lesions have distinct microRNA (miRNA) expression patterns. Some of the dysregulatedmiRNAs, including miR-181, have been suggested to be involved in controlling inflammation.Herein, we evaluated the expression of miR-181 in CNS tissue of mice affected by experimental autoimmune encephalomyelitis (EAE), ananimal model of MS.**Methods:**EAE was induced in C57BL6 mice by subcutaneous injections of MOG35-55. miR-181a2 and miR-181b2 expression was measured in the CNS of EAE miceat three different phasesof disease (post immunization, acute and chronic phase) by real-time PCR. Inflammation was assessed byquantifying IL-1, IL-6, TNFa and CD3e transcript levels in CNS. GFAP levels were also analyzed as a marker astrocyte proliferation in CNS.**Results:**Expression levels of all inflammatory cytokines as well as CD3e lymphocyte marker was significantly increased in both acute and chronic phases of disease, whileGFAP levels were more enhanced at the chronic phase of disease. miR-181aexpression levels were upregulatedin postimmunization and chronic phases whereas miR-181b increase did not show a significant changeduring disease.**Conclusions:**MiR-181 upregulation in pre- clinical phase of disease might point to its potential involvement in initial stages of leukocyte infiltration or microglial activation in EAE. Targeting miR-181 expression in leukocytes might show beneficial effects in the context of autoimmune neuroinflammation.

Keywords:EAE , MS, MicroRNA , MiR-181

1845 P

The Potential Role of iNKT Cells in Multiple Sclerosis and its Animal Model

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Multiple sclerosis (MS) is an autoimmune disorder associated with neurological signs and chronic inflammatory demyelination of the central nervous system (CNS). Although MS has been primarily thought as Th1 (T helper) and Th17 lymphocytes mediated disease, however cells of the innate immune system play an important role both in the initiation and progression of MS. The invariant Natural killer T(iNKT) cells are the unique innate lymphocytes subtype involved ininflammation and autoimmune disorders by secreting cytokines such as interferon gamma (IFNγ), Interleukin (IL)-10, IL-4, IL-13. A reduction in number or defect in function of iNKT cells has been associated with an increased prevalence of autoimmune disorders indicating iNKT cells have an immune-regulatory role in autoimmune disorders. Also the protective role of iNKT cells has been extensively studied in MS animal model and the results of these studies show that iNKT cells might be used for therapeutic purposes, but the use of

these cells for treatment of MS needs extensive studies and understanding the biology of these cells. In this review, we will attempt to show the protective role of iNKT cells in pathogenesis of MS and its animal model.

Keywords: Multiple sclerosis; MS; EAE; NKT cells

2850 P

The effects of honey bee venom on the serum interleukin 1-β level in EAE Lewis rats

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Background:Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Experimental autoimmune encephalomyelitis (EAE) is an animal model that mimics many aspects of MS In this study we hypothesized that honey bee venom has a potential role for the treatment of EAE by decreasing of $IL_{1\beta}$ level.**Methods**: Female Lewis rats were immunized with emulsion of guinea pig spinal cord and complete Freund's adjuvant. EAE sign was developed 9 days after immunization. Experimental rats were divided into 4 groups including; Control group, EAE induced rats treated by 1mg/kg of HBV, EAE induced rats treated by 2mg/kg of HBV and sham group. Clinical sign of EAE was recorded daily and serum cytokines was analyzed by ELISA. Significance in comparing multiple means was tested with one-way ANOVA.**Results**: The results indicated that treatment with BV reduces the penetration of inflammatory cells in the brain of EAE rats. Results of ELISA test showed that treatment with BV in experimental groups(102pg/ml)causes decreasing $IL_{1\beta}$ in comparison to the sham group200pg/ml.**Conclusions**: Based on the results, we showed that BV can block $IL_{1\beta}$ as a pro-inflammatory cytokine. EAE rats which were treated by BV showed a decrease in the symptoms of disorder. Therefore, anti inflammatory effects of BV on cytokines levels may be modulate MS sings and then it could be used for MS treatment in future.

Keywords: Experimental Allergic Encephalomyelitis, Multiple Sclerosis, Honey bee venom Interleukin-1β.

2520 p

Effects of Varicocelectomy on Anti-sperm Antibody in Patients withVaricocele

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Background: Anti-sperm antibody (ASA) can decrease sperm motility and, therefore,it is a cause of male infertility. The aim of this study was to evaluate the effects of varicocelectomy on anti-sperm antibody in patients with varicocele. **Methods:** This observational study was

conducted on 90 patients with varicocele at Sina and Imam Khomeini hospitals during 2006 to 2009. All varicocelectomy candidates were selected for ASA assessment both in semen and serum before and after surgery. ASA level was measured using a direct method for semen and an indirect method of Sperm MAR test, for serum. Paired t-test and McNemar's test were used for data analysis, and $p<0.05$ was considered statistically significant. **Results:** ASA level in semen was 13.7% before, and 15.7% after three month of varicocelectomy ($p=0.881$). Serum level of ASA before and after surgery were13.6% and 21.7%, respectively ($p=0.033$). Three parameters including sperm count, motility and morphology showed recovery following, varicocelectomy, but only the difference in sperm motility was significant ($p<0.05$). **Conclusion:** This study showed that varicocelectomy has no effect on semen ASA. Although serum antibody has been shown to increase after varicocelectomy but sperm motility will improve. Varicocelectomy seems to have a beneficial effect on semen parameters in infertile men with varicocele.

Keywords: Anti-sperm Antibody, Infertility, Sperm Motility, Varicocelectomy.

1950 P

Determination of Serum Selenium in Rheumatoid Arthritis patients

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Background: It has previously been reported that rheumatoid arthritis is a systemic disease characterized by inflammatory polyarthritis involving small,larj joint and constitutional featurs as result of polymorphonuclear lymphocyte and monocytes and activation and release of prostanoid and concomitant generation of reactive oxygen species. Selenium is an essential trace element with well established role as a component of enzyme glutathione peroxidase. selenium has a remarkable protective effect against apoptosis induced by superoxide anion. As a low serum antioxidant level is a risk factor for rheumatoid arthritis. **Methods:**Blood samples were collected from 50 healthy subjects (the subjects included 25 males and 25 females) and 47 patients with rheumatoid arthritis. Informed consent was obtained from all patients . Serum selenium content was determined by spectrofluorometric method described previously by Laloned et al). Selenium in rheumatoid arthritis patients were decreased in comparison with the control group.**Results:**The results indicate that there is a significant difference between serum selenium content in healthy (183.5 ±9.6 in man and 171.7 ± 8.3 in woman, µg/l) population and patient with rheumatoid arthritis(152.4±7.8 in man and 139.0 ± 6.7 in women , µg/l).**Conclusion:**This study demonstrates a strong association between selenium and rheumatoid arthritis.Such finding and more knowledge regarding the importance role of selenium in rheumatoid arthritis.

Keywords: Rheumatoid arthritis ,Selenium spectroflurometry

3168 P

Evaluation of immunological effects of hyperforin on EAE mice model

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Background: Multiple sclerosisand its animal model, experimental autoimmune encephalomyelitis(EAE) are a central nervous system disorder, mainly characterized by demyelinated changes and axonal injury. Hyperforinis, fluoroglucinol derivative of the medicinal plant Hypericumperforatum (HP).It has been strongly implicated as an anti-inflammatory and neuroprotectiveagent.**Methods:**Female C57BL/6 Mice aged 8-10 weeks, divided into four groups: 1-hyperforin treated,2-HPE-treated, 3-control and 4-normal groups.EAE induced by subcutaneous injection of an emulsion of MOG peptide in CFA. Each mouse received pertussis toxin by intraperitoneal injection. The mice were treated with Hyperforin(5mg/kg),Hypericum perforatum extract (HPE, 150mg/kg).Clinical assessment of EAE were performed daily and the mice scored for disease severity.The mice were sacrificed on days 21.The brain was removed quickly and spleen cells were isolated.The levels of inflammatory and regulatory cytokines (IL-4, IL-10, IL-17, IL-23, TGF-β and IFN-γ) were measured by ELISA. Furthermore, the mRNA expressions Foxp3, T-bet، GATA3, ROR-γt were assessed by Real Time assay. Analysis of Tregs was performed by flowcytometry. **Results:**The treatment of C57BL/6 mice withhyperforin and HPE significantly reduced the clinical severity of EAE.Furthermore, cytokine profiles revealed thedecrease of IL-17, IFN-γ, IL-23in hyperforin and HPE-treated groups.Analysis of Tregs by flowcytometry showed the increase of Tregsinhyperforin and HPE-treated groups versus vehicle treated mice. Furthermore, the mRNA expression of the transcription factor srevealed decrease T-bet,RORγt expression and increase Foxp3, GATA expression in Hyperforin and HPE-treated groups.**Conclusion:**This study indicates that Hyperforin and HPE possess anti inflammatory effects and could deviate immune system toward Treg and Th2 responses.Thus it can be beneficial in the management of EAE.

Keywords:EAE,MS,Treg,Hypericumperforatum,Hyperforin

2993 P

Clostridium perfrin gens epsilon toxin and risk of multiple sclerosis

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Background: Multiple Sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system(CNS) with unknown etiology that results from a complex contribution of environmental factors and genetic susceptibility.Among the environmental risk factors proposed for MS, an infectious etiology is the most important in epidemiological observations. One of these risk factors is infection with*Clostridiumperfringens*. *C. perfringens*is a gram-positive bacterium, which causes a wide range of diseases in both humans and animals, by producing a large number of toxins. *C. perfringens* bacteria are classified into five toxinotypes (A-E) depending on their ability to produce the major toxins: Alpha-, Beta-, Epsilon- and Enterotoxin. Up to now, there was no study on *C. perfringens*risk in MS disease. In this study, we

investigated *C. perfringens* type B, an epsilon toxin-secreting bacillus,and its relation with MS that may assist us for knowingmore details about the environmental etiology of MS.**Methods:** This article has been developed by review of several studies on topics of Multiple Sclerosis,*C. perfringens* type B.**Results:***C. perfringens* epsilon toxin may be a candidate causative toxin for nascent lesion formation in MS worthy of further investigation.**Conclusion:**In the present study, it is proposed that environmental conditions might have increased the susceptibility to MS, but more studies in different parts of the world are needed to evaluate this claim.Some studies indicate relationship between MS and inflectional agents.

Keywords: Multiple Sclerosis, Clostridium perfringens type B epsilon toxin.

3282 P

Alpha1-antitrypsin activity and its correlation with antineutrophil cytoplasmic antibody in Behcet's syndrome

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Background: Alpha-1 antitrypsin is a proteinase3 inhibitor, an autoantigen for antineutrophil cytoplasmic antibody. This protein has been associated with a variety of autoimmune diseases, for instance Behcet.Behcet's syndrome is a rare disorder that causes inflammation in blood vessels throughout body that gives rise to numerous symptoms. In this study sera from iranian patients with Behcet's syndrome were investigated to understand whether or not there is any correlation between Alpha-1 antitrypsin activity with antineutrophil cytoplasmic antibody (ANCA).**Methods:** The activity of Alpha-1 antitrypsin as its potential inhibitory capacity of trypsin (TIC) was spectrophotometrically measured by BAPNA substrate and ANCA were detected in patient's sera using the standardized indirect immunofluorescence test. **Results:** 30% of patients were c-ANCA and none of them were p-ANCA positive. The mean of TIC were 2.4±0.2 µM/mL/min. **Conclusion:**We conclude that there is a significant relation between Alpha-1 antitrypsin activity and ANCA positive test in Behcet's syndrome.

Keywords: Behcet's syndrome,Alpha-1 antitrypsin

2968 P

Evaluation of proinflammatory cytokine gene polymorphisms in autoimmune hepatitis

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Background: Autoimmune hepatitis is a disease in which the body's immune system attacks

liver cells and presenting usually with chronic hepatitis. Researchers think a genetic factor may make some people more susceptible to autoimmune disease. We investigated the pro-inflammatory cytokines gene polymorphisms in a group of pediatric patients with AIH and compared the results with a group of healthy individuals.**Methods:** The study group was conducted in 57 pediatric patients with AIH who was referred to the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Tehran, Iran. The studied alleles and genotypes include TNF-α (A/G −308, A/G −238), IL-1α (C/T −889), IL-1β (C/T −511), IL-1β (C/T +3962), IL-1 receptor (IL-1R; C/T Pst-I 1970), IL-1RA (C/T Mspa-I 11100), and IL-6 (C/G −174 and A/G nt565).**Results:** The frequencies of the following alleles were significantly higher in AIH patients, compared to healthy controls: TNF-α A allele at position -308 (23.7% in AIH patients *vs.* 14.2% in controls, p=0.035), IL-6 A allele at position nt565 (35.1% in AIH patients *vs.* 18% in controls, p=0.00042). The most significant differences in genotype frequency between AIH patients and healthy controls belong to TNF-α AA, GA and GG genotypes at position -238 (17.5% in AIH patients *vs.* 0.7% in controls, p<0.0001 for AA genotype, 3.5% in AIH patients *vs.* 41.6% in controls, p<0.0001 for GA genotype, and 79% in AIH patients *vs.* 57.7% in controls, p=0.0081 for GG genotype).**Conclusions:** IL-6 and TNF-α polymorphisms are shown to have significant differences between AIH patients and healthy controls, but IL-1 family has no significant correlation.

Keywords: Autoimmune hepatitis, Proinflammatory cytokine, Gene polymorphisms

3199 P

Serum and cellular levels of caspase-1 in RRMS patients compared to healthy controls

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Background: Caspase-1 is a cysteine protease which can proteolyticly cleave proteins. Among these proteins are inflammatory cytokines such as IL- 18 and IL-1b which turn to their active forms by this cleavage.Caspase-1 interacts with another protein named PYCARD and leads to inflammasome formation and inflammation. These inflammatory cytokines play an important role in development of many inflammatory diseases such as Multiple sclerosis which is an inflammatory demyelination disease of CNS.It is characterized by inflammatory infiltrates and progressive axon demyelination and subsequent complete axon lost. The aim of the present study was to compare between two existing methods for measuring caspase-1. **Methods**: In this study we used an ELISA assay to measureserum and cellular caspase-1 levelsin Relapsing-Remitting multiple sclerosis patients (n=23) andhealthy age-and gender-matchcontrols (n=19).**Results**: weobserved that caspase-1 concentrationof peripheral mononuclear cells lysate was higher than serum in RRMS patients and controls.In addition, Caspase1 levels were significantly increased in serum from patients with multiple sclerosis compared with healthy controls (p<0.05).Moreover, no difference was found in cellular levels of caspase-1 between groups.**Conclusion**:Our results suggest thatserum caspase-1 increased in serum following inflammation. Therefore, measurement of caspase-1 concentration in serum could be a reliable assay.

Keywords: Caspase-1, serum caspase-1, cellular caspase-1

3227 P

A randomized study to compare topical 1% pimecrolimus cream and topical corticosteroid in the repigmentation of vitiligo

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Background: Vitiligo is an acquired disease resulting from loss of normalmelanin pigments in the skin. It can be considerable as a cosmetic and psychiatric problem with no preference for gender or race. Topical medications are one of the main therapeutic options.We performed a studyto evaluate the efficacy of the pimecrolimus and corticosteroid in the treatment of vitiligo.
Methods: Fourty-two patients with symmetrical lesions of vitiligo were included. 1% pimecrolimuscreamover the lesion on one side of thebody and 0.01% fluocinolone cream on the other was applied twice daily for three month.**Results:**Results indicate that both treatmentmodalities resulted in a comparable rate of repigmentation. Efficacy of treatment was different according to the anatomical location of treated lesions. Topical pimecrolimus except for head and neck area was as effective as fluocinolone to restore skin pigments in lesions. Overall efficacy of topical pimecrolimuswasbetter than fluocinoloneanddoes not develop any serious adverse effect. Therefore, topical 1% pimecrolimusmight be a favorable option for the long-term treatment of vitiligolesions.**Conclusion:**Further studies investigating the safety and efficacy of topical 1% pimecrolimus is needed in larger population. Moreover, evaluating its efficacy in combination with other treatments would be worthy.
Keywords: Vitiligo, Topical pimecrolimus, Topical corticosteroid, Repigmentation

3213 P

Investigating the involvment of microRNA-320 family in the pathogenesis of multiple sclerosis using EAE animal model

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Background: MicroRNAs have recently emerged as a new class of modulators of gene expression. miRNAs are small non-coding RNA molecules whichcontrol protein synthesis through translational repression or degradation of mRNA transcripts. Recent studies have shown that d sregulation of immunity-related miRNAs might contribute to autoimmune disorders such as multiple sclerosis. Based on these observations, we investigated the contribution of miR-320 and its target gene, HSP20, in the pathogenesis of multiple sclerosis using EAE animal model. **Methods:** EAE was induced in C57/BL6 mice, and CNS tissue was isolated from EAE and control mice at three different time points after disease induction. The levels of different inflammatory mediators such as TNF-a, IL-1a, IL-6, F4/80 and CD3e were measuredby real-time RT-PCR. The expression levels of two isoforms of miR-320, miR-320a and miR-320b, as well as HSP-20, a validated target of miR-320,were also quantified using real-time RT-PCR method.**Results:**The levels of proinflammatory cytokines as well as the CD3e lymphocyte marker and F4/80 monocytoid cell marker were significantly enhanced in acute and chronic phases of disease. Expression of one isoform of miR-320was significantly increased in different phases of EAE whereas the level of HSP-20 showed a decreasein the spinal

cords of mice in acute and chronic phases of disease. **Conclusions:** Our findings suggest that increased expression of miR-320 might be involved in the pathogenesis of multiple sclerosis through targeting and suppression of HSP-20, a protective gene in multiple sclerosis.
Key word: Multiple Sclerosis, miR-320, HSp-20

2988P
Serum Levels of IL-17A and IL-17F in Patients with Multiple Sclerosis

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Background: Multiple sclerosis (MS) is an inflammatory condition of the central nervous system, with genetic and environmental factors having a role in its etiology. The condition is characterized by demyelination, acute inflammation, and chronic and acute lesions in the central nervous system. Human and experimental studies have shown that T-helper cells and pro-inflammatory cytokines have a major role in the pathogenesis of MS. Recent researches have shown that IL-17 secreting T cells have a role in inflammation and demyelination of the central nervous system. In the present study, the role of IL-17A and IL-17F in the immunopathogenesis and follow-up of the MS diseasehas been evaluated. **Methods:** Thirty-five MS patients were included in the present study. The subjects were selected from the patients referring to the Neurology Research Center, Tabriz University of Medical Sciences. Blood samples were taken from 35 MS patients and 35 healthy individuals as controls. Enzyme-Linked Immunosorbent Assay (ELISA)was used to determine IL-17A and IL-17F serum levels. **Results:** A statistically significant increase was noted in the serum levels of IL-17A and IL-17F in MS patients compared to the controls ($P<0.05$); however, there was no significant relationship between the serum levels of these cytokines and Expanded Standard Disability Stated Scale (EDSS) and disease Progression Index (PI). **Conclusion:** The results of the present study confirm our previous findings indicated an increase in the expression of IL-17A, and IL-17F mRNA in MS patients compared to healthy individuals. The results of the present study might have a great role in pathophysiology, immunotherapy and follow-up of MS by indicating the role of these cytokines in the ever-increasing MS disease.
Keywords: Multiple Sclerosis, Th17, IL-17A, IL-17F

2910 P
Evaluation of Interleukin 12 in serum of the patients with Relapsing-Remitting Multiple Sclerosis and Behcet syndrome compared to healthy control in Isfahan Province

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Background: Multiple Sclerosis (MS) is a common disease of the central nervous system (CNS) in which the interplay between inflammatory and neurogenerative processes. It has been reported that cytokines such as IL12 play an important role in pathogenesis of multiple

sclerosis and Behcet by shifting the T-cell response to the Th1 type. Evaluation of interleukin 12 in serum of the patients with relapsing-remitting multiple sclerosis and behcet syndrome compared to healthy control. **Methods:** In a case-control study, venous blood was collected from MS patients (n=45), patients with behcet syndrome (n=10) and healthy subjects (n=35) as control group. All selected MS patients were clinically diagnosed as having relapsing remitting multiple sclerosis (RRMS). The plasma levels of the cytokine IL-12 were measured using ELISA method. **Results:** Elevated of IL-12 plasma level has been observed in patient with RRMS and Behcet syndrome in comparison with control group (*P* value<0.05). **Conclusion:** We found a highly significant increase of IL-12 plasma levels in MS patients and Behcet syndrome in comparison with the control group. The results suggested the involvement of IL-12 in immunopathogenesis of MS and Behcet especially in the acute phase of the diseases.

3067P

Molecular Analysis of Interleukin-25 Gene Polymorphisms and serum levels in Patients with Multiple Sclerosis

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Background: Multiple sclerosis (MS) is an inflammatory autoimmune disease of central nervous system. Over-expression of IL-17 has been shown in MS patients. IL-25 (IL-17E), as a member of IL-17 family, induces IL-13 expression and impedes Th17/IL-17 responses. In the present study potential polymorphisms of IL-25 gene, in exons 1 and 2, and its serum levels have been investigated in MS patients and control group; for identifying the role of IL-25 in autoimmune diseases, like MS. **Methods:** In this case-control study, blood samples were obtained from forty Relapsing-Remitting MS patients referring to the Neurology Research Center of Tabriz University of Medical Sciences, and forty controls. Serum levels of IL-25 were measured by ELISA method. DNA extracts amplified by Polymerase Chain Reaction (PCR). Two primer pairs were designed for two regions of IL-25, consisting exons 1 and 2. Sequence analysis of exons 1 and 2 of IL-25 gene, was the final step of evaluation. **Results:** The results of this study demonstrate significant reduce in IL-25 serum levels in MS patients compared to the controls ($p < 0.005$). Molecular analysis of IL-25 showed significant differences in polymorphisms of exon 2 between MS patients and control group. However, no significant differences were found in polymorphisms of exon 1. **Conclusion:** Current study demonstrated that serum levels of IL-25 are significantly lower in MS patients, compared to controls. Considering the role of IL-25 in suppression of IL-17A effects and active phase of Experimental Autoimmune Encephalomyelitis (EAE), *in vivo*, this cytokine seems to have therapeutic potentials for autoimmune diseases like MS. Investigating the role of IL-25 gene polymorphisms as a predisposing or preventive factor for MS disease would be useful in future studies with larger sample size.

Key word: Multiple Sclerosis (MS), gene polymorphism, IL-25, IL-17E

Transplantation & Stem cells

Oral Presentations:

1817O

Intravenous adipose-tissue mesenchymal stem cells injection alleviate experimental autoimmune encephalomyelitis (EAE) by affecting of IL17

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Background: Due to their immunomodulatory and anti-inflammatory competence, mesenchymal stem cells (MSCs) have been considered as a suitable candidate for treatment of autoimmune diseases. Earlier studies have shown that treatment with bone marrow-derived MSCs may modulate immune responses and reduce disease severity in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis.IL 17 has established to owning harmful effects in exaggerating of EAE. **Methods:** We induced EAE model in 15 C57BL/6 mice. We removed abdominal adipose tissue of healthy C57BL/6 mice, exclude their MSCs and cultured them. Then 20 days after immunization with MOG peptide, one groups of EAE mice were injected by 1×10^6 AT-MSCs intravenously. Then we evaluated the IL17 production, as a detrimental inflammatory cytokine in EAE, in splenic suspension 60 days after EAE induction in all mice by ELISA technique. **Results:** The level of IL17 and clinical scores of EAE was significantly decreased in AT-MSCs treated mice compared to PBS-treated EAE mice. **Conclusion:** Our data show that, due to their immunomodulative effects, and their role in shifting inflammatory cytokine profiles to anti-inflammatory profiles, AT-MSCs may be a proper candidate for stem cell based MS therapy.

Keywords: Experimental autoimmune encephalomyelitis(EAE), Mesenchymal stem cells (MSCs), IL 17, Intravenously

1813O

Intraperitoneal adipose-derived messenchymal stem cells administration abrogate experimental autoimmune encephalomyelitis (EAE) by up-regulating of splenic TCD4+CD8+FOXP3+ cells

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Background: Multiple sclerosis (MS) is a chronic autoimmune disease targeting CNS resulting in axonal pathology. Experimental autoimmune encephalomyelitis (EAE) is a proper model for MS. Current treatment for MS usually target immune system and ignore regeneration of neural system. Stem cell therapy recently has attracted attentions for this purpose. In this regard, mesenchymal stem cells (MSCs), multipotent cells with anti-inflammatory and immunomodulatory properties, demonstrating an attractive therapeutic tool for regenerative medicine. T regulatory has an important role in modification of immune responses. **Methods:** We induced EAE model in 15 C57BL/6 mice, removed abdominal adipose tissue of healthy C57BL/6 mice, exclude their MSCs and cultured them. Then 20 days after immunization with MOG peptide, one groups of EAE mice were injected by 1×10^6 AT-MSCs intraperitonealy.At finall we removed spleens in all groups and evaluate percentage of splenic TCD4+CD8+FOXP3+ cell population by flowcytometery test. **Results:**We found out percentage of splenic TCD4+CD8+FOXP3+ cells was significantly increased in AT-MSCs treated EAE mice compared to PBS treated EAE mice.**Conclusion:** We indicated that intraperitoneal adipose tissue MSCs injection improve course of EAE in C57BL/6 mice by modifying of T regulatory.

Keywords: Experimental autoimmune encephalomyelitis(EAE), Mesenchymal stem cells (MSCs), TCD4+CD8+FOXP3+ cells, T regulatory

1818 O

Evaluation of mRNA expression and promoter DNA methylation status of apoptosisrelated genes during ex vivo expansion ofcord blood CD34$^+$ cells

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Background: Ex-vivo expansion of cord blood (CB) CD34$^+$ cells has important applications in cell-based therapy. Several studies indicated that ex-vivo expansion of CB CD34$^+$cells in cytokine liquid culture condition induced cellular defects such as apoptosis. In this study, we evaluated the mRNA expression and promoter DNA methylation status of *BCL2, MCL1* andsurvivin genes in ex-vivo expanded CD34$^+$cells.**Methods:** CB CD34$^+$cells were cultured in cytokine supplemented liquid culture with and without mesenchymal stromal cells (MSCs) feeder layer. After 14 days culture,the resulting expanded cells were evaluated for TNC, CD34$^+$cells, CD34$^+$/CD38$^-$cells and CFU-C activity. Moreover, mRNA expression and promoter DNA methylation changes of *BCL2, MCL1* andsurvivin genes in expanded CD34$^+$cells were

analyzed by Real Time RT-PCR and MSP technique, respectively. **Results:** Expansion fold in TNC, $CD34^+$cells, $CD34^+/CD38^-$cells and CFU-C count was higher in co-culture condition than cytokine liquid culture condition. In cytokine liquid culture, the mRNA expression of *BCL2* and *MCL1* genes in expanded $CD34^+$cells decreased significantly, while the mRNA expression of survivin gene increased significantly. However, in co culture condition, the decrease in mRNA expression of *BCL2* and *MCL1* genes was minimal. Moreover, promoter DNA methylation status of *BCL2, MCL1* andsurvivin genes remained unmethylated during expansion in two culture conditions.**Conclusion:** Co culture of CB $CD34^+$ cells with MSCs improved expansion of CB $CD34^+$ cells while prevented transcriptional suppression of *BCL2* and *MCL1* genes in expanded $CD34^+$cells. Moreover, promoter DNA methylation was not involved in transcriptional repression of *BCL2* and *MCL1* genes inexpanded $CD34^+$cells.

Keywords: Cord blood, Ex vivo expansion, DNA methylation, Apoptosis, $CD34^+$ cells

2698O

Monitoring of Mesenchymal Stem Cell migration in STZ- diabetic mice by *in vivo* imaging system

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Background: To chase Mesenchymal Stem Cells (MSCs) for entry into injured tissues in autoimmune diseases, the technique of choice is non-invasive fluorescence-based *in vivo* imaging method. The aim of this study is labeling of MSCs with DiDa florescent lipophilic dye with high emission spectra to monitor their recruitment into damaged pancreas of STZ-diabetic mice. **Methods:** Bone marrow derived MSCs were prepared and characterized according to the standard technique. The cells labeled with 5mM of DiD. Diabetic mice were prepared by multiple low-dose intraperitoneal injection of 50 mg/kg body weight streptozotocin (STZ) to Balb/c mice for five consecutively days. One million DiD-labeled MSCs were injected intraperitoneally once or three times with 7 days interval into streptozotocin-induced diabetic Balb/c mice to check the suitability of DiD-labeling for *in vivo* imaging. **Results:** DiD integrated into the membrane of MSCs. *In vivo* imaging system (IVIS) revealed the presence of DiD emission spectra at anatomic site of pancreases of mice up to six week after last injection, although it decreased over time. Following each imaging procedure the existence of DiD signals in the pancreas of scarified animals were confirmed by IVIS. Evaluation of frozen pancreatic sections by fluorescent microscopy was also depicted the presence of DiD-labeled MSCs in the pancreas of treated diabetic mice. The presence of MSCs at injured site was accompanied with reduction of glucose levels in diabetic mice.**Conclusion:** The results showed that DiD-labeled MSCs can be traced by IVIS for assessment of successful cell engraftment over time at injured tissues.

Keywords: Mesenchymal Stem Cells, Tracking, *In vivo* imaging system, DiD, Diabetes.

2329O

MicroRNA expression as a biomarker for prediction of Acute Kidney Transplant Rejection

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Background: The microRNAs (miRNAs) are small noncoding RNA that regulate the expression of target genes in a post-transcriptional manner. Kidney transplantation treat end-stage renal disease. But acute rejection (AR) is still a strong risk factor for chronic rejection in recipients of renal. There are large amounts of evidence showing that miRNAs play critical roles in the modulation of innate and adaptive immune responses.**Methods:**Studies that have reviewed in this article, describe a comparison between miRNA expression profile by using human microarray panel representing and quantitative RT-PCR confirmation in kidney tissue rejection and peripheral blood mononuclear cells and urine samples of acute rejection and the controls. **Results:**The miRNAs differentially expressed samples from renal transplant recipients by comparing normal allograft with AR samples. Among the differentially expressed miRNAs, 21 were found to be downregulated while 13 were upregulated. MiRNA profiles were predictive of renal allograft function. The acute rejection samples showed increased expression six miRNAs were also confirmed by quantitative real-time PCR using an independent set of biopsies had elevated expression in peripheral blood mononuclear cells of patients.In urinary cell pellets miR-10b and miR-210 were downregulated while miR-10a was upregulated in patients with AR. **Conclusion:**During AR, elevated levels of this miRNA could be detected in renal transplant recipients with a specificity and sensitivity of 90 and 92%, respectively, for diagnosing AR. Thus miRNAs could potentially be used to noninvasively monitor patients for the diagnosis of AR and possibly onset as well as response to treatment.

Keywords: Acute rejection, Kidney transplantation, MicroRNA

1909 O

Co-transplantation of mouse pancreatic islets with human embryonic stem cell derived mesenchymal stem cells to ameliorate alloxan-induced diabetes in Nude mice

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Background: Pancreatic islet transplantation has emerged as a promising treatment for type I diabetes. However, its efficacy is severely hampered due to poor islet engraftment and revascularization, which have been resulted to partially loss of transplanted islets. It has been shown that mesenchymal stem cells have some paracrine effects, such as soluble factors or cell-cell signaling, which improve both engraftment and vascularization process of Neighboringtransplanted cells. In this study we investigated the paracrine effects of a new source ofmesenchymal stem cellswithincollagen-fibrin hydrogel on transplanted mouse islet function and revascularization. **Methods:** RH6 human embryonic stem cell derived mesenchymal stem cells (ES-MSCs) and mouse isolated islets have been co-transplanted through collagen-fibrin

hydrogel in the omental pouch of alloxan-induced diabetic nude mice. Thereafter the blood glucose, body weight, glucose tolerance and serum C-peptide was measured after 28 days. As control group, islets were transplanted alone with the same approach. **Results:** The results showed improved islet functionality and micro-vessel density, compared with control group. Blood glucose and body weight of co-transplantation group return to normal range and serum C-peptide and glucose tolerance test were closer to normal pattern, despite the control islet alone group.**Conclusion:** We conclude that co-transplantation of ES-MSCswith pancreatic isletscould enhance islet functionality and revascularization. This result can be used to improve the outcome of clinical islet transplantation.

Keywords: Pancreatic islets, Mesenchymal stem cells,Alloxan-induced diabetes

2386 O

Differentiation capacity of Mesenchymal Stem Cells (MSCs) to Neural Progenitor Cells (NPCs)and comparison immunosuppressive factors production between MSCs and NPCs .

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Background: Regarding the capability of MSCs for generation of different mesodermal cells, investigators have been demonstrated that MSCs differentiate to ectodermal lineage including neuronal precursor cells. They also have immunosuppressive activities.The aim of this study was to evaluate MSCs differentiation to NPCs and measurement of immunosuppressive factors in these two cell population. **Methods:** For differentiation of mice BM-MSCs towards NPCs, MSCs were cultured in supplemented Neurobasal media. To confirm differentiation of MSCs to neural progenitor cells, nestin expression, and decreased MSCs surface marker expression in NPCs and also the levels of PGE2 and IL-10 were evaluated. **Results:** NPCs showed feature ‘’neurosphere morphology ‘’with small spheres of floating cells in suspension. Nestin was upregulated 2-fold. NPCs showed a markedly lower expression of MSCs cell surface markers such as CD44 and Sca-1 compare to MSCs. NPCs also showed significantly higher PGE2 production compared to MSCs after 24- and 48-hours of culture in the same conditions, while IL-10 levels were not different between these two cells. **Conclusion:** This study showed that MSCs have the capacity to differentiate to NPCs. Moreovere, NPCs produced surprisingly more PGE2 compare to MSCs. To recapitulate, these findings can be useful in selection of the best cell therapy in MS.

Keywords: MSCs, NPCs

1997 O

Increase of IL-21 expression level in acute rejection experiencing liver transplant patients

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Background:IL-21 as a T cell-derived cytokine involved in innate and adaptive immune responses and also acts in support the developmentand maintenance of Th17 cells. Balanced acts of IL-21 in affecting the activated Tcells as well as guiding the evolution of regulatory Tcellsregulate several aspects of immune activationduring the solid organ allograft response. Therefore, in this study the expression pattern of IL-21was evaluated in liver transplant patient's experienced acute rejection. **Methods:**In this study, expression level of IL-21 gene was determined in different times (three 72 hours periods consecutively) early post-liver transplantation. Blood samples were collected from each 97 liver transplant patients and the expression level of IL-21 genewas evaluated by an in-house comparative Real-time PCR method and also by using Livak analysis method between acute rejected and non-acute rejected liver transplant patients.**Results and Conclusion:** The expression level of IL-21 was increased in different time periods in acute rejected compared with non-acute rejected liver transplant patients. Also this increase was significantly found in third 72 hour sampling time in acute rejected patients (p=0.007).Determination of significant increase of IL-21 in acute rejected patients emphasize on the important effect of IL-21 on induction and development of inflammatory responses in liver transplant patients experience acute rejection need to confirm in completed further studies.

Keywords: IL-21, Rejection, Liver, Transplantation

1483O

The Immunological Barriers to Regenerative Medicine

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Increase in incidence of non-communicable diseases, with a chronic or degenerative etiology, represents a significant challenge of the twenty-first century. Such changes in modern medical needs have created an intensive demand for new treatments capable of harnessing the properties of stem cells to replace diseased or non-functional cell types, or that rejuvenate tissues from within, through the activity of endogenous stem cells. While the routine derivation of human embryonic stem cells (hESC) has made pluripotency accessible in man for the first time, the immune response directed at stem cell-derived tissues to be a fundamental roadblock to progress. Although the early days of regenerative medicine were accompanied by speculative optimism that tissues differentiated from hESC might prove to be poorly immunogenic, it is now widely accepted that cell therapies pose no fewer immunological challenges than whole organ transplantation. Indeed, unlike conventional transplants, the propensity for tumorigenesis of pluripotent stem cells, suggests that long-term immune suppression is unlikely to offer a solution to rejection in this particular setting.Various approaches have been suggested to help lessen the risk of rejection including: genetic manipulation to reduce MHC-I and in-

creased FasL or serpin 6 expressions in graft cells, induction of hematopoietic tolerance, and personalized production of isogenic cell lines. The production of patient specific (isogenic) pluripotent cell lines have been done through somatic cell nuclear transfer, parthenogenesis, and induction of pluripotent stem (iPS) cells from somatic cells by delivery of transcription factors. Overall, these strategies may facilitate development of stem cells suitable for regeneration of many different tissue types.

2908 O

Upregulation of miR-223 in peripheral blood mononuclear cells from renal transplant patients with acute rejection

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Background: Acute rejection of renal allografts is a life-threatening complication and is associated with alterationsin the expression levels of proteinencoding genes.Predictive biomarkers can forecast acute rejection before it occur. Because microRNAs (miRNAs) play a pivotal role in manybiological processes, as well as regulate the expression of genes implicated in adaptive immunity, we investigatedwhether acute rejection (AR) is associated with changes in miR-223expression within peripheral blood mononuclear cells (PBMCs) and whether its expression levels could be predict acute rejection of renal allografts.**Methods:**We studied expression levels of miR-223 in PBMCsfrom subjects with acute rejection (N=14) as well as subjects with normal biopsy results as controls (N=16). Levels of miR-223 were measured in subjects with AR as well as using TaqMan MicroRNA Assays that allow absolute quantification of miRNAs. We examined associations of miR-223 with acute rejection. **Results:**In our study, expression levels of miR-223increased in PBMCs of the patients with acute renal allograft rejection compared with patients with normal biopsy results.We found a significantassociation between expression of miR-223 and AR, and also renal allograft rejection, could be predicted with a high level of precision by measurement of levels of miR-223 within PBMCs.**Conclusion:**Measurement of miR-223 in PBMCs may serve as a biomarker of acute rejection of renal transplants.

Keywords: MicroRNA, Renal Transplantation, Acute Rejection, Biomarker

2824 O

Matched donors in patients' relatives other than siblings

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Background: The most available matched donors are patients' siblings. In western populations, about 70% of patients have no matched sibling donor and they should rely on unrelated donors and cord blood unites.There is high frequency of consanguineous marriagesin Iran.

This phenomenon increases the chance of finding matched related donors other than siblings. **Methods**: During November 2006to January 2014the patients referred to Hematology, Oncology, and Stem Cell Transplantation Research Center; ShariatiHospital for allogeneic stem cell transplantation and had no matched sibling donor were studied for the possibility of finding other related donors.The parents and second degree relatives (uncles, aunts, grandparents) of patients who were progeny of consanguineous marriages were HLA typed. In addition the children of any mother's relatives who had marriage with a father's relatives were studied. HLA typing was performed in A and B loci. The patients and phenotypically A and B matched donors were typed in five loci (A, B, C, DRB1, and DQB1) at low resolution level and high resolution typing performed for 10/10 matched patient-donor pairs to confirm genotypic matching. **Results:** 150 matched donors were found: 82 parents (54.6%), 33 uncles and aunts (22%), 17 grandparents (11.3%); the rest had diverse relations with the respective patients (5 cousins, 5 nephew and niece, 2 offsprings …_up to 5th degree relatives).**Conclusion:** Matched other related donors are a precious available source of stem cell donors in Iran that should be studied before unrelated donor search.

Keyword: Stem cell, Other related donors.

2447 O

Cyclosporine and mycophenolatemofetil effects on the percent of natural regulatory T cells and gene expression of FOXP3 in renal transplant recipients

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Background: Regulatory T cells (Tregs) have been suggested to be deeply associated with immune tolerance and long-term graft survival in transplantation. Immunosuppressant drugs provided the opportunity to efficiently suppress the immune responses of the recipient to the kidney donor. However, long-term treatment with these agents is associated with side effects that significantly alter regulatory T cell population and lessen the life quality and affect graft and patient survival. The purpose of this study was the survey of cyclosporine A and mycophenolatemofetil effects on the percent of CD4+CD25+FOXP3+ Treg and gene expression of FOXP3 in renal transplant recipients.**Methods:** The percent of CD4+CD25+FOXP3+ peripheral regulatory T-cells and FOXP3gene expression of thirty renal transplant recipients was studied by flow cytometry and Real time PCR, before and 6 months after transplantation. Patients were receiving cyclosporine A and mycophenolatemofetil after transplantation. **Results:** Percent of CD4+CD25+FOXP3+ Tregswas significantly decreased in the peripheral blood of female renal transplant patients 6 months after transplantation compared to before transplantation ($P = 0.032$), while there wasn't any significant difference in the FOXP3 gene expression ($P = 0.218$). Percent of CD4+CD25+FOXP3+ Tregs represented positive correlation with FOXP3transcript expression in patients before transplantation ($rs = 0.634$, $P < 0.001$), but wasn't seen any significant correlation 6 months after transplantation ($rs = 0.062$,

P = 0.725).**Conclusion:** Immunosuppression alters T cell population and induces a decrease of circulating Tregs in renal transplant recipients especially in females. Nevertheless, the FOXP3 gene expression doesn't predict the percent and frequency of CD4+CD25+FOXP3+ Tregs in these patients.

Keywords: Transplantation, Gene expression, FOXP3, Regulatory T cells

2127 O

Interleukin 35 Gene Transfer to Humanumbilical-cord-derivedmesenchymalstemcells Using Lentiviral Vectors

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Background: Human umbilical-cord-derived mesenchymal stem cells (hUC-MSCs) are easy available cells compare to bone-marrow-derived MSCs for prospective clinical applications. Human UC-MSCs are easier to isolate and expand without having the rejection reaction problems and thus ethical concerns.They showed immunosuppressive properties.They also have therapeutic effects in several different preclinical animal models of Parkinson's disease, type 1 diabetes, spinal cord injury, retinal disease and cerebral global ischemia. HUC-MSCs may be able to play an important role in autoimmune disorders.IL-35 induces proliferation of regulatory T cell (Treg cells) populations but reduces activity of Th17 cell and Th1 cell populations. A recent study identified IL-35 as a new inhibitory cytokine required for the suppressive function of Treg cells. Methods: We isolated hUC-MSCs with explants culture method.Interleukin 35-harbouring lentiviral particles were produced in HEK-293T cells by transient co-transfection of three-plasmid expression system. Aftercollecting andconcentratingthe virus's preperations, hUC-MSCs transducted at MOI = 50 in the presence of 8 µg/ml Polybrene. Total RNA was isolated from the transduced MSCs to detect IL-35 expression by real-time PCR. Expression of IL-35 inhUC-MSCswere quantified by an IL-35 ELISA kit. IL-35 bioactivity analyzed by inhibiting mouse Splenocytes proliferation using CFSE Cell Proliferation Kit.Results: There was an up to 80 % GFP positive transduction rate and our cells successfully released IL-35 in culture media.Conclusion: Our data suggest that transplanted hUC-MSCs overexpressing IL-35 may provide useful tool for basic research on gene therapy for autoimmune disorders.

Keywords: Human umbilical-cord-derived mesenchymal stem cells, IL-35, Lentiviral transduction.

2207 O

The effect of miR-375 in producing Islet Like Cell Clusters

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Background: Development of renewable sources of islet-replacement tissue for treatment of type I diabetes mellitus is an interesting worldwide issue. Placental tissue derived mesenchymal stem cells (MSCs) are a great promising source for regenerative medicine due to their plasticity, and easy availability. They have the potential to differentiate into insulin producing cells. MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression through post-transcriptional mechanisms. The miRNAs play a central role in control of many biological activities. The miR-375 is expressed in the pancreas and is involved in islet development. **Methods:** Human placental decidua basal is **(**PDB-MSCs**)** cells were cultured from full term human placenta. The MSCs (P3) were chemically transfected with hsa-miR-375. Total RNA was extracted on the fourth and seventh day after transfection. The expressions of insulin, NGN3, GLUT2, PAX4, PAX6, KIR6.2, NKX6.1, PDX1, Glucagon genes were evaluated by real- time qPCR. On the seventh day, the potency of the clusters in response to high glucose challenge was tested. **Results:** Morphological changes were followed from the second day, and during the sixth day cell clusters were formed. Insulin producing clusters showed a deep red color with DTZ. The expression of pancreatic specific transcription factorswere remarkably increased during the four days after transfection and significantly increased on the seventh day. In response to different glucose concentration (2.8 mM and 16.7mM) the C- peptide and insulin secretion were increased. **Conclusion**: In conclusion, the MSCs could be programmed into functional insulin producing cells by transfection of miR-375.

Keywords: Pancreas, Mir375, MicroRNA, Insulin

2126 O

Lentivirus-modified human umbilical cord mesenchymal stem cells maintain their immunomodulatory property

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Background: Transduction of MSCs (mesenchymal stem cells) by genetically engineered lentiviral particles has recently been shown to be a highly efficient method for gene delivery. Human umbilical-cord-derived mesenchymal stem cells (hUC-MSCs) are easy available cells compare to bone-marrow-derived MSCs for potential clinical applications. HumanUC-MSCs are easier to isolate and expand without having the rejection reaction problems and thus ethical concerns.They showed immunosuppressive properties.It was unclear whether

the UC-MSCs would retain their immunomodulatory characteristics after LV (lentivirus)-mediated gene transductionor not. **Methods**: We isolated hUC-MSCs with Explants culture method.Recombinant lentiviral particles were produced in HEK-293T cells by transient co-transfection of three-plasmid expression system. Aftercollecting andconcentratingthe virus's preparations, hUC-MSCs transducted at MOI = 50 in the presence of 8 µg/ml Polybrene. Transducted and untransducted hUC-MSCs surface molecules such as CD31, CD43, CD45, MHCII, MHCI, CD40, CD80, CD86, CD90, CD117and CD73 were determined using FACS analysis system. Genomic DNA was isolated from the transduced and untransducted MSCs to detect IL-10, HGF, VEGF, HLA-G expression by real-time PCR. Cell Proliferation assay analyzed by CFSE Cell Proliferation Kit.**Results**:There was no difference between the transduced and untransducted hUC-MSCssurface markers and IL-10, HGF, VEGF, HLA-G gene expression. Both of transduced and untransducted hUC-MSCs inhibited proliferation of human PBMC. **Conclusion**: These findings suggest that lentiviral transduction may be used to deliver therapeutic genes to human UC-MSCs and this may have a wide range of applications in gene therapy.

Keywords: Human umbilical-cord-derived mesenchymal stem cells, Lentiviral transduction, Immunomodulatory property.

2798 O

Longitudinal analysis of alloantigen-specific Tregs in skin transplanted animals revealed the relevance of antigen-specificity.

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Background: Regulatory T cells (Tregs) were identified several years ago and are key in controlling autoimmune diseases and limiting immune responses to foreign antigens. These cells have been used successfully in animal models first and more recently in the clinic to prevent Graft vs Host disease and transplant rejections. However, their locations in vivo, their migratory abilities and their in vivo survival have not been extensively investigated. **Methods:** Imaging of the human sodium/iodide symporter via Single Photon Emission Computed Tomography (SPECT) has been used as a reporter gene to image various cell types in vivo. It has several advantages over other imaging techniques including high sensitivity, it allows non-invasive whole body studies of viable cell migration and localisation over time and lastly it may offer the possibility to be translated to the clinic. **Results:** Treg lines derived from CD4+CD25+FoxP3+ cells were retrovirally transduced with a construct encoding for the human Sodium Iodide Symporter (NIS). NIS expressing Tregs were specifically radiolabelled in vitro with Technetium-99m pertechnetate (99mTcO4-). Later, Treg lines with direct/indirect alloantigen-specificity were imaged in skin transplant models using the SPECT. It was observed that adoptively transferred Tregs migrate to the site of transplant at early time points and then migrated to various lymph nodes. **Conclusions:** The data presented here suggests that SPECT/CT imaging can be utilised in preclinical imaging studies of adoptively transferred Tregs without affecting Treg function and viability thereby allowing longitudinal studies within disease models. Moreover, new insight into pattern of migration of Tregs was

identified.

Keywords: Regulatory T cells, Immunotherapy, Transplantation tolerance, T cell imaging, whole body imaging, non-invasive imaging, SPECT/CT, Adoptive transfer therapy.

2018 O

Generation of insulin-producing cells from adult stem cells by lentiviral PDX1

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Background: Type 1 diabetes mellitus, an autoimmune disorder is an attractive candidate for gene and cell based therapy. β-cell replacement is effective approaches for treatment of type 1 diabetes. The adult adipose tissue- derived stem cells (hASCs) offered as an attractive source for generation of surrogate β-cell. Pancreatic duodenal homeobox 1 (PDX1) is a transcription factor that plays key roles in β-cell gene expression. This study investigated whether human adipose tissue –derived stem cells (hAMSCs) could be transdifferentiated into insulin- producing cells (IPCs) *in vitro* by lentiviral PDX1.**Methods:** Characteristics of β-cell were evaluated with immunocytochemistry, Dithizone- staining and quantitative reverse transcription polymerase chain reaction (RT-PCR). Insulin release in response to glucose challenge was detected with chemiluminescence enzyme immunoassay (CLIA).**Results:** Results demonstrated that PDX1 gene was expressed in hAMSCs and induced transdifferentiation of these cells toward the β-cells. Islet like cell cluster appeared about 10 days after transduction. Introduction of PDX1 induced its own expression (auto- induction), number of islet related gene like Ngn3 and Nkx2-2 and β-cell function like Insulin. The insulin positive cells were detected in the induced cells. The induced cells secreted insulin hormone and very responsive to glucose challenge. **Conclusion:** These findings, suggest a therapeutic potential for human PDX1-expressing AMSCs in β-cell replacement in patients with type 1 diabetes.

Keywords: PDX1, Mesenchymal Stem Cells, Insulin, β-cell.

3056 O

Impact of toll like receptor 2 and 4 mRNA expression in renal allograft outcome

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Background:Toll-like receptors play a critical role in determining allograft outcome. TLR-2 and TLR-4 are frequently expressed on renal cells and they expression is up regulated following renal ischemia reperfusion and episodes of rejection. TLRs recognize both exogenous microbial components and endogenous molecules to promote immune response activation. **Methods**: TLR-2 and TLR-4 expression was analysed in 60 human renal allograft biopsies with commercial realtimetaqman primer probe sets. From 60 samples 25 was acute rejection, 33 was chronic rejection and 2 was stable graft function.**Results:** mRNA level of TLR2 was significantly higher in acute rejection while TLR-4 mRNA level was decreased. mRNAlevel

of TLR-4 was significantly upregulated in chronic rejection. mRNA level of TLR4 were highly accurate in distinguishing AR from CR. TLR mRNA levels correlated to inflammatory parameters according to the Banff '07 classification and to cellular influx. **Conclusion**: The elevated mRNA levelof TLR 2 inrenal transplant biopsies of patients with acute rejection indicatea pro-inflammatory state, which may contribute touncontrolled inflammation.TLR4 is markedly up-regulated in human chronic renal allograft rejection. Ourdata suggest a role for TLR2& 4 during allogen-dependentgraft damage after renal transplantation.

Keyword: Transplantation, Toll like receptor 2, Toll like receptor 4, Acute rejection, Chronic rejection.

1832O

The Effects of Human Bone Marrow-Derived Mesenchaymel Stem Cell on Acute Pancreatitis in Mice

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Background: Acute pancreatitis AP) is a common disease characterized by local pancreatic necrosis as well as systemic organ failure. So far, there are only few treatment options for patients with AP. many studies have shown that bone marrow-derived mesenchaymel stem cell(BM-MSCs) have potential of differentiation into many different cell types and are known as immunomodulatory cells.in various conditions. We aimed to investigate the anti-inflammatory of these cells in cerulean-induced AP in mice. **Method:** we induced AP in C57BL/6 mice by intraperitoneally administration of cerulean (100ug/kg/h×7dose) at intervals of 1hour. BM-MSCs was isolated from human bone marrow and characterized. 6h after the last cerulean injection, $2x10^5$ BM-MSC were in injected in the AP mice through tail vein. Mice were sacrificed at 12h after injection of cells, and blood samples were obtained by direct intracardiac puncture. Pancreas was removed immediately and used for pathological analysis. **Results**: we observed BM-MSCs express all markers of MSC cells such as CD27, CD105, but do not the markers of hematopoietic stem cells (CD45 and CD34). The AP mice showed elevated levels of Amylase and Lipase as well as histological alternations. Interestingly, when treated the AP mice with BM-MSC, we observed that the levels of Amylase and lipase were significantly reduced. In addition, BM-MSC markedly attenuated cerulein-induced histopathological alternations and water contents. **Conclusion**: Cell therapy using BM-MSC could be a suitable approach for treatment of AP. However, further investigation employing more animal models is required.

Poster Presentations:

2140 P

Adipose-derived mesenchymal stem cells inhibit anti-islet proliferation of C57BL/6 diabetic mouse splenocytes and protect beta-cell function

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Background: Type one diabetes (T1D) is a T-cell mediated autoimmune disorder in which pancreas beta-cell destruction causes insulin deficiency and hyperglycemia. In addition to daily insulin treatment, allogeneic islet transplant inT1D is another therapeutic way that needs immunosuppressive drugs to control autoimmunity and graft rejection. Since life-long application of these drugs is associated with serious side-effects, we proposed local immunomodulatory effects of mesenchymal stem cells (MSCs). The aim of this study was to investigate the influence of adipose-derived MSCs on anti-islet C57BL/6 diabetic mouse splenocytes proliferation and protection of pancreatic islet cell function. **Methods:** MSCs were extracted from abdominal fat tissue of healthy C57BL/6 mouse and cultured to proliferate. Identity of MSCs was verified by immunophynotyping and trans-differentiation protocols. Diabetes induced by sterptozotocin in C57BL/6 mice and model was confirmed. Pancreatic islets were isolated from healthy mouse and splenocytes prepared from healthy and diabetic mice.MSCs were co-cultured with diabetic splenocytes in the presence of islet lysate and proliferation assayed by MTT technique. The impact of MSCs on pancreatic islet cell function assayed by insulin mesuerment in direct co-cultures of diabetic splenocytes with normal singled-cell islets.**Results:** Our results indicated that MSCs significantly decreased diabetic splenocytes proliferation when stimulated with islet cell lysate ($P<0.05$). Moreover, MSCs in direct co-culture experiments increased insulin content secreted by islet cells ($P<0.05$).**Conclusion:** These results suggest that MSCs exert anti-proliferative effect on autoreactive splenocytes and present some protective factors to islet cells. These findings would be important in allogeneic islet transplantation and clinical practices.

Keywords: Islet of pancreas, Immunomodulation, Mesenchymal stem cells, Type one autoimmune diabetes

1816P

The Effect of silymarin on mesenchymal stem cells proliferation by affecting endogenous IL10 secretion in vitro

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Background: Mesenchymal stem cells (MSCs),a subset of adult stem cells, can differentiate in vitro into multiple cell lineages .MSCs due to anti-inflammatory and regenerative properties are more susceptible for regenerative medicine than other stem cells. Considering the little amount of MSCs in bone marrow, to maximize the clinical potential of MSCs, expansion of MSC invitro is essential to therapeutically applications. Silymarin,a mixture of flavonolignans, has proved to owning cytoprotective and anti-inflammatory properties that can act on cytokine production. IL10 is an inhibitory cytokines secreted by MSCs that can affect cell proliferation. **Methods:** We measure MSCs proliferation following exposure of MSCs to 50, 75 and 100 µg/ml silymarin in a 14 day period by MTT test and we also evaluate variations in the IL10 secretion in conditioned medium of MSC by ELISA test in different doses of silymarin. **Results:** We observe that in the cells treated with 50 and 1ooµg/ml of silymarin, secretion of IL10 by MSC was significantly increased while MSC proliferation was decreased in all days but in dose 75 µg/ml the level of IL10 was decreased and MSC proliferation was increased over time. **Conclusion:** Silymarin by decreasing secretion of IL10 in MSC culture in dose 75 µg/ml can increase MSC proliferation.

Keywords: Silymarin, Mesenchymal stem cells (MSCs), Cytokine, IL10

1467P

Immunomodulatory effects of adipose derived mesenchymal stem cells on gene expression of major transcription factors in T cell subsets

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Background: Based On the immunomodulatory properties of mesenchymal stem cells(MSCs), it has been proposed that these properties of MSCs play a crucial role in establishment and leading of T lymphocytes, especially Th cell subsets, toward different functional subsets. **Methods:** In order to find the effect of immunomodulatory and regulatory function of adipose derived-MSCs(AD-MSCs) on C57BL/6 spleen isolated mononuclear cells (Spleen-MNCs), gene expression of well-known effector and regulatory Th cells-related transcription factors i.e. t-bet, GATA-3, Ror-γt, Foxp3 and their related cytokines, i.e. IFN-γ for Th1 cells, IL-4 for Th2 cells and IL-17 for Th17 cells and IL-10, TGF-β for regulatory T cells respectively, were studied under the co-culture condition system. Proliferation index of Spleen-MNCs were analyzed by cell proliferation assay kit using CFSE staining method. **Results:** Our findings have indicated that the AD-MSCs have a great impact on the up-regulation of immunomodulatory cytokines

such as TGF-β ($p<0.005$), down-regulation of inflammatory cytokines IFN-γ ($p<0.005$), and transcription factors such as t-bet ($p<0.005$).**Conclusion:** Considering the immunomodulatory effects of MSCs in the differentiation of Th cell subsets, understanding and harnessing this property of MSCs could be a powerful strategy in the treatment of inflammatory autoimmune diseases such as multiple sclerosis.

Keywords: AD-MSCs, Immunomodulation, Cell subsets, Autoimmunity

1862P

The Comparison of Immunoregulatory properties of Stem cells from human exfoliated deciduous teeth and Bone Marrow-derived Mesenchymal Stem Cells.

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Background: Mesenchymal stem cells (MSCs) have remarkable capacities. Stem cells from human exfoliated deciduous teeth (SHED) are introduced recently and possess characteristics similar to MSCs. Because of their convenient accessibility, safety of harvest, being ethically uncontroversial, SHED can be a preferable source for the ever-increasing MSCs application in experimental and preclinical settings. While they are new, compared to other MSC populations, their immunoproperties have not been studied yet, as much as necessary. In this study, we explored the effect of SHED on T lymphocytes as the chief executives of the immune response and compared to conventional MSCs (BMMSCs).**Methods:** The isolated T lymphocytes were activated both specifically (by allogenic PBMCs) and nonspecifically (by phytohemagglutinin (PHA)) in vitro and cocultured with SHED or BMMSCs under same conditions, subsequently their proliferation and cytokine secretion (two indicator of T cell activation) were measured. **Results:** In our experiment, BMMSCs and also SHEDcould inhibit the proliferation and cytokine production of both PHA and alloantigen stimulated T lymphocytes in a dose-dependent manner. Although the inhibition decreased by the separation of lymphocytes and MSCs by a semipermeable membrane, but it was not abolished. **Conclusion:** This study showed that SHED can suppress the activation of human T lymphocytes in vitro like other MSCs. However, compared to BMMSCs, this suppression was distinctly alleviated. Moreover, in the equal condition the pattern of immune-modulation of BMMSCs and SHED was different, suggesting that SHED do not exert the exact mechanisms of BMMSCs' immunosuppression. However, this finding should be verified by further studies conducted to identify the detailed mechanisms responsible for the immunomodulation of SHED and also BMMSCs.

Keywords: Immunoregulatory, SHED, BMMSC, T lymphocyte.

2495P

Characterizationof cancer stem like cell in HT-29 colonic adenocarcinoma cell line

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Background: Tumors contain a small population of cancer stem cells (CSC) proposed to be responsible for tumor maintenance andrelapse. Aldehyde dehydrogenase 1 (ALDH1) activity has been used as a functional stem cell marker to isolate CSCs indifferent cancer types including colorectal cancer. Our aim was todetermine the utility of ALDH1 activity along with CD44 and EPCAM in identifying stem cell –like cells in human HT-29colonicadenocarcinoma cell line. **Methods:** HT-29 cell line was cultured in serum-free medium containing growth factors for sphere formation. q-RT PCR was performed to evaluate stemnessgenes in parental and spheroid cells .Colon cancer stem cell markers include CD44,EPCAM and ALDH1 were analysed by flowcytometry in both parent and sphere populations. In order to further evaluate the tumorigenic property of spheroid cells, *in vivo* tumorigenicity was performed in nude mice. **Results:** Our results demonstrated that~ 96% of spheroid cells were positive forCD44/EpCAM,While 37% of parent cells were positive for CD44/EpCAM. Controversly, we found that ALDH activity was at least 2-fold higher in the parental cells as compared to spheroid cells.RT-PCR data showed an increased expression of "stemness" genes like C-Myc, Oct4, Nanog, Klf4, Sox2 in colonospheres than parental cells.Additionally,we showed that as few as 2500 spheroid cells were sufficient to obtain tumor growth, whereas $1x10^6$ of parental cells capable to form tumor. **Conclusion:** These finding reveal that colonospheres formed by HT-29 are highly enriched in CSCs and ALDH1 activitydoes notappear to identify cancer stem cells population in HT-29 cell line, however, colonosphere with low ALDH1 activity indicated increased tumorigenic potential and stemness properties.

Keywords: Colorectal cancer, Cancer stem cell, HT-29, ALDH

2753P

Neural induction β-Mercaptoethanolon synovium-derived mesenchymal stem cell in vitro

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Background: Because of the unique attributes of plasticity and accessibility of mesenchymal stem cells (MSCs), it seems they are promising candidates for cell-based therapy of degenerative diseases. Stem cells have great potential as therapeutic instruments in the treatment of neurological diseases as diverse as multiple sclerosis, stroke, spinal cord injuries, etc… MSCs derived from different sources but superiority of synovium as a cell source has reported in previous studies. **Methods:** We isolated Synovium mesenchymal stromal cells (SMSCs) from knee joint of baker cyst and ACL(Anterior cruciate ligament) patient.We used β-Mercaptoethanol(BME) to induce SMSCs to the neural cells. Exposure of SMSCs for 24 hours to BME were done, then Mesenchymal cell were amplified with FBS and DMEM in

vitro. Alizarin red and Oil red O staining were done to investigate stemness property of cells. RT-PCR was done to prove neural gene expression. **Results:** In this study, we analyzed the expression of neuralgenes by SMSCs in vitro and demonstrated the proliferation ability and multi lineage maintenance of Synovium- derived mesenchymal stem cells in vitro.We detected the presence of lipid vacuoles accumulation and calcium deposits, by Oilred O and alizarin red staining also We did reverse transcription polymerase chain reaction (RT-PCR) analysis and showed that mRNA levels encoding for Glypican-4(GPC4)and Neurogenic differentiation 1(NeuroD1), were increased in induced SMSCs. **Conclusion:** According to our finding and pervious research results,further investigation are necessary to know synovium tissue position as a different potential source for clinical application.

Keywords: Synoviummesenchymal stromal cells (SMSCs), Differentiation, Neural Cells

2042P

Intrathecal mesenchymal stem cell therapy in Multiple Sclerosis: a follow-up study for five years after injection

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Background: Mesenchymal stem cell therapy has been used in multiple sclerosis (MS) in order to modulate the course of the disease in previous studies. One of the major concerns in such cases is long term safety or efficacy of this type of therapy.This study was conducted to report the clinical status of five patients with secondary progressive multiple sclerosis and one patient with neuromyelitis optica, five years after an autologous intrathecal mesenchymal stem cell (MSC) injection. **Methods:** The patients (three male, three female) had a progressive course nonresponsive to the conventional immunomodulatory treatments with Expanded Disability Status Scale (EDSS) score of 3.5 to 6. They received the MCSs after discontinuingother treatments. They were examined annually to assess the disease activity and possible complications. **Results:** Two patients had no change in their EDSS scores. One was diagnosed to have Devic's disease decreased one score in the EDSS,but experienced four relapses during these five years. Three patients had an increase in EDSS scores by 1-2 scores after five years. Two experienced relapses after injection. There was no significant adverse reaction, infection, or neoplasm during this period of follow up. **Conclusion:** Intrathecal mesenchymal stem cell therapy for MS is generally safe and did not result in any adverse reaction like malignancy for a relatively long period of time. At least half of the patients had no change in their EDSS and the remaining patients had a delay in disease progress.

Keywords: Multiple Sclerosis, Stem Cells, Mesenchymal Stem Cell Transplantation, Tissue Therapy

2044P

Human placental mesenchymal stem cells (hPMSCs) effects on gene expression profile of cytokines in peripheral blood mononuclear cells (PBMNCs)

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Background: Immunosuppressive and multipotent differentiative capacity of bone marrow derived mesenchymal stem cells (BM-MSCs) are being exanimate in numerous studies. Although BM is a major source of MSCs, however differentiation capacity of these cells decline with aging and procedure of collecting BM is highly invasive. Therefore an alternative source of MSCs is required. **Method:** In this study we tried to isolate cells with MSC properties from human placenta by enzymatic digestion. Flowcytometery analysis has done for evaluation MSCs cell surface markers. Also the effect of irradiated hPMSCs on the growth pattern of PBMNCs was examined at co-culture system. Finally evaluation of gene expression profile of PBMNCs cytokines, including IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, TNF-α and TGF-β was done by real time-PCR technique. **Results:** Flowcytometery results showed that hPMSCs express CD90, CD105, CD106, HLA-G and CD349L markers. Butin the case of CD73 marker, maybe there are two different populations in placental MSCs. Furthermore assessment of irradiated hPMSCs effect on PBMNCs growth patterndemonstrated a dose dependent inhibitory effect of hPMSCs on PBMNCs growth. Finally, the results of gene expression profile of PBMNCs cytokines showed that hPMSCs, caused the PBMNCs to decrease cytokines gene expression including IL-2, IL-6, IL-12, IFN-γ and TNF-α and caused to increase gene expression of other cytokines including TGF-β and IL-10. But the results of changes in gene expression of IL-4, IL-5 and IL13 were not reportable. **Conclusion:** In co-culture system, direct cell-cell interaction of PMSCs and PBMNCs, led to cytokine gene expression profile progressed to the production of anti-inflammatory cytokines.

Keywords: Stem cell, Mesenchymal stem cell, Placenta, Immunomodulation, cell therapy

2267P

The Effect of TNF-α in Differential Gene Expression Pattern of CXCR4 on Human Marrow-Derived Mesenchymal Stem Cells

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Introduction: Cell therapy and tissue repair are used in a variety of diseases including tissue and organ transplantation, autoimmune diseases and cancers. Now mesenchymal stem cells (MSCs) are an attractive and promising source for cell-based therapy according to their individual characteristics. Soluble factors which are able to induce MSCs migration have a vital role in cell engraftment and tissue regeneration. Tumor necrosis factor α (TNF-α) is a major cytokine present in damaged tissues. **Methods:** We have investigated the pattern of gene expression of chemokine receptor CXCR4 in nine groups of human bone marrow-derived MSCs stimulated with TNF-α in different dose and time manner. **Results:** Comparison of TNF-α treated with untreated MSCs revealed the highest expression level of CXCR4 after

treatment with 1 ng/ml, and 10 ng/ml of TNF-α in 24 hours, and the production of CXCR4 mRNA was regulated up to 216 and 512 fold, respectively. Our results demonstrated the differential gene expression pattern of chemokine receptor CXCR4 in human marrow-derived MSCs stimulated with inflammatory cytokine TNF-α. **Conclusion:** These findings suggest that *in vitro* control of both dose and time factors may be important in stem cell migration capacity, and perhaps in future-stem cell transplantation therapies.

Keywords: Mesenchymal stem cell, Chemokine receptor CXCR4, Tumor necrosis factor α, Gene expression pattern, Real Time PCR

2269P

The Immunoregulatory Effects of Human Marrow-Derived Mesenchymal Stem Cells

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Background: The ability of mesenchymal stem cells (MSCs) to differentiate into many cell types, as well as their high ex vivo expansion potential, makes these cells an attractive therapeutic tool for cell transplantation and tissue engineering. Due to the fact that MSCs could reduce the incidence severity of graft versus host disease, we have investigated the immunologic properties of human marrow-derived MSCs.**Methods:** Bone marrow were obtained from healthy human donors of bone marrow to a related patient at Bone Marrow Transplantation Center, Nemazi Hospital, after obtaining approval of the Ethics Committee and Written informed consent. The Mononuclear cells derived over the Ficoll-Paque density-gradient, and plated in tissue cultures dish. The adherent cells expanded rapidly and maintained with periodic passages until a relatively homogeneous population was established. The MSCs were characterized by immunophenotyping and differentiation into osteoblast and adipocytes. Alloreactivity was studied after adding the MSCs to allogeneic lymphocytes in mixed lymphocyte reaction cultures. **Results:** Flow cytometric analysis, and the differentiation potential into osteoblast and adipocytes showed that more than 90% of human MSCs were positive by specific markers and functional tests. Indeed, The MSCs expressed CD90, and CD73. But not CD80, CD40, and HLA class II. They also were negative for the hematopoietic markers CD34, and CD45. The MSCs do not induced proliferation of allogenic lymphocytes and suppressed them. **Conclusion:** The human marrow-derived MSCs do not elicit alloreactive lymphocyte proliferation. These results suggest that these cells have potentials for allogenic transplantation.

Keywords: Mesenchymal stem cells, Immunoregulatory effects, Bone marrow

1767P

Effect of *Helicobacter pylori* infection on stromal-derived factor-1/CXCR4 axis in bone marrow-derived mesenchymal stem cells

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Background: Recent studies have demonstrated that during chronic *Helicobacter* pylori*(H. pylori)* infection bone marrow-derived-mesenchymal stem cells (BMD-MSCs) migrate to the gastric tissue and could be also the origin of gastric adenocarcinoma. The chemokine CXCR4 through binding to its ligand stromal-derived factor (SDF-1) plays a crucial role in migration of inflammatory and stem cells. However, the possible effect of *H. pylori* infection on the SDF-1/CXCR4 axis has not yet been elucidated. **Methods:** Gastric epithelial cell line, AGS, and BMD-MSCs were cocultured with *H. pylori* for 24 h. The expression of CXCR4 was examined in BMD-MSCs by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and flow cytometry, and SDF-1 expression in AGS cells was detected by qRT-PCR and enzyme-linked immunosorbent assay. Further, migration of BMD-MSCs toward SDF-1 was evaluated by chemotaxis assay. **Results:** We found that coculture of *H. pylori* with BMD-MSCs or AGS: (i) enhanced CXCR4 expression on the cell surface of BMD-MSCs and (ii) increased SDF-1 secretion by AGS cells. Consistently, we observed that *H. pylori*-treated BMD-MSCs showed a higher capability to migrate toward SDF-1 gradient compared with untreated cells. **Conclusion:** We found that *H. pylori* upregulates CXCR4 expression in BMD-MSCs and enhance their migration toward SDF-1. This study provides the first evidence that *H. pylori* infection may enhance BMD-MSC migration through acting on the SDF-1/CXCR4 axis.

Keywords: Helicobacter pylori, Mesenchymal stem cells, CXCR4

2304P

Comparison of TGF-β production by mesenchymal stem cells derived from murine lung and adipose tissues

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Background: Mesenchymal stem cells (MSCs) are therapeutic stem cell source for replenishment of damaged tissues. They are recognized by being positive for CD73, CD90, and CD105 and devoid of CD11b, CD34 and CD45 together with mesodermal differentiation potential. These tissue resident stem cells have the potential to regulate the immune system via excess mechanisms, e.g. release of soluble factors which result in the immunosuppressive activities.Transforming grows factor-β (TGF-β) secretion from MSCs plays a role in immune system suppression. TGF-β inhibits proliferation and cytotoxicity of neighboring immune cells. MSCs have been disclosed in lung and Adipose tissues but the comparison of adult adipose tissue stem cells and lung resident MSCs properties is still unclear. We therefore compared secretion of TGF-β in isolated MSCs from adipose tissue and lung. **Method:** Mesenchymal stem cells were isolated from murine lung and adipose tissues. Specific differentiation and the expression of surface antigens were compared in both cell populations. TGF-β productive levels were assayed by ELISA kit according to manufacturer's guideline and OD values were measured using a microplate reader. **Results:** Both adult stem cells had CD73, CD105 and CD90 and no expression of CD34, CD45 and CD11b and adipocyte and osteocyte differentiations were observable. No significant difference was found in TGF-β production

between two stem cell populations. **Conclusion:** TGF-β secretion in lung-derived MSCs is comparable to adipose tissue-derived MSCs and both stem cell sources will probably be apply in treatment of lung diseases.

Keywords: Mesenchymal Stem Cells, Adipose tissue, Lung, TGF-β

2303P

Comparison of NO secretion by mesenchymal stem cells derived from murine lung and adipose tissues

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Background: Mesenchymal stem cells (MSCs) are reservoir for tissue turnover. They are characterized with mesodermal differentiation potential and expression of CD73, CD90, CD105 and lack of CD34, CD45 and CD11b. MSCs release large amounts of biomolecules such as nitric oxide (NO). NO is one of eminent candidate in the immunosuppression mechanisms by MSCs. These stem cells can be found in lung and adipose tissues but the comparison of both cell population properties is still unclear. Herein, a comparison was conducted between NO secretion in lung and adipose tissue derived MSCs. **Methods:** Mesenchymal stem cells were isolated from murine lung and adipose tissues. Mesodermal differentiation and surface protein antigens expression were compared in both cell populations. Finally, Nitric oxide levels in cell cultures were evaluated by standard Griess reaction. **Results:** Both tissue resident stem cells expressed CD73, CD105 and CD90 as well as lack of CD34, CD45 and CD11b and osteogenesis and adipogenesis were deceted. Adipose-derived MSCs and lung-derived MSCs produced insignificant difference of nitric oxide. **Conclusion:** NO secretion in lung-derived MSCs is similar to adipose tissue-derived MSCs and both stem cell sources will probably be apply in the treatment of lung disorders.

Keywords: Mesenchymal Stem Cells, Adipose tissue, Lung, NO

2174P

Correlation of Cytokine Changes and Myocardial Repair after Mesenchymal Stem cell Transplantation in Myocardial Infarction

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Background: Mesenchymal Stem Cells (MSCs) are derived easily from bone marrow and other adult tissues. Due to cytokine secretion, MSCs have potentials for many clinical applications such as myocardial infarction (MI). In the present study the relationship between TNF-α, TGF-β cytokines and myocardial repair are investigated after administration of mesenchymal stem cells in experimental MI. **Methods:** Experimental MI was induced in 16 rabbits divided in test and sham control groups (8 in each). Rabbits in test group were treated

by MSCs derived from human bone marrow. Animals in sham group received culture media instead of MSCs. Blood samples were collected every week. Cytokine assay was conducted using ELISA and cardiac tissue samples prepared after eight week were used for histological studies. **Results:**We indicated that MSCs transplantation in rabbit model of MI leads to new angiogenesis, lower collagen synthesis and more myocardiocyte survival in ischemic area. Furthermore, TGF-β measured in different time points demonstrated a significant decrease in the first week, followed by the increased levels of this cytokine during the rest time of the study. We also found that Serum levels of TNF-α had enhancement in the first week of the study.**Discussion:** We conclude that the local induction of TNF-α and TGF-β after MSC treatment of MI, correlates with cardiac repair and cardiomyocyte regeneration.
Keywords: Mesenchymal stem cells, Myocardial infarction, TNF-α, TGF-β

1389P

HIF-1α confers resistance to induced stress in bone marrow-derived mesenchymal stem cells

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Background: The major limiting factor in therapeutic application of mesenchymal stem cells is their high vulnerability during the early days of transplantation. Hence, researchers of this field have been encouraged to find various strategies to make the cells resistant to different stresses before and after transplantation. Over- expression of HIF-1α in MSCs to confers resistance under harmful conditions was the aim of this study. **Methods:** In an in vitro approach, we engineered MSCs to overexpress HIF-1α in order to evaluate their viability following exposure to hypoxic and oxidative stresses. On the other hand, the inherent expression of HIF -1α was down-regulated by siRNA. Viability and apoptosis of the MSCs were then evaluated in vitro following their exposure to hypoxic and oxidative stress conditions. **Results:** While over-expression of HIF-1α in MSCs protected them against cell death and apoptosis triggered by hypoxic and oxidative stress conditions, its down-regulation increased apoptosis and death rate. **Conclusion:** Our study is the first to demonstrate how human MSCs can be manipulated to gain protection against stresses which potentially limit their clinical application.
Keywords: Mesenchymal stem cells, HIF-1α, HIF-1α-siRNA, Oxidative stress, Apoptosis

1784P

Calcitriol modulate the effect of supernatant of bone marrow-derived mesenchymal stem cells on neutrophil functions

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Background: Mesenchymal stem cells (MSCs) in bone marrow form a niche which has inevitable interactions with neutrophils. Moreover, previous documents have shown that calcitriol has an important role in regulating cell growth of MSCs. This study was set out to investigatethe effects of calcitriol on interaction between bone marrow-derived MSCs and neutrophil functions. **Methods:** MSCs are isolated from bone marrow of rats and pulsed with different concentrations of calcitriol (50,100 and 200 Nm) at different times (24, 48 and 72 h). Next, the supernatants of MSCs co-cultured with neutrophils for 4h and neutrophil functions were assumed. **Results:** Data showed that the supernatant of MSCs treated with calcitriol could significantly increase the phagocytosis of *Staphylococcus aureus* by neutrophils andconversely, decrease respiratory burst intensity of neutrophils. Moreover, treatmentof MSCs with calcitriol can cause a significant decrease in percent of neutrophils apoptosis. These findings were concurrent with a significant increase in IL-6 levels in the supernatant of calcitriol treated MSCs. **Conclusion**: As a result, supernatant of bone marrow-derived MSCs was pulsed with calcitriol, while exertion of a protective role against potentially harmful reactive oxygen species production preserves phagocytosis and survival rate of neutrophils.

Keywords: Mesenchymal Stem cells, Calcitriol, Neutrophil.

1788p

Effect of bone marrow-derived mesenchymal stem cell pulsed with vitamin D3 on peripheral blood neutrophil functions in rat

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Background: Previous studies indicated that mesenchymal stem cells microenvironments can be designed function of related neutrophils. The present study was done to investigate the effects of bonemarrow-derived mesenchymal stem cell pulsed with 1α, 25-dihydroxyvitamin D3 on neutrophil function in rat. **Methods:** After isolation of mesenchymal stem cells from bone marrow of rats, these cells pulsed with different concentration of vitamin D3 (50,100 and 200 nM) at different time (24T48 and 72 h). Then mesenchymal stem cells co-cultured with neutrophils for 1 h. Finally, neutrophil evaluated for phagocytosis activity against opsonized yeast, respiratory burst by NBT reduction assay and viability using acridine orange/propidium iodide. **Results:** Phagocytosis ability of neutrophils in all treatments was significant in ≥100 nM concentration. Respiratory burst intensity showed significantly an increase in ≥50 nM. The data indicated that treatment with vitamin D3 at the minimum concentration of 50nM in all time caused a significant increase in percent of neutrophils survival (p<0.05).**Conclusion:** Altogether, treatment of bone marrow derived mesenchymal stem cells with vitamin D3 potentiated the mesenchymal stem cells effects on survival, phagocytic ability and respiratory burst of neutrophil.

Keywords: Mesenchymal Stem cells, Calcitriol, Neutrophil.

1962p

Evaluation of the relationships between genetic polymorphism of CTLA-4 gene with outcome of TTV infection in bone marrow transplant patients.

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Background: Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a co-stimulatory molecule plays an important role in the negative regulation of T-cell proliferation and activation which has been affected by genetic polymorphisms in bone marrow transplant patients. Also single nucleotide polymorphisms of CTLA-4 may interact with viral infections like Torque Teno Virus (TTV) with unknown pathogenesis. Therefore in this study the relationship between CTLA-4 gene polymorphisms with TTV infection was evaluatedin bone marrow transplant patients. **Methods:** In this cross sectional study EDTA-treated blood samples were collected from 71 allogenic bone marrow patients between years:1383-1392. The genetic polymorphisms of CTLA-4 gene +49A/G were analyzed by an in-house-PCR-RFLP protocol. The prevalence of TTV infection was also evaluated by an in-house-semi-nested PCR technique.**Results:** TTV infection was detected in 24 of 71(33.8%) bone marrow transplant patients. The AG genotype of the CTLA-4(+49 A/G) gene were significantly more frequent in TTV infected allogenic bone marrow transplant patients (P= 0.01, OR =3.57, 95% 1.07-2.21). Significant association was also found between GG genotype of the CTLA-4(+49 A/G) gene with TTV infection in bone marrow transplant patients (P= 0.02, OR =0.11, 95% 0.0-1.5). **Conclusion:**Determination of the higher frequent pattern of CTLA-4(+49 A/G) genepolymorphism in TTV infected bone marrow transplant patients, present the importance of this format of CTLA-4 gene in viral and clinical outcomes occurred post allogenic bone marrow transplant need to confirm in completed studies.

Keywords: TTV, Co-stimulatory molecules, Bone marrow transplantation.

1961p

Study of the Relationships between ICOS gene polymorphisms with polyomavirus BK infection in autologus bone marrow transplant patients

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Background: ICOS is a co-stimulatory molecule plays an important role in positive regulation of T helper cell differentiation which has been affected by gene polymorphisms in bone marrow transplant patients. Single nucleotide polymorphisms in ICOS gene may interact with viral infections like polyomavirus BK has important role in post bone marrow transplant outcomes. Therefore in this study the relationship between ICOS gene polymorphisms with polyomavirus BK infection was evaluatedin bone marrow transplant patients. **Methods:** In this cross sectional study EDTA-treated blood samples were collected from 59 Autologus bone marrow patients between years 1383-1392. The genetic polymorphisms of ICOS gene (1720-

T/C) were analyzed by an in-house-PCR-RFLP protocol. The prevalence of polyomavirus BK infection was also evaluated by an in-house-nested PCR technique.**Results:** Polyomavirus BK infection was found in 32 of 59(52.5%) bone marrow transplant patients. In this study Significant association was found between CT genotype and allele T of ICOS gene (1720-T/C) were more frequent in polyomavirus BK infected bone marrow transplant patients.(P =0.02, OR =0.22, 95% CI: 0.04-1.02, P= 0.03, OR =0.26, 95% CI: 0.05-1.10) .Also The genotype CC of ICOS gene (1720-T/C) was significantly less frequent in polyomavirus BK infected bone marrow transplant patients(P =0.02, OR =4.58, 95% CI: 0.98-24.21). **Conclusion:**The results suggest that ICOS (1720-T/C) gene polymorphism associate with selection inflammatory pressure in the infection progression. Therefore, this biomarker can be used in following of the viral related clinical outcomes post bone marrow transplantation.

Keywords: Co-stimulatory molecules, Polyomavirus BK, Bone marrow transplantation

1578p

The role of HLA-DRB1 molecules on susceptibility to renal disease and kidney transplant outcome

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Background: Kidney transplant is the best treatment option for the patients with end-stage renal disease.Genetic factors such as matching of HLA especially HLA-II molecules are the greatest cause of kidney transplant failure. HLA-DRB1 is the most important molecule that play role in tissue compatibility and susceptibility to acute kidney transplant rejection. The aim of the present study is to analyse the influence of HLA-DRB1 molecules and HLA-DRB1 sharing in the survival of kidney transplant has been studied. **Methods:** In this study, 41 kidney transplant pairs (recipient and donor) were investigated using HLA typing. HLA-DRB1 typing by molecular techniques (sequence- specific primers with low resolution) was performed using commercial kit from BAG (Germany). After sequence amplification has been finished, Separation of the amplification products is done by electrophoresis via a 2% agarose gel and the results were interpreted using BAG typing software **(**BAG,Germany) and re-checked manually by typing worksheet. Then Epi Info software was used for statistical analysis. **Results:** statistical analysis of the allele frequencies in 41 recipients of kidney transplant didn't show any significant association between HLA-DRB1 allele and susceptibility to acute kidney transplant rejection ($p>0.05$). Comparing HLA-DRB1 allele frequencies between normal and patient population showed that HLA-DRB1*14 allelic group was more frequent in normal group and HLA-DRB1*04 allelic group was more frequent in patient group (P=0.03 and P=0.05 respectively). In addition the result of HLA-DRB1 allele sharing in rejection and non rejection group didn't show any significant relation between allele sharing and kidney transplant outcome. **Conclusion:** results of the present study did not showed relation between HLA-DRB1 alleles and kidney transplant rejection. But considering the significant role and relation of HLA-DRB1 molecules in tissue compatibility and protective or susceptibility to renal diseases, show that HLA-DRB1 typing for both recipients and donors is highly recommended.

Keywords: HLA-DRB1, Kidney transplant, allograft rejection

1763p

Serum starvation induces down-regulation of HLA-class I in Human peripheral Blood Mononuclear cells: A potential tool for lymphoid tissue transplantation.

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Background: Since VanRood discovered that HLA-matching between organ donor and recipient leads to more survival of the organ, the HLA-matching has been still performing worldwide. However in some conditions like bone marrow transplantation finding of matched donor is often problematic. Here we bring some evidence indicating that serum starvation could be a potential tool in down-regulation of HLA-class1expression.**Methods:** PBMCs were cultured inRPMI-1640 + FBS10% (control) as well as in mediumonly (starvation) for 16, 24, 48, 72, 96 hrs then the cells were counted. The pattern of cell death was determined and HLA-I expression was assessed. Then the culture was continued for another 96hrs inthe medium+ 10%FBS (re-feeding). Again the cells were counted and HLA-I expression levels were determined. At three different time points the HLA-I expression were analysed on fresh PBMC. **Results:** After 96hrsthe starved cell numbers significantly decreased ($p<0.05$). The 16hrs starved PBMC showed maximum apoptosis cell death, After re-feeding the number of these cell was dramatically decreased while 72hrsand 96hrs starved PBMC showed almost the same survival rate of non-starved control ($p=0.048$).Photomicrograph obviously supports this finding. There was a negative trend in HLA-I expression during the first 72hrs ($p<0.01$) however this trend changed to significant increase during the next 24hrs ($p<0.01$).Interestingly this reduction was stable after re-feeding. HLA-I expression did not significantly change by culturing. **Conclusion:** HLA-I expression could be stably decreased in human PBMC by starvation for 72hrs while the viability rate of the cells is enough for possible clinical application.

Keywords: Human mononuclear cell, Serum starvation, HLA-I, Transplantation

1584P

Molecules expressed on Mesenchymal stem cell-derived microvesicles induce tolerogenic signaling in auto-reactive cells

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Background: Generation and maintenance of immunological tolerance is a pivotal aim in

the field of autoimmunity. Regulatory molecules of Programmed Death Ligand-1 (PD-L1), galectin-1 and TGF-β are described as key mediators of peripheral tolerance that actively suppress auto-reactive cells and inhibit their mediated tissue damages. Accordingly, biological intervention in host immune system for induction of peripheral tolerance is pivot to many of the recent studies. Mesenchymal stem cell-derived microvesicles (MVs) are viewed as potential mediators to shed peripheral tolerance toward auto-reactive cells via bearing of tolerogenic molecules. **Methods:** Here, MVs were isolated from mesenchymal stem cell (MSC) cultures' conditioned medium. They were explored for the expression of PD-L1, galectin-1 and membrane bound TGF-β through flow cytometry. The immunoregulatory effects of MVs on splenic mononuclear cells (MNCs) derived from experimental autoimmune encephalomyelitis (EAE) affected mice were investigated using MTT assay, ELISA and flow cytometry. **Results:** MVs derived from MSCs expressed PD-L1, galecin-1 and membrane-bound TGF-β. MVs exhibited the potential to inhibit auto-reactive lymphocyte proliferation and also they can promote them to secret anti-inflammatory cytokines of IL-10 and TGF-β. Interestingly, inducing inflammatory setting on MSCs, revealed the enhancing regulatory effects of MVs via increased expression of some regulatory molecules, specifically PD-L1 and TGF-β. Induction of tolerogenic signaling, promotion of CD4+ CD25+ Foxp3+ regulatory T cells generation and apoptotic activity towards activated T cells are shown to be possible mechanisms involved in MV-mediated regulation. **Conclusion:** Recent study suggests MSC-derived MVs as potent organelles for induction of peripheral tolerance and modulation of immune responses.

Keywords: Mesenchymal stem cell-derived microvesicle, Experimental autoimmune encephalomyelitis, Tolerogenic Molecules

1754p

The effect of cyclosporine blood level on cytomegalovirus reactivation in hematopoietic stem cell transplant recipients

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Background: The immunosuppressive drug cyclosporine is the main compound used for preventing graft rejection and GVHD in transplant recipients. However, the excessive amount of this drug in patients' blood can cause severe immunosuppression and may result in opportunistic infections, especially with cytomegalovirus, which is the most important pathogen that causes morbidity and mortality in hematopoietic stem cell transplant recipients. Therefore, in present study, the effect of cyclosporine blood level on cytomegalovirus reactivation was analyzed in a cohort study. **Methods:** a total number of 1179 successive blood samples were obtained from 82 patients who underwent allogeneic transplantation. Cyclosporine blood level was measured using the Cyclosporine direct RIA KIT. Cytomegalovirus reactivation in blood samples was detected by the indirect immunofluorescence detection of pp65 antigen using the CMV Brite Turbo Kit. Viral reactivation was also analyzed by the Real-time PCR assay. **Results:**

Based on the results of the Mann-Whitney U test, the mean and median of cyclosporine level in cytomegalovirus positive samples was higher than negative samples booth by the pp65 antigenemia (P= 0.001) and the Real-time PCR (P< 0.001) methods. Furthermore, when the level of cyclosporine was compared among patients, it was shown that the patients with higher mean level of the drug showed more cytomegalovirus reactivation episodes (P= 0.038 by the Student's t-test).**Conclusion:** The blood level of cyclosporine in hematopoietic stem cell transplant recipients should be monitored and adjusted to be in normal range in order to prevent cytomegalovirus reactivation.

Keywords: Cyclosporine, Cytomegalovirus, pp65 antigenemia, Real-time PCR, Transplantation

2323p

Hematopoietic chimerism analysis after allogeneic stem cell transplantation

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Background: Chimerism analysis after allogeneic stem cell transplantation (allo-SCT) allows detection of early marrow engraftment, disease relapse, and graft rejection. Several approaches have been published for the detection of chimerism. FISH with X- and Y-chromosome specific probes and PCR-based amplification of a single variable number of tandem repeat (VNTR) or short tandem repeat (STR) markers are frequently performed method. STRs, which are standard tools for genotyping in parentage testing and forensic human identity testing, provide an excellent tool for this purpose because of their high degree of polymorphism and relatively short length. The study was aimed to analyse the chimeric status of peripheral blood leukocytes in recipients of allo-SCT with the use of short tandem repeat (STR) microsatellite markers. **Methods**: We have investigated allo-SCT in patients suffering from different types of leukemia or non-malignant hematologic disorders by close molecular monitoring during 15 days to 24 months after transplantation. A STR-PCR was performed on DNA isolated from whole white blood cell of donor and before and after SCT in patients. A set of twelve STR markers (ARA, ADA, D4S2366, D16S539, D7S820, D13S317, F13A1, FES/FPS, VWA, CSF1PO, TPOX and TH01) with the amelogenin marker were used in four-multiplex PCR.**Results:** The multiplex PCR showed a sensitivity of about 5% when analyzed on polyacrylamide gel. In this study, amelogenin marker with three STR markers of D4S2366, D16S539 and TH01 which were designed for multiplex 1, showed the highest degree of discrimination between donor and recipientin 95% of the cases. Also, these markers have high sensitivity in showing the mixed chimerism in patients. On the other hand, chimerism assessment using these four markers in one multiplex, has Low cost and high speed to determine engraftment status after allo-SCT. **Conclusion:**STR analysis using a multiplex PCR can provide an accurate, rapid and quantitative assessment of chimerism in patients with post-allogenic SCT. These studies also showed that the analysis of mixed chimerism could be useful for patients in guiding early implementation of additional treatment designed to circumvent graft failure or suppression of relapse.

Keywords: Short Tandem Repeat (STR), Allo-SCT, Chimerism, Multiplex PCR

2178 P

Do HPA-1 and HPA-5 discrepancies affect aGVHD after Hematopoietic Stem Cell Transplantation

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Background: HPA-1 and HPA-5 are suggested as minor histocompatibility antigens which are involved in acute graft versus host disease (aGVHD) after HSCT. The aim of this study was to evaluate HPA-1 and HPA-5 alleles frequencies in some Iranian patients in need of HSCT and related healthy donors, using PCR-SSP method. **Methods:** DNA was extracted from the peripheral blood samples taken from 48 healthy individuals as HSCT donors and 48 related HSCT recipients in two groups of aGVHD^{+} and without GVHD. All the recipient-donor pairs were HLA-A/B/DR full-matched siblings. **Results:** The HPA-1 frequencies were identified as HPA-1a=0.987, HPA-1b=0.013 whereas the HPA-5 frequencies were obtained as HPA-5a=0.99, HPA-/5b=0.01 in all cases. We found three HPAs discrepancies among donors and recipients as follow:

Donor	Recipient	GVHD
HPA1aa/5aa	HPA1aa/5**bb**	+
HPA1**bb**/5aa	HPA1aa/5aa	+
HPA1aa/5aa	HPA1**bb**/5aa	_

Conclusion: It seems there is no significant difference between GVHD+ and GVHD- groups and HPA-1,-5 discrepancies may not affect GVHD occurrence after HSCT (P=0.55).

Keywords: HA-1, HPA-1, HPA-5, aGVHD, Hematopoietic Stem cell transplantation (HSCT)

1859 P

The effect of cocultured umbilical cord blood hematopoietic stem cells with mesenchymal stem cells on GATA-1, GATA-2, GATA-3 and FOG-1expression in differentiated cells to megakaryocyte progenitor cells

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Background: Human umbilical cord blood(hUCB) is well known as a rich source for hematopoietic stem cell transplantation in recent decades. Co-culture of HSCs with MSCs is considered as an effective way for ex vivo expansion of HSCs.In this study, we examined the effects of hUCB-MSCs on the differentiation of CD34+ cells-derived UCB to megakaryocyte lineage and then surveyed the expression of GATA-1 ,GATA-2, GATA-3 and FOG-1 in differentiated cells.**Methods:**The CD34^{+} subpopulation of UCB samples was separated using direct CD34 microbead kit and immunomagnetic cell sorting (MACS) system.Confluent Human CB MSCs were seeded in a 24-well tissue culture plate as a stromal layer. Purified Human CB CD34^{+} cells were plated in the transwell and cultured in the presence of serum-free stemspan medium and the cytokine cocktail.Total RNA from co-culture group and

control group separately were extracted.RNA was reverse-transcribed with random-hexamer as primer. Complementary DNAs was generated by reverse transcriptase-polymerase chain reaction (RT-PCR) by using Beta2M primer as a house keeping gene. cDNAs were used as templates in the quantitation of GATA-1,GATA-2,GATA-3 and FOG-1.For real-time PCR, reactions involved the fluorescent dye SYBR green and absolute gene-expression levels were calculated by generating standard curves for each gene. **Results & Conclusion:** The results showed that MSCs can upregulate GATA-1, FOG-1 and GATA-2 and down regulate GATA-3 expression in co-cultured CD34+ cells-derived UCB to megakaryocyte progenitor cells.

Keywords: Hematopoietic stem cells, Umbilical cord blood

2344 P

Association of IL-17 genetic polymorphism with post hematopoietic stem cell transplant clinical outcomes.

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Background: IL-17 is important proinflammatory cytokine may have role in presentation of immunologically related clinical outcomes post hematopoietic stem cell transplantation. Gene polymorphisms also have significant role on types of IL-17 effects on transplant outcomes. Therefore in this study the correlation between IL-17 cytokine genetic polymorphism with clinical outcomes were evaluated in hematopoietic stem cell transplant (HSCT) patients. **Methods:** In a cross sectional study EDTA-threated blood samples were collected from each 60 HSCT patients. These patients divided to two groups including 35 and 25 patients' not experiencing or experiencing graft versus host disease (GVHD), respectively. The genetic polymorphisms of IL-17 (-197, A/G) cytokine gene were evaluated by RFLP-PCR method. The serum level of IL-17 cytokine was also analyzed using ELISA method.**Results:** IL-17 cytokine (-197, A/G) GG genotype was found to be significantly higher in patients with GVHD compared to non-GVHD HSCT patients (P=0.04). However, IL-17 (rs3819025) G allele was found significantly higher frequent in grade 0-I of GVHD compared to sever grades of GVHD in HSCT patients (P=0.05). In addition, according to sex,IL-17 -197 GG genotype showed a significance higher frequency in non-GVHD male patients (P=0.05). IL-17 serum levels did not show any significant difference between patients with GVHD compared with non-GVHD ones.**Conclusion:** Finding of significant higher frequency of IL-17 -197 GG genotype and G allele in transplant patients and also, sex dependency of IL-17 -197 GG genotype is can candidate to increase the risk of GVHD in HSCT patients need to more evaluate in further completed studies.

Keywords: Hematopoietic stem cell transplantation, Graft versus Host Disease, Gene polymorphism, IL-17.

2043 P

Human umbilical cord blood mesenchymal stem cells (hUCB-MSCs) altered gene expression cytokine profile in peripheral blood mononuclear cells (PBMNCs)

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Background: Although bone marrow is the main source of mesenchymal stem cells but harvesting mesenchymal stem cells from bone marrow is invasive procedure and the number of mesenchymal stem cells and potency of differentiation considerably reduce with aging and there is infectious disease contamination risk in isolated samples frombone marrow, so there isa needto searchalternative resources. Umbilical cord bloodisavaluable resourcefor the isolation ofmesenchymal stem cells. **Methods:** In this study, we successfully isolated MSCs from hUCB. The morphological phenotypes and differentiation ability have done by alkaline phosphatase and Oil red O test. Cell surface markers were examined by flowcytometery. Furthermore inhibitory effect of irradiated hUCB-MSCs on phytohaemaglutinin activated human PBMNCs proliferation, have examined in co-culture system. Finally IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, TNF-α and TGF-β gene expression have evaluated by real time-PCR technique in human PBMNCs at co-culture with hUCB-MSCs. **Results**: Flowcytometery results showed that hUCB-MSCs did not express CD34, CD45, CD14, CD19 and CD3 but they did express strongly CD73, CD90, CD105, CD106 markers and at average rate they expressed HLA-G molecule. Co-culture of PBMNCs with irradiated hUCB-MSCs demonstrated a dose-dependent inhibitory effect of irradiated hUCB-MSCs on phytohaemagglutinin activated human PBMNCs proliferation. Finally the results of gene expression showed that irradiated hUCB-MSCs caused to reduce IL-2, IL-6, IFN-γ and TNF-α gene expression levels in PBMNCs and caused to increase in IL-4, IL-10 and TGF-β, whereas the results of gene expression levels of IL-5 and IL-13 didn't changed. **Conclusion**: UCB-MSCs could alter pro-inflammatory response of activated PBMNCs to production anti-inflammatory cytokines with no detectable changes in IL-5 and IL-13 gene expression levels.

Keywords: Stem cell, Mesenchymal stem cell, Umbilical cord blood, Immunomodulation, Transplantation

2466 P

Osteogenic and Adipogenic Cells Differentiated from Lentivirus-modified human umbilical cord mesenchymal stem cells sustain their immunomodulatory function

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Background: Human umbilical-cord-derived mesenchymal stem cells (hUC-MSCs) are easy available cells compare to bone-marrow-derived MSCs for potential clinical applications.

HumanUC-MSCs are easier to isolate and expand without having the rejection reaction problems and thus ethical concerns. They showed immunosuppressive properties.Transduction of MSCs (mesenchymal stem cells) by genetically engineered lentiviral particles has recently been shown to be a highly efficient method for gene delivery.It was unclear whether the transductedhUC-MSCs would retain their immunomodulatory characteristics after differentiation into osteogenic and adipogenic cells or not. **Methods:** We isolated hUC-MSCs with Explants culture method. Recombinant lentiviral particles were produced in HEK-293T cells by transient co-transfection of three-plasmid expression system. HumanUC-MSCs transducted at MOI = 50 in the presence of 8 μg/ml Polybrene. Transduced and untransductedhUC-MSCs were assessed for osteogenic and adipogenic differentiation potentials.The transduced and untransductedosteogenic and adipogenic Cells surface molecules such as CD31, CD43, CD45, MHCII, MHCI, CD40, CD80, CD86, CD90, CD117, CD73 were determined using FACS analysis system. Genomic DNA was isolated from the osteogenic and adipogenic cells differentiated from Lentivirus-modified hUC-MSCs to detect IL-10, HGF, VEGF, HLA-G expression by real-time PCR. Cell Proliferation assay analyzed by CFSE Cell Proliferation Kit.**Results:** There was no difference between the transduced and untransductedosteogenic and adipogenic Cells surface markers and IL-10, HGF, VEGF, HLA-G gene expression. Both of them inhibited proliferation of human PBMC. **Conclusion:** These findings suggest that lentiviral transduction doesn't alter the immunomodulatory property of hUC-MSCs after differentiation into osteogenic and adipogenic cells.

Keywords: Human umbilical-cord-derived mesenchymal stem cells, Lentiviral transduction, Immunomodulatory property, Osteogenic and Adipogenic differentiation.

2629 P

Evaluation of LPS treatedbone marrow-derived mesenchymal stem cells on NK cellsactivity against Yac-1 cells

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Background: Mesenchymal stem cells (MSCs) are multipotent, non-hematopoietic precursor cells that could befound in many adult textures. Multipotency and immonumodulatory potential of MSCs makes these cells as a remarkable tools for treatment of some diseases. It seems that stimulation of toll like receptors expressed on the surface of mesenchymal stem cells may be potentiated immonumodulatory potential of these cells. This study was done to investigate the effects of polarized bone marrow-derived mesenchymal stem cells of mouse on the cytotoxicity activity of natural killer (NK) Cells. **Methods**: MSCs were isolated from bone marrow of femur and tibia of NMRI-mice. Third passage of cells were treated with LPS (10 ng/ml) for one hour. Then the effects of polarized MSCs evaluated on cytotoxicity activity of natural killer (NK) Cells on lymphoid cancer cells Yac-1 (as target cells) using flowcytometery after 48 h. **Results**: MSCs treated with LPS an decrease in the percent of survival Yac-1 cells and incresed the percent of dead cells (necrosis and apoptosis) in compared to un-treated MSCs. **Conclusion**: Pervious research indicated that MSCs inhibit expansion and cytotoxicity

effects on NK cells on tumor cells such as Yac-1.LPS:This results indicated that MSCs treated with LPS decreased the inhibitory effects of MSCs on NK cells.
Keywords: Mesenchymal Stem Cells (MSCs), Natural Killer (NK), LPS

2632 P

The effects of poly I-C treatedbone marrow-derived mesenchymal stem cells on NK cells activity against Yac-1 cells

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Background: Mesenchymal Stem Cells (MSCs) are multipotent, non-hematopoietic precursor cells that could be found in many adult textures. Multipotency and immonumodulatory potential of MSCs makes these cells as a remarkable tools for treatment of some diseases. It seems that stimulation of toll like receptors expressed on the surface of mesenchymal stem cells may be potentiated immonumodulatory potential of these cells. This study was done to investigate the effects of polarized bone marrow-derived mesenchymal stem cells of mouse on cytotoxicity activity of natural killer (NK) Cells. **Methods**: MSCs were isolated from bone marrow of femur and tibia of NMRI-mice. Third passage of cells were treated with Poly:IC (5µg/ml) for one hour. Then the effects of polarized MSCs evaluated on cytotoxicity activity of Natural Killer (NK) Cells on lymphoid cancer cells Yac-1 (as target cells) using flowcytometery after 48 h.**Results**:MSCs treated with poly I-C an increase in the percent of survival Yac-1 cells and decreased the percent of dead cells (apoptosis and necrosis) in compared to un-treated MSCs**.** **Conclusion**: Previous research indicated that MSCs inhibit expansion and cytotoxicity effects on NK cells on tumor cells such as Yac-1.POLY I-C: In this regard, MSCs treated with poly I-C potentiated this effects of MSCs on NK.
Keywords: Mesenchymal Stem Cells (MSCs), Natural Killer (NK), Poly I-C

2209 P

The Expression some ofthe embryonic stem cell markers inthe full term Human Placenta

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Background: OCT4 is a POU domain–containing transcription factor encoded by Pou5f1. In the absence of OCT4, pluripotent cells in vivo (epiblast) and in vitro (stem cell) both revert to the trophoblast lineage. This implicates OCT4 as an important regulatory molecule in the initial cell fate decisions during mammalian development. Nanog, a homeodomain–containing protein, was identified as a factor that can sustain pluripotency in stem cells even in the absence of leukemia inhibitory factor (LIF). Although the transcription pathways are

independent, Nanog and OCT4 work in concert to support stem cells potency and self-renewal. OCT4 and Nanog are transcription factors required to maintain the pluripotency and self-renewal of embryonic stem (ES) cells and also Mesenchymal Stem Cells (MSCs).Placenta is a temporary organ that accompanies pregnancy connected to the fetus via the umbilical cord. It plays a fundamental and essential role in fetal development, nutrition, and tolerance and has different cells.**Methods:**Placenta at term were obtained following cesarean section of healthy mothers. Total RNA was extracted from Placenta tissue using Mini-RNase RNA extract kit. RT-qPCR was used to identify the gene expression of Nanog and OCT4.**Results:**Gene expression of Nanog and OCT4 was documented by RT-qPCR analysis.**Conclusion:**This report demonstrating that placenta cells express some embryonic stem cell markers such as Nanog and OCT4. Therefore, some placenta cells have pluripotent properties.

Keywords: Placenta,Stem cells, OCT4,Nanog

2208 P

Differentiation of Human-Induced Pluripotent Stem Cellsinto Insulin Like cell Clusterswith growth factor

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Background: In diabetes mellitus type 1, beta cells are mostly destroyed;while in diabetes mellitus type 2, beta cells are reduced by 40% to 60%. We hope that soon, stem cells can be used in diabetes therapy via pancreatic beta cell replacement. Induced pluripotent stem cells are a kind of stem cell taken from an adult somatic cell by “stimulating” certain genes. iPS cells may be a promising source of cell therapy. This study sought to produce isletlike cell clusters of insulin-producing cells taken from induced pluripotent stem cells.**Methods:**A human-induced pluripotent stem cell line was induced into isletlike cell clusters via a 4-step protocol, by adding insulin, ransferring, and selenium (ITS), N2, B27, fibroblastgrowth factor, and nicotinamide. During differentiation, expression of pancreatic beta cell genes was evaluated by RT-qPCR; the morphologic changes of induced pluripotent stem cells toward isletlike cell clusters were observed by a light microscope. Insulin produced by these clusters was evaluated by radio immunosorbent assay, and the secretion capacity was analyzed with a glucose challenge test. **Results:** Differentiation was evaluated by analyzing the morphology and immunocytochemistry. Gene expression of insulin, glucagon, PDX1, NGN3, PAX4, PAX6, NKX6.1, KIR6.2, and GLUT2 were documented by analyzing RT-qPCR. The islet like cell clusters significantly produced insulin. The isletlike cell clusters could increase insulin secretion after a glucose challenge test. **Conclusion:** This work provides a model for studying the differentiation of human-induced pluripotent stem cells to insulin-producing cells.

Keywords: Pancreas, Islet Like cell, Induced pluripotent stem cells, Insulin

2432P

Sustained expression of homing marker in hematopoietic stem cells following expansion with cytokine and mesenchymal stem cell as feeder

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Background: Hematopoietic Stem Cell (HSC) transplantation using umbilical cord blood has been improved during last decade and several researches are focused on the ex vivo expansion of HSCs. HSCs capacities in hemostasis regulation is due to many migration and adhesion molecules such as CXCR4 and CD49d. The aim of the current study is to evaluate the effect of different expansion conditions on HSCs surface marker expression. **Methods:** HSCs and MSCs were isolated from UCB and placenta respectively. HSCs were cultured in different culture conditions with and without MSC feeder. After 7 days, expression of CXCR4 and CD49d on HSCs were evaluated and data were compared by *t*-test and Anova. **Results:** The results showed expression of CXCR4 in groups of HSCs that had been expanded in presence of feeder and/or cytokine is relatively higher in comparison with control group but differences were not significant. On the other hands, expansion of HSCs in presence of feeder solely or plus with cytokines leads significant increase of CD49d expression ($P<0.05$). Also, cocktails of cytokines have been increased CD49d expression but differences were not remarkable ($P=0.06$)**Conclusion:** Present results shows that ex vivo expansion of HSCs with cocktail of cytokines with or without mesenchymal stem cells as feeder do not affect the HSCs homing capacities and in contrast increase some HSCs surface markers such as CD49d.

Keywords: Hematopoietic Stem Cell, Ex vivo expansion, Cytokine, Surface marker, CXCR4, CD49d

2743P

Studies on immunomdulatory effect of Human Mesenchymal stem Cells in Rhesus kidney allotransplantation

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Background: Numerous in vitro studies have indicated that MSCs modulate innate and adaptive immune system by promoting generation of regulatory cells leading to immune tolerance induction. MSCs are able to block pro-inflammatory and increase anti-inflammatory cytokines secretion. So such unique immunomodulatory features make MSCs as a good candidate to induce immunological tolerance in solid organ transplantation.Examine and compare the immune regulatory effect of human BM and AD-MSCs in experimental model of renal allograft. **Methods:** MSCs, isolated from BM and AD tissues, were characterized for specific surface antigen markers using flow cytometry. Functional differentiation was performed by

differentiating MSCs into adipogenic and osteogenic linage. MLR was performed to assess the immune regulation effect of hMSC *in vitro*. Also flow cytmetry was done to assess *in vivo* T cells differentiation toward inflammatory and regulatory cells after hMSC injection into Rhesus. In addition, *in vivo* cytokine production of inflamed and regulatory T cells was measured by RT-PCR after hMSC injection. **Results:** MLR data showed the suppressive effect of hBM-MSC and hAD-MSC on Rhesus activated T cells. These data also suggested that proliferative suppression of hMSC is individual-dependent. In addition, human AD and BM-MSC injection showed high level of Treg induction for only up to 24hrs and not more than that. INF-γ activated MSCs increases their immunosuppressive capacities *in vivo*. **Conclusion:** Our *in vivo* and *in vitro* studies suggest that hAD-MSCshave great immunosuppressive potential which gives immune privilege to them for clinical applications.

Keywords: Human AD-MSC, Human BM-MSC, Human MSCs, Regulatory T cells, Immunomodulatory

1756P

Ascorbic acid and trehalose increase thaw-survival and proliferation of human wharton's jelly stem cells after cryopreservation

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Background: Human Wharton's jelly stem cells (hWJSCs) which are derived from the gelatinous substance within the umbilical cord are using for cell based therapies in regenative medicine. Efficient methods of cryopreservation that increased Thaw-survival after thawing are an important aspects of a stem cell storage. The most important factor contributing to decreased Thaw-survival is apoptosis as a result of cryoinjury. The aim of this study was to assess the effect of Trehalose and Ascorbic acid on the Thaw-survival and proliferation of stem cells. **Methods:** Treated hWJSCs with Ascorbic acid (0.06, 0.125, 0.25, 0.5 mM) or Trehalose (35, 75, 125 mM) and Untreated hWJSCs were frozen using a rapid freezing method and stored at-196 in liquid nitrogen for 90 days. After thawing Thaw-survival (live/dead counts), cell proliferation (MTT Assay), apoptosis (Annexin V-FITC Assay) was evaluated.**Results and Conclusion:** Treated hWJSCs with Ascorbic acid or Trehalose showed increased Thaw-survival, increased cell proliferation and no evidence of apoptosis compared to Untreated. Expansion of hWJSCs number would be useful for their storage in cord blood banks for regenerative medicine.

Keywords: Human Wharton's jelly stem cells, Thaw-survival, Proliferation

1685 P

The investigation of rs7903146(C/T) in TCF7L2 gene and post-transplant diabetes in liver transplant recipient

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Background: Post-transplant diabetes mellitus(PTDM) is a frequent complication in organ transplantation. PTDM is a form of type 2 diabetes mellitus, which is thought to develop in response to a relative insulin deficiency resulting from impaired insulin production or increased insulin resistance. The association between variants of TCF7L2 gene and PTDM was showed in many populations. The aim of this study was to investigate association between rs7903146 (C/T) polymorphism in the TCF7L2 gene and PTDM in Iranian liver transplant population. **Methods:** Seventy patients with PTDM and 70 controls without PTDM were studied. The PCR-RFLP method was used for genotyping rs7903146 (C/T) polymorphism. **Result and conclusion:** The genotype frequency in control group was CC=27.14%, CT=52.85%, TT=20% and in patient was CT=51.42%, CC=27.14%, TT=21.42%, (p-value=0.83).The allele frequency in control group was C allele=0.535 and T allele=0.464 and in patient group was C allele=0.528 and T allele=0.471, (p-value=0.96). The results of this study showed that there was no significant different between this polymorphism and PTDM. This is the first study that investigates the association of TCF7L2 polymorphism with PTDM in Iranian population that showed no relation betweenrs7903146 (C/T) polymorphism in TCF7L2 gene and PTDM.

Keywords: PTDM, polymorphism, TCF7L2

1482 P

Association of Glutathione S-transferase *M1* and *T1* genetic polymorphisms and risk of GVHD after bone marrow transplantation

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Background: Glutathione S-transferases (*GSTs*) are a large family of multi-function proteins and play an important role in phase II of biotransformation of many substances. GST has major protective roles in cell against reactive oxygen species (ROS) and detoxification of many xenobiotics including carcinogens, environmental pollutions and anticancer agents. **Methods:** In this study 86 patients with bone marrow transplantation from Namazi Hospital were assessed; Among 86 patient, 33 patients with GVHD after bone marrow transplantation and 53 patients without GVHD after transplantation were enrolled in the study. The *GSTT1* and *GSTM1*polymorphisms were identified by multiplex PCR method.**Results:** The results of present study show that no significant relationship between *GSTT1* and *GSTM1* genetic polymorphism and GVHD after bone marrow transplantation. **Conclusion:** In this study there isn't observed any significant relationship between *GSTM1* and *GSTT1* and outbreak of GHVD.

Keywords: *GSTT1*, *GSTM1*, GVHD, Bone Marrow Transplantation.

1869 P

The effects of selected physical activities on Interleukin 17 and 23 and the body composition of renal transplant patients

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Background: The purpose of current research was to review the effects of 10 week of selected physical activities in the Interleukin 17 and 23 changes and the composition of renal transplant patients. **Methods:** In order to do so we collected 44 patients who had recently had the renal transplantation and then we randomly divided them in two groups of training group (consisting of 29 individuals) and control group (of the remaining 15 patients). All the patients took blood and Anthropometric test two days prior to the trainings. Then all the training group subjects participated in a 10 week training program including a 60-90 minutes training sessions three times a week. Each training session included three sections: warm-up, main stage and cool down. The control group did not participate in any physical activity during this 10 weeks period. At the end of this period the same blood and anthropometric tests were taken. **Results and Conclusion:** The resulting data were analyzed using the dependent and independent T tests. The results revealed that the 10 week physical training program was pretty efficient in reducing the body fat and body mass index in the training group but no significant changes were observed in Interleukin 17 and 23 levels in the blood samples. In general it can be said that physical trainings will improve renal transplant patient's body composition and as a result, it would improve patient's health.

Keywords: Physical Training, Body Composition, Interleukin 17, Interleukin 23, Renal Transplant

1998 P

Evaluation of themRNA level of TLR2 in acute rejectedliver transplant patients

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Background: Toll-like receptors (TLRs) have been identified to augment innate immune defense mechanisms and furthermore play an important role in initiation and modulation of adaptive immune responses, T-helper subset differentiation, and immune tolerance. TLR2 forms heterodimers with TLR1, TLR6, allowing it to recognize a wide range of exogenous or endogenous molecules from pathogen and host, respectively. On the other hand, TLR-mediated signals play a role in acute allograft rejection. In this study, the expression level of TLR2 was evaluated in liver transplant patients with acute rejection in comparing with non-rejected patients. **Methods:** Adult patients who received orthotopic liver transplant enrolled in this study and divided in two groups including: 54 non-acute rejected and 51 acute rejected liver transplant patients. The EDTA-treated blood samples were collected in 1st, 3rd and 7th days post liver transplantation. In house comparative Real-time PCR method was designed for analysis of the expression level of TLR2 compared withβ-actin control gene. The expression level of

TLR2 was evaluated by Livak method (2-ΔΔcT).**Results and Conclusion:** The expression level of TLR2 was highly increased in acute rejected compared with non-acute rejected liver transplant patients all days post transplantation. Also this increase was significantly found in first 72 hour sampling time in acute rejected patients (p=0.05).Based on these results, significant increase of TLR2 was found in acute rejected patients emphasize on the important effect of IL-21 on development of pro-inflammatory responses in liver transplant patients experience acute rejection need to confirm in completed further studies.

Keywords: TLR2, Rejection, Liver, Transplantation

1872 P

Polymorphisms of the CD40, IL-18 genes and kidney transplant rejection in Iranian patients

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Background: Cytokines and costimulatory molecules are important factors determining the outcome of transplantation. Since host ability may be affected by cytokine and costimulatory genes polymorphisms, the aim of the present study was to investigate the effect of IL-18 and CD40 gene polymorphisms in outcome of kidney transplantation. **Methods:** A total of 240 kidney transplant recipients were included in this study. Alleles and genotypes frequencies for IL-18 (rs1946519) and CD40 (rs1883832) were determined in 79 acutely rejected (AR group) and 161 non-acutely rejected (non-AR group) kidney transplant recipients. IL-18 and CD40 gene polymorphisms were evaluated by PCR-RFLP methods: **Results and Conclusion:** There were no found significance associations between IL-18 and CD40 polymorphisms with acute kidney graft in AR group compared to those of non-AR group. Also, after grouping the kidney recipients according to living and cadaver donors there were no found significance associations between IL-18 and CD40 polymorphisms with acute kidney graft in AR group compared to those of non-AR group. In addition, after categorization of kidney recipients according to their gender there were no found significance associations between IL-18 and CD40 polymorphisms with acute kidney graft in AR group compared to those of non-AR group. The mentioned results indicate that there is no correlation between all genotype and alleles of CD40, IL-18 with outcome of kidney transplantation. This subject need to be studied in different population, race and ethenicity.

Keywords: IL-18, CD40, Kidney allograft, Gene polymorphism

3138 P

Immunomodulatory Effects of Adipose Tissue Derived Mesenchymal Stem Cells in Mouse Model of Type 1 Diabetes Mellitus

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Background: Type 1 diabetes mellitus is an autoimmune disorder in which insulin-secreting β-cells in pancreatic islets of Langerhans are irreversibly destroyed. As diabetes is caused by the loss of a single cell type, in recent years, cellular replacement therapy for Type 1 diabetes mellitus, has received much more attention. More recently, additional efforts have focused on the use of stem cells as sources of new β-cells. **Methods:** Since mesenchymal stem cells have immunomodulatory effects, they could be a good candidate for immunotherapy of autoimmune disease. In this study mouse model of type 1 diabetes mellitus, multiple low-dose streptozotocin (MLD-STZ), was used and the regenerative and immunomodulatory effect of adipose tissue derived mesenchymal stem cells (AD-MSCs) were analyzed. **Results:** The results indicated that intraperitoneal injection of AD-MSCs could eliminate hyperglycemia, promote islet regeneration and increase the number and size of insulin positive islets. Furthermore, the immunological analysis of splenocytes and serum cytokines indicated that intra-peritoneal injection of AD-MSCs resulted in a marked reduction of Th17 and increased Th2 cytokines. In addition, an increased CD4+CD25+FOXP3+ regulatory T cells and downregulation of the Th1 immune response were detected after intraperitoneal injection of AD-MSCs in the mouse model of type 1 diabetes. **Conclusion:** These data demonstrate that intraperitoneal injection of AD-MSCs or CM could provide a new strategy for the treatment of type 1 diabetes and other autoimmune diseases.

Keywords: Immunomodulatory effects, Type 1 Diabetes, Mesenchymal Stem Cells, Mouse Model

3175 p

High expression of perforin and granzyme B in urinary cells from renal transplant recipients during acute rejection episodes after renal transplantation

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Background: Solid organ transplantation is the most effective treatment for end-stage organ failure, but the rejection of transplanted organs still remains as a main factor which impacts onits effectiveness. Several immunological processes involvedin the occurrence ofacute rejection.Perforin and granzyme B are cytotoxic proteins of cytotoxic T lymphocytes (CTLs) which can play an important role in allograft rejection. In this study we aimed to investigate the hypothesis thatmRNA profiles of these proteins, could be used as diagnostic biomarkers during development of acute rejection. **Methods:** In this cross-sectional study we collected urine specimens from renal transplanted patients before they underwent core needle biopsy. Then, we examined the mRNA profiles of perforin and granzyme B in urinary cells of those patients using TaqMan Gene Expression Assays. **Results:** Perforin and granzyme B mRNA levels was higher in urinary cells of patients with acute rejection than recipients with other histological findings. **Conclusion:** Our results shown that measurements of mRNA levels forperforin and granzyme B could be useful tool for differential diagnosing of acute rejection of renal allografts.

Keywords: Renal Transplantation, Acute rejection, Perforin, Granzyme B

3192 P

Study of effect of supernatant of *Lactobacillus acidophilus* on proliferation of mesenchymal stem cells of rat

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Background: Nowadays, the use of probiotics harvests has been current and in the variety of fields and researches has been surveyed. For example in the immunity and cancer has been numerous researches. The effect of probiotics on the increasing the embryonic cells has been surveyed and published. Mesenchymal stem cells have pretty less growth and confined number of passages. In this research the supernatant survey of *Lactobacillus acidophilus* bacteria in terms of one of the most effective probiotics on the increasing proliferation of the mesenchymal stem cells due to enhance of the increasing them in the case of the treatment of the patients who need the graft by mesenchymal stem cells has been done. **Methods:** The survey has been done on mesenchymal stem cells separated from bone marrow of rat that it leads the second passage to the divisible stage due to admission of their mesenchymal. Afterwards, the cells treated the supernatant of *Lactobacillus acidophilus* which has been separated beforehand in the second passage and the number of the cells was measured by MTT test equated with the standard. In addition, we also brought treatment ambiance of medium of MRS broth with cells disparately and the statistical results were surveyed. **Results:** The curve of the cell's growth in different amounts was drawn and we converted the results of MTT test to the number of cell by standard curve. Then by using statistical analysis we could determine the rate of data with SPSS and their significancy with ANOVA. Our discovery represents the over effect of supernatant of *Lactobacillus acidophilus* on the light effect of the ambiance of medium of MRS broth. **Conclusion:** The use of supernatant of *Lactobacillus acidophilus* is a practical and economical method for increasing of proliferation the mesenchymal stem cells which are isolated from bone marrow.

Keywords: *Lactobacillus acidophilus*, Mesenchymal stem cells, Cell Proliferation

3087P

Effect of genetic on development of new-onset diabetes after transplantation in liver transplant recipients

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Background: New-onsetdiabetesaftertransplantation (NODAT) is a polygenic disease and KCNJ11 E23K polymorphism is considered as a diabetes-susceptibility gene. In this study, the association between KCNJ11 (rs5219) variants and the risk of developing NODAT after liver transplantation is assessed. Methods: One hundred twenty liver transplant recipients that received tacrolimus-based immunosuppressive drug were included. Patients were genotyped using polymerase chain reaction–restriction fragment length polymorphism and the incidence of NODAT was compared between different groups. Other nongenetic risk factors were also considered. Results:The KCNJ11 KK variant was associated with an increased risk for NODAT with respective odds ratios of 6.03 [95% confidence interval (CI) = 15.4-2.37; P<0.001]. Other risk factors included sex, age and body mass index. Conclusion: The polymorphism in KCNJ11

might predispose patients being treated with tacrolimus to the development of NODAT after liver transplantation.

Keywords:KCNJ11, NODAT, Polymorphism, Transplant

3308P

Immunomodularory function of Balb/c mice mesenchymal stem cells (MSC) and 3T3 fibroblasts: difference and resemblance

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Background: MSCs with inhibitory mechanism are imperative for treatment of autoimmune diseases. Contamination of MSC cultures with fibroblasts has been reported. The aim of this study was to compare the immunomodulatory function of MSCs and fibroblasts. **Methods:** Proliferative response was detected by dilution of CFSE in CFSE-labeled splenocytes in two-way ***mixed lymphocyte reaction*** (MLR). In addition, supernatants of MLR were collected and the levels of PGE2, IL-10, IFN-γ and IL-17 were analyzed by ELISA. **Results:** Physical contact of splenocytes with MSCs or fibroblasts inhibits proliferation compared to MLR in the absence of them (22.78±5.90, 40.68±6.02, and 71.15±7.25%, respectively; p=0.0001 for both). MLR supernatants in the presence of 3T3 or MSCs showed significantly higher amount of PGE2 and also reduction of IL-17 compare to control MLR, while the decrease in the levels of IFN-γ were mediated by MSCs. In the presence of culture supernatant (CS) of 3T3 or MSCs, MLR inhibition was occur only by MSCs compared to MLR without any CS (60.46±10.91, 54.95±4.63 and 71.15±7.25%; p=0.06, p=0.001; respectively). The decrease in the levels of IFN-γ were not detect in presence of CS of 3T3 cell unlike MSC compare to control MLR (4572±566 and 3220±200 versus 3903±222pg/ml; p=0.26 and p=0.05, respectively). Surprisingly in the presence of CS of 3T3 or MSCs, significant higher amount of IL-10 compare to control MLR were detected. **Conclusion:** Fibroblasts can suppress MLR only after contact with splenocytes, less significant than MSCs. Interestingly; 3T3 fibroblasts unlike MSCs were not able to reduce IFN-γ level in the MLR.

Keywords: Mesenchymal Stem Cell, Fibroblasts, Immunomodularory function,MLR.

2883P

NK Cells Activity and aGVHD in Pediatric Hematopoietic Stem Cell Transplantation

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Background: Hematopoietic Stem Cell Transplantation (HSCT) has proved to be a successful treatment for pediatric malignancies such as Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid Leukemia (AML), immune deficiency diseases as well as bone marrow disorders. Graft-Versus-Host-Disease (GVHD) has been widely known as the major obstacle to HSCT and is the main cause of mortality after transplantation. Since NK cells have a significant role in the pathophysiology of aGVHD, the present study endeavored to explain these cells activities and possible prognostic role of NK cells. **Methods**: Peripheral blood samples of 18 HSCT recipients (12 boys and 6 girls, aged between 2 and 16) were collected on (-8), (+7) and (+14) days of transplantation. 3 out of the 18 patients were diagnosed with aGVHD. On (-8), +7days and (+14) days, PBMC were collected by means of ficoll separation and analyzed by Flow Cytometry using triple antibody staining as well as control antibody . The cells were gated by CD45 antibody. Within the CD45 positive population, CD56, as NK specific marker and CD69, as NK activation marker were investigated. The collected data were analyzed by Flow Max software.**Results**: Statistical analysis of data obtained from Flow Cytometric analysis indicate that the percentage of activated NK cells in patients with aGVHD compared with the control group is significantly higher. Mean of CD56+ and CD69+ cells on -8 days in patients withaGVHDand without aGVHD showed no significant difference.

of CD56+ NK cell ٪			
CD56+	-8 day	7 day+	+14 day
aGVHD	1.56	29.2	4.57
nonGVHD	1.59	3.89	1.98

of CD56+ CD69+ NK cell ٪			
CD69+	-8 day	7 day+	+14 day
aGVHD	0.30	1.4	1.83
nonGVHD	0.37	1.28	0.64

Conclusion: NK cells are interfaces between innate and adaptive immune systems. While, the activity of NK cells can contribute to the occurrence of aGVHD, a large population of such cells in HSCT can prevent aGVHD. Hence, based on the findings of the present study, NK cell activity marked by cell surface CD56 and CD69 may predict the occurrence of aGVHD and necessary prophylactic intervention are required in such cases.

Keywords: Natural Killer Cells (NK-Cell), Acute Graft-Versus-Host-Disease (aGVHD), Cell Surface Markers CD56 and CD69, Hematopoietic Stem Cell Transplantation (HSCT).

2339P

Survival of peripheral blood neutrophils following treatment with soluble factors from rat Mesenchymal Stem cells

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Background: Mesenchymal Stem Cells have extensive potential to proliferate and differentiate into different cell lineages. This study was done to investigate the effect of supernatant of MSCs on the neutrophils survival. **Methods:** MSCs was isolated from rat (6-8 weeks) femoral and tibial bone marrow and cultured in DMEM. Then maturation MSCs, its supernatant were incubated with neutrophils isolated from peripheral blood of rat at 37 ° C for 1 h. Neutrophil survival was measured at 6 and 24 h incubation with supernatant of MSCs by flow cytometric analysis using An/PI. Data were analyzed by SPSSsoftware, one-way ANOVA followed by Tukeytest at a significance level of ($P<0.05$).**Results:** 6-hour incubation of neutrophils with a supernatant of MSCs, the healthy cells in the cell percentage was significantly increased and decreased the amount of necrosis, but there was no significant decrease in apoptosis compared to controls ($p<0.05$). The 24-hour incubation of neutrophils with cell supernatant significantly increased the percentage of healthy cells and apoptosis was reduced compared to the control group, and reduced cell necrosis was not significant in the treated groups than in control ($p<0.05$).**Conclusion:** The interactions between MSCs and immune cells and provide mechanisms likely involved with the in vivo MSC-mediated could be therapeutic implication for immune-mediated, immunodeficiency diseases and cell therapy.

Keywords: Soluble factors MSCs, Neutrophil, Survival

2338P

Evaluation of peripheral blood neutrophil survival following treatment with Mesenchymal stem cells in rats

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Background: Because of theirmultipotency and ease of purification, amplification and immunomodulatory properties Mesenchymal stem cells (MSC), are anideal stem cell source for cell therapies. This study was done to investigate the effect of Rat MSCs on the survival neutrophils.**Methods:** MSCs was isolated from rat (6-8 weeks) femoral and tibial bone marrow and cultured in DMEM. Then maturation MSCs were incubated with neutrophils isolated from peripheral blood of rat at 37 ° C for 1 h. Neutrophil survival was measured at 6 and 24 h incubation with of MSCs by flow cytometric analysis using An/PI. Data were analyzed by SPSSsoftware, followed by Tukeytest at a significance level of ($P<0.05$).**Results:** The 6-hour incubation of neutrophils with MSCs, significantly reduced the percentage of normal cells and increased apoptosis compared to controls and increased cell necrosis was not significant in the treated group than in control.The 24-hour incubation of neutrophils with MSCs, significantly increased the percentage of healthy cells and apoptosis was reduced compared to the control

group, and reduced cell necrosis was not significant in the treated groups than in control ($p<0.05$).**Conclusion:** In spite of the clinical importance of MSCs, some biological aspects of them including their self-renewal, proliferation and immune modulatory effects are of great therapeutic potential for cell therapy.
Keywords: Mesenchymal stem cells, Neutrophil, Survival

2926 P

Evaluation of Different Mesenchymal Stem Cells Passages on the Expression of Specific Cell Surface Markers in Bone Marrow, Cord Blood and Adipose Tissue

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Background: Mesenchymal Stem Cells (MSCs) found in many adult tissues are an attractive stem cell source for the regeneration of damaged tissues in clinical applications.Although no significant differences concerning morphology in Mesenchymal Stem Cells derived from different sources were obvious, differences might be observed concerning Immunophenotypic and functional properties during different passages. **Methods:** Mononuclear Cells (MNCs) were isolated from Umbilical Cord Blood, Bone Marrow and Adipose Tissue. Adhered cultured cells in DMEM were isolated and purified and stained with CD34, CD45, CD29, CD90, CD105 and HLA-DR and analysed via flow cytometry during three passages. **Results:** According to flow cytometry analysis Human Mesenchymal Stem Cells isolated from three sources were highly positive for CD90, CD105 and CD29 and there were no significant differences during three passages.MSCs were also negative for hematopoietic lineage markers such as CD34, CD45 and HLA-DR during different passages. **Discussion:** In summary we can conclude Immunophenotypic characteristics of all three sources of MSCs had no significant differences along three passages and all three sources represented identical features. According to advantages and disadvantages of three sources, seems Adipose Tissue is a promising source that can be obtained by a less invasive method and in a larger quantity that grow easily.
Keywords: Mesenchymal Stem Cell, Bone Marrow, Adipose Tissue, Cord Blood, Cell Surface Markers, Passage

3052 P

Mouse AD-MSC isolation & surface markersanalysis by flowcytometry&differentiation toosteogenic and adipogenic

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Background: Mesenchymal stem cells (MSCs) are stromal cells with potent regenerative and immunomodulatory properties. They are found in multiple tissues, including bone marrow and adipose tissue. In this research we isolate MSCs from peritoneal fat tissue and analyze the cell surface marker and adipogenic and osteogenic differentiation. **Methods:** Mesenchymal

stromal cells (MSC) wereisolated by enzymatic digestion of peritoneal fat tissue from BALB/C mouse and were cultured in DMEM medium supplemented with 10% FBS.After two passages, cell surface markers of MSCs(CD45,sca1, CD11b, CD-90, CD-44, CD-105, CD73, CD29 and VEGFR) analyzed by flowcytometry method.For osteogenic differentiation, 20000 cells per well of four well plate were incubated in medium supplemented with 10^{-7} M dexamethasone, 50 µg/ml ascorbic acid bi-phosphate and 10 mMβ-glycerol phosphate. After 30 days the cultures were fixed with 70% cold ethanol for 1 h at room temperature, and incubated with Alizarin Red S. For adipogenic differentiation, 60000 cells per well of four well plate were incubated in medium supplementedwith 10^{-7} M dexamethasone and 0.5 Mm IBMX,66nM Insulin and 0.2 Mm Indomethacine.After 30 days, the cultures were fixed in 3% formaldehyde in PBSfor 10 minutes and stained with Oil Red O.**Results**:According to Flow cytometric analysis, 14.24%, 73.95%, 99.52%, 87%, 15.45%, 60.93%, 2.46%, 10.26%, 19.79% of AD-MSCs express CD11b, CD105, CD29, CD44, CD73, CD93, CD45, Sca-1 and VEGFR respectively. Culture of MSCs in adipocyte-differentiation media showing cells containing drops of fat revealed by Oil Red O & in osteogenic-differentiation media showing the formation of calcium containing precipitates stained by Alizarin Red S.**Conclusion:**In this experiment we confirmed the quality of isolated AD-MSCs according to the cell surface marker and differentiation potential.

Keywords: Osteogenic, Adipogenic

3173 P

Effect of progesterone on membrane-bound HLA-G in adipose tissue mesenchymal stem cells

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Background: Mesenchymal stem cells (MSCs) are multi-potent progenitor cells with remarkable immunosuppressive properties. The immunomodulatory effects of MSCs is mediated by indoleamine 2, 3-dioxygenase (IDO), prostaglandin E2 (PGE2), nitric oxide (NO) and histocompatibility leukocyte antigen-G (HLA-G). Progesterone (P4) effects have been profound on the regulation of immune responses, but theP4 effects on the MSCs remains unknown. Therefore, in this study, we investigated the P4effects on membrane-bound HLA-G ofMSCs.**Methods:** AT-MSC cells were isolated fromhuman adipose tissue. Cells were cultivated in 75 cm2 flasks.100,000 cells were placed into eachwell of 24-well plates and cultured with or without P4at the concentrations of 1×10^{-5} and3×10^{-5} M for 12, 24, and 48 hr in phenol red-free DMEM/F12, 10% fetal bovine serumand 1% antibiotic. ForHLA-G analysis, we used the mouse anti HLA-G1/G5MEMG/9 FITC antibody andisotypic controls for all samples.**Results**:We found thatmembrane-bound HLA-G can be detected on 10.7±1.3% of MSCs by flowcytometrywithout P4; HLA-Gpositive cells were increased about 13-18% at the1×10^{-5} and3×10^{-5} Mconcentrations of P4 within 3 days. P4 enhanced immunomodulatory

function ofMSCs through Up-regulation of HLA-G and it was dose-dependent.**Conclusion:** It seems that HLA-G expression can be modulated by hormones. These results emphasize the potential effects of MSCs as a relevant therapeutic candidate in transplantation.
Keywords: Mesenchymal stem cell, Immunosuppression, Histocompatibility locusantigen-G (HLA-G), Progesterone

3112 P

Production of insulin producing cells from human bone marrow mesenchymal stem cells for treatment of type I diabetes

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Background: Type I diabetes is an immunologically-mediated devastation of the insulin producing cells (IPCs) in pancreatic islet. The use of stem cells for producing β cells is new promising tool. Adult stem cells such as mesenchymal stem cells (MSCs) are multipotent cells and have self-renewal capacities that capable them differentiating to ectodermal ,mesodermal, and endodermal tissues. Pancreatic and duodenal homeobox factor 1 (PDX1), is a master regulator gene for the embryonic development of the pancreas and is crucial for normal pancreatic islet function in adult. **Methods**: In this study to generate IPCs we induced the overexpression expresses of PDX1 in hMSCs by lentivral vectors. After being infected with Lenti-PDX1, hMSCs were successfully induced to differentiate into IPCs.**Results:** 21 days after induction, islet-like clusters containing insulin producing cells (IPCs) were confirmed by dithizone (DTZ) staining. The differentiated PDX1^{+} hMSCs expressed multiple islet cell genes like, Insulin, and glucagon that detected by qRT-PCR. In addition insulin and C-peptide expression was confirmed by immonocytochemistry staining. Glucose challenge test was performed at different concentrations of glucose so extracellular and intracellular insulin and C-peptide assayed using ELISA. Conclusion: These finding demonstrate the utility of manipulating PDX1 expression as an important new strategy for the efficient generation of functionally IPCs from hMSCs.
Keywords: IPCs, type I diabetes

3432 P

Generation of Human Induced Pluripotent Stem Cells by Reprogramming of Adult Human Fibroblasts with a Four Transcription Factor, Doxycycline Inducible Lentiviral Transduction System

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Background: Pluripotent cells, such as embryonic stem cells, can potentially serve as a source of cell- and tissue replacement therapy. Rejection after transplantation of cells and tissue derived from embryonic stem cells is a significant obstacle to their clinical use. Recently,

human somatic cells have been reprogrammed directly to pluripotency by ectopic expression of four transcription factors (Oct4, Sox2, Klf4 and Myc) to yield induced pluripotent stem (iPS) cell. Human iPS cells are a potential source of patient-specific pluripotentstem cells that would bypass immune rejection. **Methods:** Human induced pluripotent stem cells were generated by reprogramming of patient-specific fibroblasts through ectopic expression of four transcription factors (Oct4, Sox2, Klf4 and Myc) bydoxycycline inducible lentiviral transduction system. IPS cell colonies werecharacterized by ICC, RT-PCR, Alkaline Phosphatase staining, Teratoma formation. **Results:** After transduction of fibroblasts, expression of OCT4 and KLF4 were detected in cells treated with doxycycline. Sixteen days post infection iPS colonies were picked and displayed typical pluripotency marker expression of Alkaline Phosphatase, Nanog, SSEA4, Tra1-60.**Conclusion:** Type 1 diabetes (T1D) is the result of an autoimmune destruction of pancreatic β cells. Induced pluripotent stem (iPS) cells can be generated from patients with T1D by reprogramming their adult fibroblasts with 4 transcription factors (OCT4, SOX2, KLF4, c-MYC). T1D-specific iPScells have the hallmarks of pluripotency and can be differentiated into insulin-producing cells. These results are a step toward using DiPS cells in T1D disease modeling, as well as for cell replacement therapy.

Keywords: Induced pluripotent stem (iPS) cells

3254 P

Secretom derived from Nrf2- engineered mesenchymal stem cells protects MSCs against oxidative stress

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Background: Recent studies have proposed cell therapy as a promising therapeutic strategy for treatment of many types of disease. Among different cell types, MSCs have been explored as therapeutic tools for variety of diseases including neurodegenerative diseases. However, massive cell death during few days after transplantation limits their effectiveness and clinical usage. Hence, development of strategies to improve cell survival in vivo is a major challenge. For overcoming this limitation, genetically modification of MSC by cytoprotective genes has recently been shown to be highly efficient method but there are still concerns to use them in clinical trials. Therefore, we hypothesized that culture of MSCs in the presence of secretome of genetically manipulated cells by one of the cytoprotective gene i.e. Nrf-2 maybe improve cell survival.**Methods**: In this study, we manipulate bone marrow derived mesenchymal stem cells with nuclear factor erythroid 2-related factor 2 (Nrf2) gene. Then we cultivate another group of MSCs in the secretome derived from Nrf2 manipulated cells. Next, the viability and apoptosis of these cells have evaluated following oxidative stress exposure. **Results:**Secretome derived from Nrf2 manipulated cells protect MSCs against cell death and the apoptosis induced by oxidative stress conditions.**Conclusion:**Our results suggested that cultivation of MSCs in the presence of secretome derived of Nrf2-MSC improve cell survival. Our findings would be used as a novel strategy for enhancing MSC-based cell therapy especially in neurodegenerative diseases such as MS and open new window for clinical application of MSCs.

Keywords: MSCs, nuclear factor erythroid 2-related factor 2

1758P

Morphologic changes in direct cover vitrified ovaries after outotransplantation

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Background: Many attempts have done to improve cryopreservation of mammalian ovaries using simple, economical and efficient technique "vitrification". The aim of the present study was to evaluate the morphology of autotransplanted mouse ovaries after cryopreservation by direct cover vitrification. **Methods:** Ovaries from 6-8 week old NMRI mice was removed with anesthesia, then the ovaries was divided 2 groups. Left ovary was outotransplanted to same mice and right ovary was vitrified with direct cover vitrification (DCV) method.
vitrification solutions be prepared in 3 concentration:
1) DPBS containing 5% Ethylen qlycol + 5% DMSP+ 0.5 mol sucrose +20% FBS (DCV1)
2) DPBS containing 5% Ethylen qlycol + 10% DMSP+ 0.5 mol sucrose +20% FBS (DCV2)
3) DPBS containing 10% Ethylen qlycol + 5% DMSP+ 0.5 mol sucrose +20% FBS (DCV3)
Ovaries were stored in liquid nitrogen for 1 week. For thawing, the ovaries were warmed at 25°c water and 1, 0.5, 0.25 ml sucrose and then equilibrate with culture medium. Then the vitrified and fresh ovarian tissues were autografted intraperitoneally. After 1 month the animals were sacrificed and ovarian tissue was evaluated by LM (Light Microscope). **Results:** Ovarian tissue frozen in DCV3 group retained a higher percentage of morphologically normal follicles or viable follicles than tissue frozen in other DCV groups after autotransplantation ($P<0.001$). **Conclusions:** Direct cover vitrification by using 10%EG appears to prevent ice crystal injury and is more efficient for cryopreservation of mouse ovarian tissue after autotransplantation.

Keywords: Ovarian tissue, Direct cover vitrification, Transplantation

Vaccine & Vaccine Development

Oral Presentations:

2107O

Improving long peptide vaccine potency by using a strategy that enhances CD4+ T help in BALB/c mice.

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Background: CD4+ T helper cells are known to play an important role in the generation of CD8+ T-cell immune responses. In previous study, we have shown that co-administration of rHER2/neu synthetic long peptide vaccine with CpG-ODN adjuvant was able to generate antitumor immunity in vaccinated female BALB/c mice. In current study we used an additional strategy to improve vaccine-specific CD8+ T-cell immune responses and enhancing the vaccine potency.**Methods:** BALB/c female mice (n=9 per each group) 3 times were subcutaneously vaccinated with rHER2/neu-specific CTLs conjugated with epitopes (p5 and p435) constructing long peptide that was designed in our previous study in combination with a strategy to enhance CD4+ T help using a universal Pan DR epitope (PADRE) and CpG-ODN. 14 days after last vaccination three of mice per each group were euthanized and immune responses studied in their spleens for assessment of TCD4 and TCD8 subpopulation by flow cytometry and IFN-g secretion by Elispot. The remaining six mice challenged by live TUBO cell line and were followed for tumor size and survival.**Results**: We observed that mice vaccinated with long peptide in combination with PADRE peptide and CpG-ODN generated better specific TCD8+ IFNγ+ and TCD4+ IFNγ+ immune responses as well as significantly improved anti-tumor effects against TUBO tumors compared to long peptide-based vaccine with either PADRE peptide or CpG alone.**Conclusion:** The combination of TCD4+-induced PADRE peptide and CpG with antigenic long peptide is capable of generating potent antigen-specific CD8+ T cell immune responses and antitumor effects in vaccinated mice.

Keywords: Her2, Long peptide vaccine, PADRE peptide, CpG

1996O

Enhanced protective efficacy of nonpathogenic recombinant Leishmania tarentolae expressing cysteine proteinases combined with a sand fly salivary antigen

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Background: Novel vaccination approaches are needed to prevent leishmaniasis. Live attenuatedvaccines are the gold standard for protection against intracellular pathogens such asLeishmania. The nonpathogenic tohuman lizard protozoan parasite, Leishmaniatarentolae, has been used effectivelyas a vaccine platform against visceral leishmaniasis in experimental animal models.Correspondingly, pre-exposure to sand fly saliva or immunization with a salivaryprotein has been shown to protect mice against cutaneous leishmaniasis. Here, wetested the efficacy of a novel combination of established protective parasite antigensexpressed by L. tarentolae together with saliva antigens as a vaccine strategy againstL. major infection. Different DNA/Live and Live/Live prime-boost vaccination modalitieswith live recombinant L. tarentolae stably expressing cysteine proteinases (type I andII, CPA/CPB) and PpSP15, an immunogenic salivary protein from Phlebotomuspapatasi, a natural vector of L. major, were tested in both susceptible BALB/c andresistant C57BL/6 mice.**Methods**: The immunogenicity and protective efficacy of the livevaccine L. tarentolae expressing CPA/CPB in the presence or absence of DNA plasmidencoding the sand fly salivary protein PpSP15 was tested in resistant and susceptiblemice strains. Both humoral and cellular immune responses were assessed beforechallenge, at 3 and 10 weeks after Leishmania infection.**Results**: In both strains of mice, thestrongest protective effect was observed when priming with PpSP15 DNA and boostingwith PpSP15 DNA and live recombinant L. tarentolae stably expressing cysteineproteinase genes. **Conclusion**: The present study is the first to use a combination ofrecombinant L. tarentolae with DNA plasmid encoding a sand fly salivary antigen(PpSP15) and represents a novel promising vaccination approach againstleishmaniasis.

Keywords: Recombinant L. tarentolae, Cysteine proteinases, Salivary Gland antigen, L. major, protective efficacy

2096O

PD-1 blockade increases telomerase activity in stimulated CD57-CD45RO+CD8+ memory T cells of patients with symptomatic Herpes Zoster infection

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Background: PD-1, an inhibitory surface receptor, is by far the most respected marker of exhausted memory and effector CD8+ T cells which hampers the development of therapeutic vaccines and adoptive immunotherapy in chronic diseases. We investigated the PD-1 levels on

the IE62- and IE63-specific CD8+ T cells of patients with reactivated zoster and the effect of PD-1 pathway blockade on the telomerase activity in CD57-CD45RO+CD8+CD3+ memory T cells.**Methods:** Heparinized blood was obtained from 9 patients and 5 asymptomatic individuals with a history of chicken pox. PBMCs were stimulated with VZV IE62 and IE63 peptide pools. The expression of CD3, CD8, CD137 and PD-1 molecules was analyzed by flowcytometry. Non-senescent CD57-CD45RO+CD8+CD3+ memory T cells were purified by MACS negative selection and stimulated with anti-CD3/anti-CD28 with or without PD-1 blockade. The telomerase activity of the stimulated cells was evaluated by a PCR-ELISA assay.**Results:** PD-1 molecule was highly expressed in CD8+ T cell population. The frequency of PD-1+ and CD137- cells in total CD3+CD8+ T cells of patients was increased compared to controls and peptide stimulation did not affect their frequency. Telomerase activity of non-senescent CD57-CD45RO+CD8+CD3+ memory T cells in symptomatic patients was lower than that of controls. Blockade of PD-1 at the time of stimulation increased telomerase activity of non-senescent CD57-CD45RO+CD8+CD3+ memory T cells. The highest telomerase accelerating effect of blocking PD-1 was observed in the presence of CD28 co-stimulatory signals accompanied by increased CD137 expression.**Conclusion:** We showed that low telomerase activity of the patients with reactivated zoster could be overcome by blocking PD-1 signaling pathway at the time of TCR stimulation. Targeting PD-1 may be useful to recover effector function of exhausted CD8+ T cells in VZV infection and may be a promising strategy for specific immunotherapy of the disease.

Keywords: PD-1, CD57-CD45RO+CD8+ memory T cells, Herpes zoster

2003O

Evaluation of recombinant L.tarentolae harboring immunogenic protein of sandfly as an experimental vaccine in BALB/C mice against L.major infection

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Background: Leishmaniasis is a zoonotic vector born disease. During transmission of infectious parasite by sand flies, some of their saliva get access into the skin of mammalian host. Sand fly saliva contains different proteins that one of them is named as PpSP15. The aim of this study is to use recombinant live non-pathogenic L. tarentolae harboring PpSP15 as a new vaccine strategy in BALB/c mice infected with L. major.**Methods**: The L. tarentolae Tar II (ATCC 30267) strain was grown and transfected. Integration of the expression cassette into the ssu locus was confirmed by diagnostic PCR. The presence of PpSP15-GFP genes in L.tar pLEXSY-neo2-PpSP15-GFP was confirmed both at the level of DNA and RNA. At the level of protein, we used western blotting. The efficacy of this novel strategy is tested in different groups of BALB/c mice vaccinated with different modalities such as DNA/Live and Live/ Live prime-boost vaccination with live recombinant L. tarentolae stably expressing PpSP15. All groups were challenged with L. major+SGH. In different time periods, both humoral and cellular immune response are measured.**Results:** In this study, we first confirmed the level of PpSP15 expression in recombinant L. tarentolae-SP15. Before infectious challenge, the level of humoral and cytokine expression in different modalities showed the immunogencity of prepared recombinant L. tarentolae-SP15. C**onclusion:** Although, we are working on

protective efficacy of this novel strategy but expected to have the enhancement of Th1 immune response in a way to control and inhibit the parasite propagation.
Keywords:L. tarentolae- SP15, vaccine

1847O
Evaluation of immunogenicity and protective efficacy of a divalent DNA vaccine encoding TOmp31 and L7/L12 Brucella antigens

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Background: Brucellosis is the most common bacterial zoonotic disease worldwide and it is endemic in many developing countries including Iran. In humans, the most common causes of brucellosis are B. melitensis and B. abortus. The Omp31 and L7/L12 are known as conserved and immunodominant antigens in human Brucella pathogens.**Methods:** This study was designed to obtain the truncated Omp31 and fusion form of it with L7/L12 (L7/L12- TOmp31) using bioinformatics tools. This constructs are designed to evaluate the immunogenicity and protective/crossprotective efficacy of DNA vaccine against human Brucella pathogens. The fusion was cloned in pcDNA3.1 and PEGFP-N1 vectors. Transient expression of the fusion was performed by transfection of the COS-7 cells with PEGFPN1-L/L12-TOmp31. Six-eight week-old female BALB/c mice were anesthetized and immunized by the footpad route+electroporation with pcDNA-L7/L12-TOmp31 or empty pcDNA as a control. Each mouse was immunized and primed at day 0 and boosted at day 21. Two Positive control groups were immunized with B. melitensis Rev-1 and B.abortus RB5, respectively. The negative control group was inoculated with PBS. Sera were obtained at days 0,21,42.
Results and Conclusion: The C-score, structure Z-score and Ramachandran Z-score of the truncated Omp31 protein structure were -0.53,-0.72 and -0.98 respectively. The C-score, structure Z-score and Ramachandran Z-score of the L7/L12-TOmp31 fusion protein structure were -3.55, -4.95 and -0.71 respectively. The fusion construct was cloned in pcDNA3.1 and the expression of the fusion protein was confirmed successfully in vitro after transfection into COS-7 cells. The resulting transfection yielded 36.6% of the COS-7 cells that expressed L/L12-TOmp31. The analysis of immune responses, including protection experiments and cytokine production are underway.
Keywords: Brucella, DNA vaccine, Fusion,L7/L12,Omp31

1608O
Evaluation of cellular immune responses to truncated ORF-2 protein derived from HEV capsid protein in the peripheral blood of individuals recovered from hepatitis E infection

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Background: Hepatitis E virus (HEV) causes acute hepatitis in one-third of the world's population, and has high mortality rate of up to 30% among pregnant women. Therefore, primary prevention of the HEV infection is essential. The aim of this study was to obtain well yield of the highly purified truncated ORF2 protein, and to evaluate its immune responses. **Methods:** Recombinant plasmid pET-30a-ORF2.2 (ORF2.2, encoding 112-608 amino acid sequence of capsid protein) was constructed and used for transformation of Escherichia coli BL21. The recombinant protein was highly expressed and purified by Ni^{2+}-chelate-affinity chromatography (Qiagen, Germany). The expressed and purified protein was used to stimulation of PBMCs isolated from HEV recovered individuals and control group. Immunological activity of the recombinant protein was evaluated by ELISA cytokine (IFN-γ, IL-4, IL-10, IL-12), IFN-γ ELISpot, and Cell proliferation assay.**Results:** The highest expression was induced by adding IPTG at a final concentration of 1 mM at 37°C for 4 hr. The SDS-PAGE analysis showed a band about 55 kDa. The recombinant protein was able to activate T-cell responses and stimulate production of Th1-type cytokines in PBMCs isolated from both recovered and control individuals. These patterns were also seen in IFN-ELISpot and proliferation assay. **Conclusion:** The optimized truncated ORF2 protein was expressed in E. coli successfully and well yield of the highly purified protein was obtained, which was able to stimulate human type 1 T helper cells. This protein may be used in the future as a potential vaccine candidate against hepatitis E infection.

Keywords: Hepatitis E virus, truncated ORF2, Optimization, Expression, Escherichia coli, Purification

2022O

Effects of immunization of cattle with salivary gland extract of Rhipicephalus(Boophilus) annulatus tick on biological parameters of ticks

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Background: Previously, several researches have been conducted to examining the feasibility of artificially immunizing cattle against tick infestation using various types of crude tick extracts.

The aim of this study was to assess the effects of immunization of cattle with immunogenic proteins of salivary gland extracts of Rhipicephalus (Boophilus)annulatus tick on biological parameters of ticks.**Methods:**From total 32 protein bands of salivary gland extract of Rhipicephalus(Boophilus)annulatus tick , recognized by SDS-PAGE, 22 bands were identified as immunogenic proteins by Western-blot analysis and 14 bands being more dominant were eluted from polyacrylamide gel. Test group was injected intradermally with eluted proteins with equivalent amount of adjuvant every two weeks for a total of three times and control group was injected with sterile PBS(pH:7.2) instead of eluted proteins. Four weeks after last injection, each cattle were infested with approximately 500 tick larvae. Engorged female ticks were collected, counted, weighed and maintained in incubator to observe tick performance

parameters.**Results:** The results indicated immunization of cattle resulted in reduction in mean tick counts, attachment, engorgement weights, feeding index, egg mass weight, hatchability and fertility index (respectively 63.1%, 62.6%, 30.2%, 36.4%, 40%, 78.7% and 13.3%) and increased duration of feeding, preoviposition and incubation period of eggs (respectively 8.6%, 45 and 31.34%). All changes were statistically significant in comparison with control group.**Conclusion:** This investigation indicates immunization of cattle with these antigens could induce a protective immune response against Rhipicephalus(Boophilus)annulatus tick that would be expected to provide a safe nontoxic means of tick control.

Keywords: Rhipicephalus(Boophilus)annulatus, immunization, salivary gland

2392O

Non variant antibodies to different allelic forms of Plasmodium vivax duffy binding protein: implication for P. vivax vaccine development

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Background: Region II of Duffy binding protein (DBP-II) is a promising vaccine candidate of Plasmodium vivax. However, due to the presence of antigenic diversity in this antigen, it may be necessary to develop a polyvalent vaccine that would increase the cost of developing vaccines. Therefore, the main objective of this study was to define the frequency of Pvdbp-II haplotypes in malaria hypoendemic regions of Iran and then to investigate variant/non variant specific antibody responses to the circulating PvDBP-II forms.Methods:Blood samples were collected from 202 P. vivax infected patients from Chabahar district. Five distinct variants of PvDBP-II antigens were cloned and expressed in E.coli M15 and naturally occurring IgG antibody to these proteins was evaluated in malaria patients' sera using ELISA. To determine antibody responses to conserved/shared sites of the selected variant forms of PvDBP-II in P. vivax-infected human sera, an antibody depletion assay was performed in 20 corresponding patients' sera.Results:The prevalence of IgG antibodies to all five PvDBP-II variant forms was equal (42.1%) in the studied patients' sera (P> 0.05, Cochran's Q test). Sequence analysis in 20 selected samples showed 8 distinct haplotypes and immunodepletion results showed that the Iranian malaria patients induced anti-PVDBP-II responses to conserved/shared epitopes of all examined PvDBP-II variants.**Conclusion:** High degree of cross-reactive antibody responses to heterologous PvDBP-II variants was found in Iranian P.vivax infected individuals. Therefore, polymorphisms in PvDBP-II antigen could be less important and one variant of this antigen may be sufficient to be included in PvDBP-II-based vaccine.

Keywords: Malaria, Plasmodium vivax, Duffy binding Protein, Vaccine, Immuno-depletion ELISA

2393O

Bacterial-expressed recombinant Plasmodium falciparum Apical Membrane Antigen 1: Impact on Functional Immune Responses to a Malaria Vaccine Candidate

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Background: The Plasmodium falciparum apical membrane antigen1 (PfAMA1) is a leading malaria vaccine candidate antigen for inclusion in a polyvalent malaria vaccine. Vaccination against immunogenic and functional molecules of the parasite life cycle has the potential to reduce malaria in the elimination and eradication programmes. Besides, subsequent alterations of antigen integrity and/or stability compared to the native molecule are likely to affect the functionality of immune response towards the recombinant protein. Therefore, to have the functional immune response to recombinant PfAMA1 in both malaria vaccine development and sero-prevalence studies, the bacterial-expressed recombinant PfAMA-1 was immunologically evaluated.**Methods:**The almost full length of the Pfama1 gene was cloned and expressed successfully in E. coli M15. The recombinant protein was purified with metal affinity chromatography and the activity of purified recombinant protein was determined by SDS-PAEG (under both reduced and non-reduced conditions), Western blot, immunofluorescence (IFAT) and ELISA methods. **Results:**Analysis of expressed PfAMA1 showed that it had the predicted primary sequence, and tertiary structure analysis, confirmed its compact disulfide-bonded nature due to correct refolded after purification. In addition, data showed that the recombinant PfAMA-1 was detected in Western blot (under reduced and non-reduced condition) and mice antibodies made to the protein recognized the native parasite PfAMA1 in IFAT. Finally, the recombinant protein was detected by >74% of patient sera in ELISA.**Conclusion:** The present result suggests that the expressed protein is immunogenic and in correct folding. Therefore, it is suitable as antigen in both malaria vaccine development and sero-prevalence studies.

Keywords: Plasmodium falciparum, Apical Membrane protein-1, Recombinant expression, Vaccine, Sero-prevalence

2461O

The oral chimeric vaccine candidate Omp19-Omp31 induces protection against Different *Brucella* spp challenges by inducing IL-17 immune response

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Background: *Brucella* (B) species are the causative agents of brucellosis, the world's most prevalent zoonatic disease. The results of brucellosis are abortion, reduction of milk production and infertility in infected domestic animals and transmission probability to human. Since entry site of *Brucella* to the body is mucosal sites, the immunization of these sites by administration of oral vaccines can be a proper strategy for protection against this pathogen. Previous studies have shown *B. abortus* Omp19 as oral vaccine can result in protection against

B. abortus, *B. suiss* and *B.melitensis*. Additionally, vaccination by *B. melitensis* Omp31 has resulted in protection against *B. ovis* and *B. melitensis* in mice and sheep. **Methods:** Omp19 and Omp31 were attached by hydrophobic linkers in chimeric construct. For high expression in *E. coli*, the construct codons were optimized. The secondary and tertiary structures, protein stability and solubility, antigenicity, primary sequence analysis, B-cell and T-cell epitopes of the construct were predicted by bioinformatics tools. Cloning, expression and purification of recombinant protein, nanoparticle preparation, characterization of antigen-loaded N-trimethyl chitosan Nanoparticles, mice immunization in oral and intraperitoneal routes, ELISA, cytokine and protection assay were performed. **Results:** The construct had many linear and spatial B-cell epitopes, MHC class I and II binding peptides. Mice orally administered with chimeric nanovaccine showed higher antibodies and cytokine titers and protection level than mice intraperitoneally administered.**Conclusion:** The results show the chimeric construct can be a proper oral vaccine candidate against different*Brucella*spp for application in domestic animals.

Keywords: Oral Vaccine, Brucellosis, Chimeric Construct

2568O

The protective effect of *Clostridium botulinum* type Aneurotoxin-associated protein (HA-33) on *Clostridium botulinum* type E recombinant candidate vaccine in oral administration

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Background: The complexes of botulinum neurotoxins (BoNTs) consist of neurotoxin and neurotoxin-associated proteins. There are two kinds of associated proteins: non-toxic hemagglutinin (NTHA),and non-toxic non-hemagglutinin (NTNHA). Hemagglotinin proteins cover the complex and apparently have the main protective effect of the toxin in gastrointestinal harsh conditions. In BoNT/A, HA-33 is placed in the outer surface of the complex. The protein has also a role in the delivery of the toxin complex. These roles persuade us to investigate the protective effect of the protein from the subunit vaccines in oral administration. So, we chose a subunit candidate vaccine of *Clostridium botulinum* type E (the recombinant binding domain) as a subunit vaccine model.**Methods:** First, HA-33 and the binding domain of *Clostridium botulinum*type E proteins were expressed and purified. Then, they were combined in a 1:1 w/w proportion and wereadministered orally and intraperitoneally.We, ofcourse, applied both positive and negative controls.The antibody titer of each group of mice was obtained by ELISA test after a weak of each administration. After immunization period, the immunized mice were challenged by activated BoNT/E.**Results:** Antibody titers,obtained by ELISA, showed a fairly good response of antibody to the candidate vaccinewhen the vaccine was administrated orally in combination with HA-33. The immunized mice were able to survive after they were fed with 10 LD50 of the activated toxin.**Conclusion:** The results show a fairly good protective effect of HA-33 protein from *Clostridium botulinum* type E recombinant candidate vaccine in oral administration.

Keywords: Oral Administration, *Clostridium botulinum*, HA-33

3270O

Expression Fusion of C-terminal fragment of Mycobacterium tuberculosis HSP70 to NA in pFASTBAC to enhances specific immune responses

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Background: Although most influenza vaccines are produced in eggs, new types of vaccines must be developed. In this study, the immunogenicity and safety of a baculovirus-expressed neuraminidase (NA)of H1N1 influenza virus and fusion with of C-terminal fragment of HSP70 ,Heat shock proteins (HSPs) are capable of promoting antigen presentation of chaperoned peptides through interactions with receptors on antigen presenting cells. **Methods:** Full-length gene NA-HSP70 was amplified by PCR and cloned into shutter vector pFASTBAC, which was cotransformated with DNA into E. coli DH10 and generated recombinant bacluvirus was produced by transfecting sf9 cell line , Expression levels were validated by western blot analysis, and the induced immune efficacy in mice were analyzed. **Results:** The integration of NA-hsp70 in the pFASTBAC and its expression were confirmed by PCR and Western-Blot assays respectively. After intranasal immunization, the serum IgG was induced at a titer of 1: 1000 and 1:100 000 in BALB/c mice at primary and secondary immunization respectively. **Conclusion:** recombinant pFASTBAC NA-hsp70 was successfully constructed and it could induce desired immune efficacy and enhance immune responses.

Keywords: neuraminidase (NA); Heat-shock protein 70; Protein vaccine; Humoral immunity: influenza virus

2979P

Comparison between memory B cell populations induced by botulinum toxin binding domain and tetanus toxin binding domain in mice model

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Background: Although, Botulinum neurotoxin and Tetanus neurotoxin produce by clostridia genus but they have different mechanism of action. Also, they have 36% identity in their proteins but differ in induction of immunological memory in human. Tetanus toxoid induces long term memory (10 years) but botulinum toxin only induces a midterm memory (2 years) after vaccination. The aim of this study is flow cytometery analysis and measurement of memory B cells populations after mice immunization with Tetanus toxin binding domain and botulinum toxin binding domain. **Method:** Recombinant Bont Hc and THc proteins were expressed in optimized conditions and then purified. Afterward, mice were immunized with rBont Hc or THc. After six months, mice spleen cells were extracted; stained with antibodies against CD19, IgD, and IgG and analyzed by flow cytometry. **Results:** SDS-PAGE gel was

confirmed expression and proper purification of rBont Hc subunit and THc protein. ELISA data was shown no difference between antibody level against rBont Hc and rTHc in mice. After six months, memory B cell populations in Bont Hc immunized mice was higher than memory B cell population induced by THc. **Conclusion:** Although, specific memory B cells was seen in Bont Hc and THc protein immunize mice; but it seems THc has a more potency to induction immunological memory in compare with Bont Hc protein. In other word, antigen nature has a pivotal role in immunological memory induction.

Keyword: Botulinum toxin binding domain (Bont HC), Tetanus toxin binding domain (THc), Memory B cell, Flow cytometry

Poster Presentations:

2110P

CpG Immunostimulatory Oligodeoxynucleotide 1826 Enhances Antitumor Effect of rHER2/neu -synthetic long peptide in female BALB/c Mice

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Background: Human epidermalial growth factor receptor 2(HER2) is one of the most attractive targets for immunotherapeutic approaches in breast cancer patients. In this study we aimed enhancement of immunogenicity and efficacy of rHER2/neu-synthetic long peptide (SLP) in combination with CpG-ODN 1826 in BALB/c mice model.**Methods:** BALB/c female mice 3 times were subcutaneously vaccinated with rHER2/neu -synthetic long peptide consisting of rHER2/neu-specific CTLs epitopes (p5 and p435) that was designed in our previous study and conjugated together by double-Arg (RR) in combination with CpG-ODN adjuvant. 14 days after last vaccination four mice per group were euthanized and Immune responses were studied in their spleens for assessment of TCD4 and TCD8 subpopulation by flow cytometry and IFN-g by ElISpot. The remaining mice per group (6 mice) challenged by live TUBO cell line and were followed for tumor size and survival.**Results:** In the mice vaccinated with p5-p435 long peptide in combination with CpG as adjuvant, ELISpot and flow cytometry analysis indicated that CD8+ IFNγ+ T cell population and level of IFNγ production were also increased in this group compared to mice without CpG and PBS administration. Tumor in the mice that received the long peptide vaccine with CpG grew slowly compared to those received the long peptide alone or PBS. Percentage of survival was significantly improved in the mice that received vaccine with CpG compared with control group.**Conclusion:** The use p5-p435 synthetic long peptide in combination with CpG is capable of generation potent antigen-specific CTL immune responses and antitumor effects in vaccinated mice.

Keywords: CpG-ODN, synthetic long peptide, Her2/neu

1911P

Seroprevalence of anti-rubella antibodies in populations aged 1 to 25 years in Ahvaz, Southwest Iran

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Background: Rubella is an infectious disease of childhood that is caused by the rubella virus. It is a teratogenic virus and congenital rubella syndrome (CRS) is an important cause of severe birth defects. To control the effects of congenital rubella vaccination program runs. The goal of this study was to evaluate the Seroprevalence of anti-rubella antibodies in Ahvaz, Iran.**Methods:** In this cross - sectional study anti-rubella IgG antibodies investigated in 865 individuals aged 1-25 years by ELISA. The cut-off values of more than 11 IU / ml was considered as immune. Data was analyzed using SPSS software version 18, chi-square test, ANOVA and Pearson correlation.**Results:** Our subjects were 563 female (65%) and 302 male (35%) with mean age 12.7±7.1 yr. Seven hundred fifty nine subjects (87.7%) were immune against rubella. Level of immunity to rubella were different in age groups (p=0.0001). Highest level of immunity (97/2%) was observed in the 19-25 years and lowest (79.0%) in the 1-6 years. Immunity rates were significantly higher in females than males (p=0.010). Pearson correlation between age and antibody titer showed a significant positive relationship. The antibody titer also increased with increasing age (r=0.365; p=0.001).**Conclusion:** The results of this study showed that immunity in the 1-to 25-year-old age group was found to be high, and the rubella vaccination program has been successful to creating appropriate immunity against rubella, especially in the high age groups, that are of high-risk group.

Keywords: Seroprevalence, rubella, Immunity, Vaccination, Ahvaz

1912P

Serum level of anti-measles antibody among populations under twenty-five-year-old in Ahvaz, Southwest of Iran

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Background: Measles is an acute and highly contagious viral disease which could be prevented by vaccination. The Ministry of Health and Medical Sciences of Islamic Republic of Iran, has launched a mass Measles vaccination campaign throughout the country from 2003. More than 32 million inhabitants between 5 and 25 years old received the measles vaccine. The purpose of this study was to determine the prevalence of positive measles antibody titers in target groups (subjects under 25 year olds) who had received mass vaccination.**Methods:** This cross-sectional study was performed on 900 healthy subjects younger than 25 years in Ahvaz

city of Iran during 2012. Measles IgG antibody of serum was measured using the ELISA method.**Results:** Anti-Measles antibody titer was positive in 821 cases (91.2%) and negative in 79 cases (8.8%). Among girls and boys, 92.2% and 89.8% were seropositive, respectively. These differences were not statistically significant between the two sexes (P = 0.133). Anti-measles antibody titer increased with the increase of age from ≤ 5 to 21 - 25 years (87.3% in ≤ 5-year-old group vs. 96.5 in 21 to 25-year-old group). These differences were statistically significant between the age groups (P = 0.021). There was a positive correlation between the age and the seropositivitye rates (r = 0.036, P = 0.274).**Conclusion:** Results showed that the present vaccination program is insufficient for immunity against measles in this area. The general population immunity was lower than the necessary level for the elimination of measles. Therefore, sero-epidemiological studies are necessary to organize national programs for control and elimination of measles.

Keywords: Seroprevalence , Measles, Immunity, Vaccination, Antibody titer

1590P

Immunogenicity of annual influenza vaccine: a necessity in patients with beta-thalassemia major

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Background: Beta-thalassemia is a hereditary anemia resulting from defects in the production of β-globin chains. A wide spectrum of immune abnormalities has been described in β-thalassemia patients with multiple transfusions. Iron overload has been implicated as the main precipitating factor of immune deficiency in β-thalassemia. Iron directs the immune response toward a Th-2 response pattern, which is unfavorable for fighting a viral infection especially Influenza. So, it has been investigated influenza vaccine immunogenicity in thalassemic patients. **Methods:** In this review, the immunogenicity of influenza vaccine in patients with thallasemia is reviewed in context of clinical and seroconversion states. **Results:** The findings of studies of the immune response to vaccine of patients indicate that several weeks after the administration of the vaccine, thalassemic children and healthy controls had seroconversion rates of about 80% and seroprotection rates of 100%. Also, their vaccine-induced immunogenicity is similar to that of healthy subjects, but the fact that vaccine failures have been reported in properly immunized subjects suggests that protection can sometimes be less than expected. **Conclusion:** In thalassemia patients, because of immune response tendency toward Th2 pattern by iron overload, it should have switched to Th1 by vaccination. It seems Influenza vaccine can be a good candidate for both immunization against influenza virus and Th1 stimulation for reinforcing immune system against other bacterial or viral infection. Therefore annual influenza vaccination can be necessary in thalassemia patients.

Keywords: Beta-thalassemia, Influenza vaccine, Seroconversion

2017P

Development of immunogenic Haemophilus influenza type b, PRP conjugate with Pseudomonasaeruginosa ExotoxinA

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Background: Haemophilus influenza type B (Hib) is an important encapsulate bacteria,which cause pneumonia and meningitis in infants. The role of Poly ribosyl Ribitol Phosphate (PRP) Polysaccharide is significant in Hib immunity. These studies demonstrated the poor immunogenicity of PRP in young children whose B cell immunity is not well developed at that age, and consequently the absence of protection, along with the fact that repeated doses of PRP vaccine failed to elicit a memory response. **Methods:** Hib purified PRP was provided by Pasteur institute of Iran, Karaj complex. Pseudomonas aeruginosa (PA-103) Exotoxin A was purified according to Liu method. PRP was conjugated to exotoxin Aby ADH as spacer and EDAC as a linker. Immunization was done, choosing 2 groups of rabbits. 50µg of PRP and 50µg of the conjugate were injected intramuscularly to groups 1 and 2, with a 15-days interval, respectively. The bleeding was performed on days 0, 15, 30, 45 and titers of sera were measured by Serum Bactericidal Assay. **Results:** The bactericidal titer of PRP was 8 there was no antibody increase in the second injection. The antibody titer of the conjugate was arisen up to 16 by the first injection and 64 in the second injection.**Conclusion:** The results to be shown, the PRP- Exotoxin Aconjugate and the pure PRP were able to stimulate the bactericidal antibody, although there was no antibody against pure PRP, in the second injection.There was a significant antibody titer against the second injection of the conjugate.

Keywords: Polyribosyl ribitol phosphate, Exotoxin A, Conjugate vaccine, Haemophilus influenza type B

2389P

Is Generative Cell Specific 1 (GCS1) of P. falciparum a potential transmission-blocking vaccine antigen?

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Background: One of the new identified malaria transmission-blocking vaccine (TBV) candidate antigens is Generative Cell Specific 1 (GCS1) located on the male gametocytes of Plasmodium species. In order to develop an efficient GCS1-based TBV, it is essential to determine the gene diversity of GCS1 in global malaria-endemic areas. Therefore, in this study, nucleotide diversity in the Iranian P. falciparum GCS1 (PfGCS1) were analyzed and epitope mapping was predicted to find conserved immunogenic epitopes. **Methods**: Molecular analysis of PfGCS1 was carried out by PCR-sequencing (n=22). The potential linear and conformational B-cell epitopes in the PfGCS1 were detected using ABCpred and DiscoTope servers, respectively. The predicted B cell epitopes were mapped on PfGCS1 antigen by using WebLab Viewer Lite 4.2 program.**Results:** The results showed 3 distinct haplotypes in

Iranian PfGCS1 with different frequencies: GCS1-A (N184/D445, 13.6%), GCS1-B (S184/D445, 68.2%), and GCS1-C (N184/N445, 18.2%). The overall nucleotide diversity (π) for all 22 sequences of Iranian PfGCS1 was 0.00063 ± 0.00016. Epitope mapping prediction of PfGCS1 showed that most of the potential linear and conformational B-cell epitopes are located in conserved regions.**Conclusion:** The present study showed a very low genetic diversity of PfGCS1 among Iranian isolates. Notably, most of the linear and conformational B-cell epitopes are located in conserved regions. This matter will help the researchers to develop a vaccine based on PfGCS1 antigen.

Keywords: Plasmodium falciparum; Generative Cell Specific 1 (GCS1); Genetic diversity; Transmission-blocking vaccine.

2510P

Immuno-informatic analysis of Apical Membrane Antigen 1 of both P. vivax and P. falciparum species to select potent immunodominant regions for multi-species malaria vaccine design

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Background: The selection of immunogenic epitopes that are more conserved would provide an easy strategy for poly-epitopes and multi-species vaccine development. To achieve this goal knowledge of B-cell epitopes and intrinsically unstructured/disordered regions (IURs) is highly desirable in the design of vaccines. Apical membrane antigen 1 (AMA1) is a leading malaria vaccine candidate antigen in both Plasmodium falciparum and P. vivax species. Therefore, the genetic diversity of these antigens was investigated and epitope mapping were predicted by using immuno-informatics analysis. **Methods:** P. vivax (n = 37) and P. falciparum (n = 21) Iranian clinical isolates were analyzed by PCR-sequencing. The potential B-cell epitopes and IURs of both PvAMA1 and PfAMA1 antigens were identified by using ABCpred and RONN servers, respectively. To visualize the surface distribution of IURs and B-cell epitopes on the three-dimensional structure, these regions were mapped on a three-dimensional structure of PvAMA-1 and PfAMA-1 proteins using WebLab Viewer Lite 4.2. **Results:** Epitope mapping prediction of PvAMA-1 showed the presence of few mutations in the potential B-cell epitopes but not in IURs. Besides, the most of SNPs in PfAMA1 were detected in B-cell epitopes with a few mutations in predicted IURs. Many of predicted IURs were overlapped with B-cell epitopes in antigens of both species that imply on immunodominant regions. Therefore, the conserved DII loop in both P. falciparum and P. vivax AMA1 was predicted as potent B-cell epitope. **Conclusion**: In silico prediction of immunodominant regions demonstrated simple approach for selecting poly-epitopes in multi-species Plasmodium vaccine design.

Keywords: Plasmodium vivax, Plasmodium falciparum, Epitope prediction, Apical membrane antigen 1, Multi-species malaria vaccine

1966P

Identification of the immunodominant regoin of Bordetella pertussis filamentous hemagglutinin

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Background: Whooping cough is a vaccine-preventable infectious disease induced by Bordetella pertussis (Bp). One of the major components of acellular pertussis vaccines is filamentous hemagglutinin (FHA) which is a principle virulence factor of Bp. This study has demonestrated the immunodominant region of FHA for human antibody response. **Methods:** Serum levels of antibody against four overlapping recombinant fragments of FHA were measured by an indirect ELISA in two groups of healthy children and adults vaccinated with cellular and acellular pertussis vaccines, respectively. Recombinant FHA fragments were expressed in E.coli and purified by affinity chromatography. **Results:** The antibody response in both groups of subjects was mainly directed against epitopes located in the fragment spanning amino acid residues 1877-2250 of the mature FHA molecule, (FHA3) ($P<0.001$). No or low antibody response was induced to other fragments of FHA. **Conclusion:** Our findings indicate that the antibody response against FHA within both cellular and acellular pertussis vaccines is restricted to immunodominant epitopes including residues 1877-2250 of the FHA molecule. Thus, this recombinant fragment could be considered as suitable candidate for substitution of the native FHA molecule in acellular pertussis vaccines.

Keywords: Filamentous hemagglutinin, Bordetella pertussis, epitope mapping, immunodominant epitopes, acellular pertussis vaccine

2587P

Cloning, expression and partial purification of a chimeric protein composed of antigenic fragments of epsilon and beta toxins of Clostridium perfringens for immunization

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Background: Since Clostridium perfringens is a major pathogen in human beings and livestock with various toxins , it has been the subject of many immunological and veterinary researches. The goal of this study was to clone, express and purify a chimeric protein of C.perfringens epsilon (ET) and beta toxins' (BT) antigenic fragments based on bioinformatics studies, and to use it as a recombinant protein with double antigenic capacity for immunization.**Methods**: Specific primers for antigenic regions of ET and BT were designed according to NCBI. The fragments were amplified by PCR and cloned into vectors. They were then digested and ligated together by a specific linker peptide designed by bioinformatics tools , based on antigenicity and secondary structure features. The fusion fragment was cloned into expression vector. The recombinant fusion protein was detected by ELISA and Western Blotting and was partially purified using ammonium sulphate and chromatography through gel filtration column. The

purification step was evaluated by ELISA. The fractions of chromatography column with most fusion protein concentration were selected for injection to mouse for antibody production. The injection was repeated every week for 4 weeks. Serum was collected and tested for the presence of antibodies against fusion protein. **Results:** The cloning step was verified using PCR and plasmid digestion.The results of immunological tests showed that the recombinant chimeric protein has double antigenic properties. The fusion protein was partially purified and the mouse serum showed positive reaction with the recombinant fusion protein. **Conclusion**: According to the data it can be suggested that this method can be used to produce novel subunit vaccines containing only the antigenic fragments of one or more toxins. This will make immunization safer, cheaper, faster and in cases even more effective.

Keywords: antigenic regions, chimeric protein, immunogenicity, purification

1656P

Epigenetic based Design of malaria vaccine

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Background: More than one millions people die from malaria infection every year, children are the most. Finding a method for preventing this disease is not well known. If we study malaria parasite in term of genetic and epigenetic, we will find interesting points which may be useful for finding a way for designing a vaccine against this parasite.**Research:** Malaria falsiparm display a protein named PfEMP1which enhances the attachment of parasit to the Red blood cell carpsule, and cause pathogenesis. The gen that displays protein as well as other proteins of malaria should be K4H3M3(lys 4, histon 3 , three mutilation) until to be activated in term of transcription. This methylation is performed by pfset10 protein which is specific for the parasite which is different with human histone lysine methylations.**Results**: If we can find a way to deactivate this protein by use nano particle containing complete protein (such as antibody) or deactivate the gen (by help of micro RNA).Probably we can control the infection caused by parasite, specially it is role in creating epigenetic memory and transferring memory infectious to its next generation.

Keywords: Malaria, Epigenetic, Methylation

1410P

Factors affecting maternal delay in vaccination of infants in Iran

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Background: Failure in accuracy and neglecting the time table of infant's immunization against vaccine preventable diseases may cause either death or disability. The present study was designed to determine the causes of parental delay in vaccination of children.**Methods:**

Through a cross-sectional survey, 202 mothers of children under 18 months were interviewed in Khorramabad city western Iran in 2011. A structured questionnaire was used for data gathering.**Results:** Overall, 84 mothers (41.6%) were not aware of vaccine names and mean delay in vaccination was 15± 8 days. Child's sickness was the most frequent reasons for delay in infant vaccination. Maternal characteristics such as low levels of education (p=0.001), increased age (p=0.01), being employed (p=0.002) and multi-parity (p=0.02) were the main significant factors associated with delay in infant vaccination in multivariate regression analysis.**Conclusion:** Delay in infant vaccination in Iran, despite being free of cost is of concern. Health education programs targeting less educated, employed, aged and multiparas mothers are recommended to reduce delay in infant vaccination.

Keywords: Infant, Iran, Delay, Vaccination
Keywords: Infant, Iran, delay, vaccination

1749P

High prevalence of non-response to HBV vaccine among children with autism spectrum disorder (ASD)

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Background: The morbidity and mortality associated with hepatitis B virus (HBV) infection are a major public health concern manifested themselves in conditions such as cirrhosis and primary liver cancer that develop slowly during chronic disease. Up to 90% infected children develop chronicity. Therefore, WHO aims to control HBV worldwide by integrating hepatitis B vaccination for all infants.The aim of study was to identify responsiveness to HBV vaccine among a group of children with autism spectrum disorder (ASD).**Methods**: A cross-sectional survey was carried out with 53 children with ASD. All children were tested for Anti-HBs and Anti-HBs using a commercially available enzyme-linked immunosorbent assay (ELISA) **Results:** According to the levels of anti-HBs, 14 (26.4%), 27 (51%) and 12(22.6%) were non-immune, relatively immune and immune, respectively (P value 0.023). Totally, 6 (11.3%) were anti-HBc positive. 1 (7%) and 5 (18.5%) of nonimmune and relatively immune were anti-HBc positive respectively. There was no association between the age and gender of children with the levels of response to vaccine.**Conclusion**: The high prevalence of nonresponsiveness to HBV vaccine (3-4 times as higher as compared to general population) highlights a public health concern. The recommended regimen for HBV vaccine administration might not be enough to protect children with ASD. Alternative immunization regimens (e.g. administration of boosters), or more effective HBV vaccines (a third generation or HBV DNA vaccines) are required.

Keywords: HBV vaccine, autism, HBV chronicity.

1465P

Magnesium Sulphate Enhancement of Measles Virus Yields for Vaccine Production in Cultured Cells

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Background: The measles infectionis the disease which is considered as the fifth cause of mortality in children in developing countries. A live attenuated measles vaccine has been effectively employed to prevent measles infection, since 50 years ago.The final bulk of vaccine is prepared base on the titer of virus in single harvest and thefinished product should contain not less than 1000 infectious virus particle per human dose and increase in virus titer in the single harvest could decrease the requirement volume ofharvested virus to prepare the final bulk. The aim of the present study was to determinethe effect of MgSO4treatment on virus titer in single harvest.**Methods:** Micro titration and macro titration plus Real time RT-PCR methods were conducted to assessalteration of virus titer due to $MgSO_4$ treatment in comparison to control group.**Results:** Obtained results utilizing micro and micro titrationmethods revealed an increase of 2.3 and 2.17(log CCID50/ml) in virus titer respectively. The increase of virus titer was also confirmed by Real time RT-PCRmethod. **Conclusion:** The Improvement of vaccine production procedure using the mentioned result can potentially be cost-effective and timebenefit.

Keywords: Magnesium sulfate, Measles virus,Vaccine

1572P

Effecting Factors on influenza vaccination in nurses

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Background: Influenza is causes of many hospitalizations and deaths annually. Center for Disease Control and Prevention recommended the flu vaccine in employee health.But studies have shown that a small part of healthcare workers are vaccinated against flu. lThis study aimed to determine the factors influencing vaccination and was designed to improve this situation.**Methods:** Present study is a descriptive y cross-sectional study on 200 nurses were selected from nurses working in hospitals in Yazd. They were selected at random. Data collctetion tool was influenza vaccination Questionnaire with incentives and disincentives factors. Questionnaires were completed through self-report and analyzed using SPSS statistical software.**Results:** Based on the findings incentives received influenza vaccine was in 95% of personal protection, 5/25% for family care, 18% comfort, 5/16% doctor Recommendation,

5/26% Tracking health workers, and Inhibition of influenza vaccine was 26% of cases, Lack of belief in the effectiveness of vaccines, 31%lack of knowledge and information about the vaccine, 21%fear of side effects and 26%Lack of risk.**Conclusion:** Results showed that needs more facilities for example immunization in the workplace, providing free vaccine and nursevaccinelaceationtors with in vaccination.acTherefore is required for facilitating the strengthening and weakening of preventive.

Keywords: Incentives Factors, inhibiting Factors, influenza vaccination, nurses

1611P

Prediction of different Bcell epitopes of L1 and L2 proteins of Human Papilloma Viruses to rational vaccine design: an in silico analysis

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Background: Human papillomavirus is one of the most common sexually transmitted infections in sexually active young women and has been implicated as a cause of the majority of cases of cervical cancer, which is the second most common cancer in women over the world. Cervical cancer is the major cancer and approximately 93% of invasive cervical cancers caused by HPVs. Five 'high-risk' types of HPVs (11, 16, 18, 31 and 45) are of particular importance. HPVs capsid proteins including L1 and L2 proteins have been shown to generate neutralizing antibodies against HPV particles in immunized cases. In this study, using in silico analysis we designed a prophylactic universal vaccine for HPVs.**Methods:** Using NCBI database of putative sequences of L1 and L2 capsid proteins and immune B Cell epitope database and also using database of high risk HPV serotypes, the best epitopes of proteins for L1 and L2 capsids were selected for B Cells to recognize. After analysis, selected epitopes in different patterns were evaluated for best statuses of proteasome cleavage sites.**Results:** As the result, 20 epitopes of 5 high-risk serotypes of HPV were selected that are conserved epitopes, have best hydrophilicity, antigenic index and surface probability. These 20 epitopes have 20 amino acids resulting in 400 fusion pepides.**Conclusion:** It seems that designing of a rational universal vaccine that immunologically cover HPV11, 16, 18, 31 and 45 strains is feasible in order to develop prophylactic universal cervical cancer vaccine.

Keywords: Human papillomavirus (HPVs), Epitope prediction, Universal vaccine

1839P

A sandwich ELISA to quantify glycoprotein content of rabies vaccines

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Background: Rabies is a lethal zoonotic disease which a major health problem in the developing countries. There is no cure for rabies but prevention is effectively possible with safe and potent vaccines. The potency of rabies vaccines could directly be correlated to the amount of rabies virus glycoprotein content of the vaccine. Sandwich ELISA technique is recommended by reference international documents as a tool to measure the glycoprotein content of rabies vaccines. The main goal of the present study is to provide a method readily adaptable to quantify rabies glycoprotein content in cell culture produced rabies vaccine. The technique has successfully been optimized for domestic as well as imported vaccines. **Methods:**Two different antibodies against Rabies Virus glycoprotein were used in this study including a mouse derived monoclonal antibody and a human derived polyclonal antibody. Purified Vero and BHK-21 cell-derived rabies vaccines, containing Pasteur strain (PV) were used as antigen along with Rabipur vaccine as reference standard in sandwich ELISA.**Results:** Our results showed a reproducible quantification method based on the established sandwich ELISA. This was confirmed by several independent experimental and different types of Rabies Vaccine for human or animal use.**Conclusion:**In this study we introduce a simple and sensitive ELISA method pertinent for quantification of Rabies glycoprotein content during manufacture of Rabies Vaccine.

Keywords: Rabies, Vaccine, Glycoprotein, Sandwich ELISA.

1688P

Cloning, expression and purification of immunogenic major25-kilodalton outer-membrane protein of Brucellaabortus encoded by omp25 gene

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Background: The outer-membrane proteins (OMPs) of Brucella spp. have been classified into 3 groups according to their molecular weight. The immunogenic major 25-kDa outer-membrane protein of Brucella abortus belonging to group 3 of OMPs which is encoded by omp25gene.The protein plays an important role in protective immune responses. The present study was aimed to clon and express the omp25 gene in EscherichiacoliBL21(DE3)host for immunological investigations and future prospects in the field of vaccine development. **Methods:**The omp25gene of B. aburtus544 was amplified by PCR method.The amplified fragment was directly cloned into pTZ57R T vector and after digestion by BamHI and SacI enzymes on T vector, subcloned into pET32a(+) vector.The expression vector pET32a(+) was transformed into E. coli BL21 (DE3) by cold calcium chloride ($CaCl_2$) method. The recombinant plasmid wasverified by PCR, enzymatic digestion and sequencing methods. Afterward the recombinant protein was expressed in E. coli cells byinduction with IPTG andwas revealed by SDS-PAGE method. At last the protein was confirmed by Western blot analysis.**Results:** PCR amplification of omp25gene resulted in an amplicon of 693bp.The gene fragment was cloned into expression vector pET32a(+), then transformed into E. coliBL21 (DE3). Several colonies containing recombinant plasmid selected on medium supplemented withampicillin

and analysed. PCR and enzymatic digestion methodsconfirmed the process of gene cloning, moreoversequencing data exhibitedthat the gene sequence was similar to the original sequenceof omp25 gene.The recombinant protein with 231 amino acid was expressed in E. colicells with significant yield. SDS-PAGE revealeda 25-kDa protein band on polyacrylamidegel.The protein was purified usingNi-NTA agarosecolumn and was recognizedbyWestern blottingmethod withpolyclonal rabbit antiserum.**Conclusion:** In this study, we cloned and expressed the B. aburtusomp25gene in E. coliandpurified the 25-kDaouter-membraneprotein as a recombinant antigensuccessfulyas well as we obtained high concentration of the protein.The protein can be used forimmunological experiments such as diagnostic antigen, evaluation of immune responsesand development of subunit vaccine.

Keywords: vaccine, B. abortus, omp25, E.coli BL21(DE3), pET32a(+)

1796P

Evaluation of adjuvant activity of alum-naloxone and alum naltrexone mixtures on efficacy of complete Plasmodium berghei blood stage vaccine in balb/c mice.

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Background: Malaria one of the most important infectious disease of humans and animals caused by parasitic protozoans of the genus Plasmodium**.** naloxone and naltroxane are an opioid receptor antagonist that are used primarily in the management of alcohol dependence and opioid dependence. In this study the role of alum-naloxone mixture and alum-naltroxane was investigated in induction of humoral and cellular immunity in response to complete Plasmodium berghei blood stage(CPB) for protection against P. berghei infections.**Methods:** mice in the experimental groups received CPB alone or in combination with the adjuvant Alum, NLT, NLX, Alum- NLT or the alum-NLX mixture in days 0, 7 and 14. Tow week after the last immunization, evalution of the immune response to challenge was performed by mortality, evaluating the proliferation of splenic lymphocytes in response to CPB. Determination of IgG isotyping titer.**Results**: data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Lymphocyte proliferation was significantly higher in AL-NLT-Vac group than control group. A significant increase in anti-P. berghei IgG2a/ IgG1 ratio was observed in mice AL-NLT-Vac group as compared to the Vac group. The survival rate of AL-NLT-Vac group was significantly higher than those of control group.**Conclusion**: Result showed immunization complete Plasmodium berghei blood stage in addition to alum-naltroxane effecatively induces protection against infection.

Keywords: Plasmodium berghei, Vaccination, Adjuvant, Naloxone, Naltroxane

1690P

The mixture of Alum and Naltrexone, elicits humoral immune responses for excreted/secreted antigens of Toxoplasma gondii tachyzoites vaccine in Balb/c murine model

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Background: Several Toxoplasmagondiiantigens, such as membrane, cytoplasmic andexcreted-secreted antigens (ESA) can be potential candidates for immunization. Among these antigens, ESA play an important role in induction of immune system responses. Dense granules, micronemes and rhoptries are secretory organelles in Apicomplexa protozoa. The aim of this study is to investigate whether Alum-NLT (Aluminum phosphate-Naltrexone) mixture, as a new adjuvant, can induce humoral immunity in response to excreted/secretedantigens (ESA) of T.gondiias a model vaccine or not.**Methods:**Six- to eight-week-old female Balb/c mice were divided into five groups. Mice in the experimental groups received either ESAvaccine alone or in combination with the adjuvant Alum, NLT or Alum-NLT mixture; Mice in the negative control group received phosphatebuffered saline (PBS). All mice were immunized, three times subcutaneously (s.c.) with a total volume of 150µl each with a 10-day interval. Ten days after the final immunization, immune response to T.gondiiwas assessed. The virulent RH strain of T. gondii was used to challenge.**Results:**Our results revealed that Alum-NLT mixture as an adjuvant during vaccination boosts the efficacy of the ESA vaccine by means ofincreasing T.gondii-specific IgG, IgG2a production and the ratio of IgG2a/IgG1 (P-value < 0.05), the highest survival rate was observed in mice that immunized with the Vac-Alum-NLT mixture.The use of this adjuvant mixtureimproved the protective immunity against T.gondii.**Conclusion:**Administration of the Alum-NLT mixture as an adjuvant in ESA vaccine enhances humoral immunity.

Keywords: Alum, excreted-secreted, humoral immunity, Naltrexone.

1795P

Effects of Artemisia annua extracts and Propranolol for the stimulation of cellular immune responses for excreted/secreted antigens of Toxoplasma gondii

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Background: Artemisia annua,a medicinal plant is native to Asia.A. annuapossesses anticancerproperties,is broadly used in the treatment of malaria. With regard to the fact that the plant possesses anti-parasite potential, we haveevaluated the ability of A.annua,and Propranolol (PRP)to enhance the protection of an ESA(Toxoplasma gondiiexcreted/secreted), which functions as a vaccine model against a T. gondii. **Methods:**For immunization of the mice, were allocated 60 female BALB/c mice (6-8 week-old) were divided in to 4 groups of 15 mice. The first, second and thirdgroups received the ESA alone, ESA-A. annuaand ESA-

PRP respectively, and the control group received PBS, all mice were immunized on days 0, 10, 20. Ten days after the final immunization, mice all groups were divided in to two groups: 5 to evaluate cellular immune responses (MTT test andmeasurement of concentration of IFN-γ and IL-5)and10to measure the survival rate.**Results:**Results showed that the mice immunized with A. annua extract, and PRPas an adjuvant lead toenhance cellular immunity,and protection against a challenge infected with T. gondiithan the mice vaccinated with the ESA, and PBS alone. But no significant difference between the effects of A. annua extract, and PRP was found.**Conclusion:** The present findings are the first evidence demonstrating the ability of the A. annua extract, and PRPas an adjuvant in combination with the ESA vaccine, can increase cell-mediated immunity and shift the immune response to ESA.

Keywords: Artemisia annua, cellular immunity, excreted-secreted, Propranolol.

1658P

Vaccine against Toxoplasma Gondii: AlumorNaltrexone, or both

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Background: Toxoplasmosisa main problem in immunodeficiency patients. Which is caused by an intracellular parasite named Toxoplasma gondii.For this reason development of a vaccine, which can prevent the complications of acute infection, is an attractive alternative.**Methods:** In this study we used, T. gondiiLysate Antigen (TLA)(as a model vaccine) the combination with the Alum-NLT (Aluminum phosphate-Naltrexone) mixture, were evaluated in immunization of 6–8 week inbred female Balb/c mice.The first group was selected as a negative control group, then the second, third, fourth, and fifth groups were immunized with Vac, Vac-Alum, Vac-NLT, and Vac-Alum-NLT, respectively. All mice were subcutaneously (SC) immunized three times at 10-day interval.Ten days after the final immunization, shifting of the immune responses against TLA toward Th_1/Th_2 was assessed via evaluating IFN-γ (Interferon-γ) and IL-5 (Interleukin-5) production by splenocytes and delayed-type hypersensitivity (DTHs). The virulent RH strain of T. gondii was used to challenge.**Results:**Responses of the DTH and cellular immune showed that in mice immunized with the Vac-Alum-NLT mixture, efficacy by increasing induction of IFN-γ and IL-5 production, immune responses shifted toward a Th1 profile (by increasing the IFN-γ/IL-5 ratios). The highest survival rate was observed in mice that immunized with the Vac-Alum-NLT mixture.**Conclusion:** Findings of the study demonstrate for the first time that administration of Alum-NLT mixture increased immunogenicity TLA vaccine against T.gondii infection and TLA is a potential vaccine candidate against toxoplasmosis.

Keywords: Immunization, Naltrexone, Toxoplasma gondii, Vaccine.

2023P

Humoral immune response of cattle immunized with salivary gland extract of Rhipicephalus (Boophilus)annulatus tick

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Background: Recently, many researches have been carried out in search of alternative harmless methods especially immunization of cattle against tick biting using various tissue extracts of ticks. The purpose of this study was to evaluate the humoral immune responses induced after immunization of cattle with immunogenic proteins of salivary gland extracts of Rhipicephalus (Boophilus) annulatus tick. **Methods:** 14 immunogenic protein bands of Rhipicephalus(Boophilus)annulatus tick salivary gland extract recognized as more dominant by western-blot analysis, eluted from acrylamide gel and injected intradermally into test group with equivalent amount of adjuvant every two weeks for a total of three times. Similarly, the control group was injected with sterile PBS (pH: 7.2). Four weeks after last injection, a tick challenge performed with about 500 tick larvae. Humoral immune responses to immunization with tick salivary glands eluted proteins measured by ELISA.**Results**: Results showed a steady increase in antibody production level of test group from the first week of immunization. Then it reached a plateau for four weeks and after tick challenge it rose sharply and peaked at week 12 and then declined and gradually stabilized at week 18. In control group, there was a period of stability during the first eight weeks. After tick infestation, it surged dramatically and reached a peak at week 12. Then, it fell slightly to a constant level. **Conclusion**: Although both groups have had a significant increase in their antibody production level, test group have expressed a constant increase in antibody production level from the first week of injection boosted after tick challenge. These results confirmed that immunization against Rhipicephalus (Boophilus) annulatus could be achieved under experimental conditions.

Keywords: Rhipicephalus (Boophilus) annulatus, Immunization, Salivary gland

2124P

Fusion protein consisting of conserved epitopes of hemagglutinin and matrix protein, as Influenza A virus subunit vaccine candidate

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Background: Influenza viruses have some conserved peptide that are candidate to universal vaccine production. One of them is hemagglutinin stalk domain (HA2) which mediates membrane fusion, and the other one is M2, a proton-selective ion channel. The extracellular N-terminal domain (24 amino acid peptide) of M2 protein (M2e) is highly conserved across human influenza A subtypes and an appropriate target for development of influenza vaccine with broad-spectrum protection. The degree of epitope density of M2e has been shown to be a critical factor influencing the magnitude of epitope-specific responses. It has been considered that the potency and immunogenicity of M2e could be improved using 3M2e –

HA2 conjugation.**Methods:** In this study, The HA2 gene segment from Influenza A virus (A/Tehran/18/2010(H1N1))[GenBank: HQ419001.1] was amplified and cloned into a prokaryotic expression plasmid pET28a. The construct (pET28a-HA2) sequence was analyzed using sequencing and enzyme digesting. Expression of this construct was confirmed using Western blotting assay by anti His-taged antibody. Then synthetic 3M2e gene subcloned into dephosphorylated pET28a-HA2 construct, upstream of HA2 gene that digested by same Enzyme (BamH1). The expression of pET28a -3M2e-HA2 in E.coli (BL21) was induced by addition of IPTG 1mM, and confirmed by SDS-PAGE and Western Blotting.**Results:** The results of sequencing revealed that the 3M2e gene was properly cloned in upstream of HA2 and in the right frame to 6xHis tag. Western blotting using monoclonal anti-M2 antibody confirmed the constant antigenic determinant of 3M2e in this fusion peptide. **Conclusion**: Strategies for development of such an antigen have included increasing peptide density, fusing M2e with Influenza HA2 conserved peptide. This chimer protein could be an appropriate subunit vaccine candidate to prevent influenza infection.
Keywords: Influenza virus A, 3M2e-HA2, Subunit vaccine

1975P

Preparation and Evaluation of Polyclonal Anti Body Against ExotoxinA- Flagellin Fusion Protein of Pseudomonas aeruginosa in Rabbit

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2574P

Prime-boost vaccination strategy based on TSA gene of Leishmania major induced immune responses in BALB/c mice

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Background: Leishmaniasis is one of the most serious parasitic diseases that is endemic in 88 countries with annual incident of 2 million cases in the world. Despite of many efforts toward vaccine against leishmania no vaccine has been approved yet. Here, in order to increase efficiency of DNA vaccine, we evaluated 2 different strategies (DNA/DNA, DNA/Protein) to achieve an effective vaccine against cutaneous leishmaniasis.**Methods:** BALB/c mice were immunized intramuscularly with 100 µg recombinant plasmid DNA, expressing Leishmania major Thiol-specific-antioxidant (TSA) antigen and boosted with (100 µg) plasmid DNA or (20 µg) TSA recombinant protein in incomplete Freund's adjuvant. As a challenge, three weeks after the last vaccination animals were infected by Leishmania- major. Finally immunologic parameters comprising , lymphocyte proliferation was measured by Brdu method ,secretion of IL-4 , IFN-γ cytokines and also total antibody, IgG1and IgG2a isotypes were evaluated with ELISA method.**Results**: Results demonstrated that both strategies induced proliferation activity, IL-4 and IFN-g cytokine secretion as compare with control groups, but DNA/ Protein vaccination strategy resulted in higher level of lymphocytes proliferation and IFN-g production and lower level of spleen parasite burden than the other approach.**Conclusion:** Finding of this study indicated that vaccination with DNA encoding leishmania TSA antigen followed by a recombinant protein booster has potency for further studies.

Keywords: TSA, Leishmania major, Immune response, Prime-boost strategy

1822P

Relationship between nucleotide diversity of small Hepatitis B surface antigen and HBV vaccine escaped in mentally retarded children hospitalized in boarding institute.

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Background: Hepatitis B virus causes one of the most prevalent infective diseases in the world. Unlike the existence of effective vaccine, HBV vaccine escaped strains are one of the important concerns in HBV prevention. In case of mentally handicapped patients, who are probably immunocompromised and receive antiepileptic drugs, this problem would be worsened. The aim of this study was to determine HBV vaccine escape mutations in mentally retarded (MR) patients who live in compressed centers.**Methods:** Serum samples were collected from 146 MR patients and tested for HBsAg using ELISA method. Then all HBsAg negative samples were tested for anti HBc by the same method. HBV DNAs were extracted from positive samples and Nested PCR for HBV surface gene were done with specific primers. Afterwards positive PCR samples were sequenced bidirectionally.**Results:** nine of isolated samples were successfully amplified and sequenced .All of them had HBV D1 genotype and

ayw$_2$ and ayw$_3$ subtype. three strain had G145R mutation among S gene, which is considered as vaccine escaped mutation. Changes taking place in the area contribute to the lack of effective response to vaccines.**Conclusion:** Since these patients have certain behaviors such as licking things. Also, are held by a small number of nurses in compact places, the infection may spread easily to other patients. Considering their weakened immune system, existence of vaccine escaped mutations pose a substantial risk to their community. More research on these isolated patients is recommended to show if we need new HBV preventive strategies for them.
Keywords: Mentally retarded patients, vaccine escaped mutation, HBV, Iran

2165P

Molecular characterization of the carboxypeptidase B2 of Anopheles stephensi as a VIMTs (vaccines that interrupt malaria transmission) target in EMR/WHO endemic malaria region

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Background: Malaria is one of the important infectious diseases in the word. The goal of global malaria eradication program will be accomplished by 2050. According to the malEra guidelines; one of the necessities for successful eradication is VIMTs development. In EMR/WHO region, An. stephensi is the main malaria vector. Our previous study (Raz et al. 2013) on An. stephensi revealed that anti-Carboxypeptidase B1 antibodies can inhibit sexual parasite development. By completion of An. gambiae genome project, it was revealed that An. gambiae has another cpb gene (cpbAg2). Our goal in this study is molecular characterization cpbAs2 gene of An. stephensi. **Methods:** First, cpbAg1, cpbAs1 and cpbAg2 genes were aligned by MEGA 5.2 software to design gene specific primers for middle part of cpbAs2 sequencing. Then, total RNA was extracted from the mosquito and RT-PCR was performed. Gene specific primers for 3'- and 5'-RACE were designed according to the middle part sequence. Next, 3'- and 5'-RACE techniques were performed to obtain the whole target sequence. **Results**: In this study, the sequence of cpbAs2 gene of An. stephensi was sequenced for the first time and its comparison with its similar gene in An. gambiae revealed that their similarity is 87%. **Conclusion:** According to the our results on cpbAs1, 2 genes similarities with respected genes in An. gambiae, those can be candidate as universal VIMTs in regions that their vectors are An. stephensi and An. gambiae. Furthermore, if anti-CPB1,2 could inhibit CPB enzymes 'activities, it can affect the mosquito fitness as well.
Keywords: An. stephensi, 3'- and 5'-RACE, carboxypeptidese gene, malaria

1916P

Cloning and Expression of Rabies Virus Proteins For a Rabies VLP Design

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Background: Rabies is the most fatal viral disease. It is caused by Rabies virus (genus Lyssavirus, family Rhabdoviridae). Rabies could be transmitted to human and other mammals mostly by a rabid animal bite. Rabies vaccine is administered as pre- and post exposure prophylaxis. Current rabies vaccines for human use are containing inactivated whole virus. However, there is always a minor concern about efficacy of inactivation procedures due to lethality of the virus. VLPs are composed of viral coat proteins that, when over expressed, spontaneously self-assemble into particles that are morphologically similar to infectious virus. These multiprotein structures mimic the organization and conformation of authentic native viruses but lack the viral genome, potentially yielding safer and cheaper vaccine candidates. **Methods:**The matrix gene (M) of the virus was cloned in pIRES2-EGFP expression vector and verified by restriction digestion followed by DNA sequencing. Glycoprotein gene (G) was commercially synthesized. The constructed plasmids were transfected into BSR cells and expression was confirmed by appropriate techniques. Formation of VLPs subsequent to viral protein co-expression was evaluated by microscopic techniques.**Results:** Amplification of M gene, its construction in pIRES2-EGFP expression vector and expression of M gene were confirmed by appropriate tests as mentioned in methods. Assembly of virus like particles was induced by adjusting expression of M and G proteins of rabies virus in BSR cell. Particles could be detected by AFM and EM after centrifugal purification.**Conclusions:** Co-expression of M and G proteins together can trigger assembly of rabies virus like particles that could be used in production of VLP based rabies vaccine.

Keywords: Rabies virus, Matrix, VLP.

2579P

Prokaryotic expression and purification of small subunite hemagglutinin glycoprotein (HA2) for universal vaccine development

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Background: Influenza A viruses cause an acute respiratory disease. The control of infection can be achieved by vaccination, however, it has limited efficacy. It is for this reason that vaccines have to be updated yearly. The influenza viruses have some conserve peptide that candidate to vaccine production. One of them is HA2 that mediates membrane fusion and it is similar among multitude of diverse strains. Antibody against this peptide can obstruct membrane fusion and virus infection.**Methods**: The HA2 gene segment from Influenza A virus (A/Tehran/18/2010(H1N1) was amplified and cloned into pET28a. The construct sequence was analyzed using sequencing. This confirmed construct transferred and induced using IPTG. Western blot assay by anti His-taged antibody was used to examine the recombinant protein

expression. The HA2 protein purificated with Ni-colmn.**Results**: Cloning of the HA2 gene segment was determined by PCR, restriction enzyme analysis and sequencing. The results confirmed the construct (pET28a-HA2) sequence and cloning. SDS-PAGE and Western blot assays on the lysate demonstrated the high expression of HA2 subunit of influenza hemagglutinin. Purification of the HA2 protein from E. coli cells takes advantage of the poly-histidine tag attached to the C-terminus of the protein.**Conclusion**: One of the disadvantages of currently vaccines against influenza are not protective humoral And cell-mediated immune response against any influenza Virus.This is due to the genetic variation of influenza viruses. So it is better for vaccine designing be used conserved regions such as HA2.
Keywords: Influenza virus, HA2 glycopeptid, purification, vaccine

2157P

Prokaryotic expression of the recombinant fusion protein influenza A virus nucleoprotein (NP) and truncated mycobacterial HSP70 as a vaccine candidate

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Background: The novel approaches in influenza vaccination have targeted more conserved viral proteins such as nucleoprotein (NP)to provide cross protection against all serotypes of influenza Aviruses. Influenza specificcytotoxic T lymphocytes (CTL) are able to lyse influenza-infected cells by recognition ofNP, the major target in virus for CTL responses. On the other hand, studies suggest that fusing of molecular adjuvants such as Heat Shock Protein 70 (HSP70),member of intracellular chaperon super families,with an antigen can induce the cellular and humoral specific responses better than the same induced by antigen alone. It is shown that the C-Terminal of HSP70 (ctHSP70) isthe main domain responsible for inducing immunity system.**Methods:** In this study theopen reading frame of NP gene from Influenza A virus (PR/8/34) and C-Terminal (359-625) domain of HSP70 gene from Mycobacterium tuberculosis was amplifiedand cloned into expression pET28a vectorindependently.Thenthe N-terminal of whole NP protein was fused to truncated HSP70 in same vector.The fidelity of cloned genes was confirmed by sequencing.All three types of cloneswereexpressed in E. coli BL21 and confirmed by SDS-PAGE and Western blot analysis.**Results:**Results showed the integrity of vector constructs and well expression of NP, ctHSP70 and fusion form of ctHSP70-NP recombinant proteins in BL21 host cells.**Conclusion:**ctHSP70-NP fusion protein produced here may be considered and evaluated as a universal influenza vaccine which its immunogenicity potential needs to be assessed in animal models along with proper control groups including recombinant NP and ctHSP70 proteins.
Keywords: Influenza virus, vaccine, Nucleoprotein, HSP70

2390P

Immuno-reactivity of Leishmania major TSA recombinant protein vaccine candidate formulated with Freund's adjuvant, Montanide, Chitosan and BCG-Alum

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Background: Leishmaniasis as an infectious disease caused by protozoan parasites of the genus Leishmania. There is no effective vaccine for Leishmaniasis. In this regard, Designing of potential vaccine candidates is highly demanded. In present study TSA recombinant protein was expressed in E.coli and its immunogenicity in combination with different formulation of adjuvants was initially evaluated in BALB/c mice.**Methods**: Plasmid encoding TSA gene was sub-cloned into the PET28a expression vector and recombinant TSA was over expressed in BL21 E. coli by addition of IPTG and confirmed with western-blotting and purification carried out with Ni-NTA column. Groups of BALB/c mice (n=10) were immunized with candidate vaccine adjuvanted in Complete Freund's adjuvant , Montanide ISI-70, BCG-Alum and Chitosan and challenged with parasite, 21 days after final immunization. Lymphocyte proliferation was evaluated with Brdu and IL-4, IFN-g cytokines and total antibody evaluated with ELISA. Wound diameter was measured with calipers and the parasite burden was assessed by spleen culture.
Results: Immunization of mice with TSA adjuvanted in Freund's adjuvant, Montanide, Chitosan and BCG-Alum led to a significant increase in IFN-γ cytokine level, lymphocyte proliferation and antibody responses. There was considerable reduction in lesion diameter in the TSA/Montanide, TSA/chitosan groups in comparison to the control groups. A significant differences observed in all vaccinated groups in parasite burden after 8 weeks challenge with the parasite as compared to the control groups.**Conclusion**: Results show that immunization with TSA antigen with different adjuvants is suitable for further study as vaccine candidate against Leishmaniasis.

Keywords: TSA, Adjuvant, Chitosan, Montanide, BCG-Alum, Leishmaniasis

2164P

Evaluation of serum level antibody to hepatitis B surface antigen in medical students and health care workers receiving HBV vaccine in Gerash, Fars province

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Background: Hepatitis B virus (HBV) is DNA virus which is a part of the Hepadnaviridae family of viruses. This virus causes hepatitis that is cause of million deaths in the world. The most effective way to prevent HBV infection is vaccination against HBV, the measurement of post vaccination serum level of anti–HBs Ab is the only simple test available to predict the waning of protection and help plan for the administration of a booster dose. The aim of this study was to evaluate vaccinated medical students and health care workers of Gerash medical sciences school and Amir-Al-Momenin hospital immune response to the hepatitis B virus

vaccination.**Methods:** A cross sectional descriptive study was implemented on university students and health care workers of Gerash medical school in 2011. 252 serum samples were collected from individuals who had received one, two or three dose of vaccine. Anti–HBs Ab was detected by using ELISA.**Results:** A total of 230 (91.27 %) individuals had protective concentration of anti–HBs Ab (anti–HBs > 10 mIU/ml) and 22(8.73%) of them were non responder (anti–HBs < 10 mIU/ml). There was a statistically significance difference between antibody level and age, and dose of vaccination.**Conclusion:** Regarding that 8.73% of the subjects did not have protective antibody, it is suggested that anti–HBs antibody level should be measured in the medical university students and health care workers and non–immune ones must be revaccinated.
Keyword: Anti–HBs, Vaccination, Medical university students, Hepatitis B

2181P

Codon-optimized expression and purification of truncated ORF-2 protein from hepatitis E virus (HEV) in Escherichia coli

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Background: Hepatitis E virus (HEV) is a causative agent for acute hepatitis among the different age group, and has high mortality rate of up to 30% among pregnant women. Therefore, primary prevention of the HEV infection is essential. The aim of this study was to obtain well yield of the highly purified truncated ORF2 protein, which can be used as a future HEV vaccine candidate.**Methods:** The truncated ORF2 gene (ORF2.1), encoding 112-660 amino acid sequence of HEV capsid protein, was optimized, synthesized and cloned into pBluescript II SK(+) vector. After subcloning in to expression vector pET-30a(+), a fragment of 193 nucleotides was deleted from the construct and the recombinant plasmid pET-30a-ORF2.2 (ORF2.2, encoding 112-608 amino acid sequence of HEV capsid protein) was constructed and used for transformation of Escherichia coli BL21 cells. After induced with IPTG and optimized the conditions of expression, the target protein was highly expressed and purified by Ni2+ chelate affinity chromatography. The expressed and purified protein was analyzed by SDS-PAGE and Western blotting.**Results:** The subcloning was confirmed by PCR, restriction enzyme digestion and DNA sequencing of recombinant plasmid pET30a-ORF2.2. The results obtained from optimizing the expression conditions showed that the highest expression of the protein was induced by adding IPTG at a final concentration of 1 mM at 37°C for 4 hr. The expression and purification of truncated ORF2 protein was confirmed by SDS-PAGE and western blotting. SDS-PAGE analysis showed a protein band about 55 kDa. SDS-PAGE of the purified protein revealed that the abundant target protein appeared in the eluate of elution buffer, pH 4.5. The yield of the purified protein was about 1 mg/l of culture media. **Conclusion:** In this study, the optimized truncated ORF2 protein was expressed in E. coli successfully and well yield of the highly purified protein was obtained, which can be used as a potential vaccine candidate and as an antigen in ELISA to diagnose HEV infections.
Keywords: Hepatitis E virus, truncated ORF2, Optimization, Expression, Escherichia coli, Purification

2253P

Persistent of Humoral and Cellular Immunity after 10 Years of Vaccination with Rubella Takahashi Strain

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Background: Rubella virus (RV) and the congenital syndrome it causes are logical objects for eradication by vaccination. Vaccination in Iran started from 1981 by Takahashi strain in form of MMR (RAZI). Immunity to rubella virus as a teratogenic agent is mainly determined by measuring specific immunoglobulin G (IgG) using serological assays (ELISA) and lymphocyte proliferation assay is a way for investigation of cell-immunity.**Methods:** The blood sample was obtained from donors who vaccinated approximately 10 years ago. Their peripheral blood mononuclear cells (PBMCs) isolated and stimulated with phytohaemagglutinin (PHA), rubella haemagglutinin antigen (HA) and rubella In-house standard (IhS) separately (50 µl / well). Then a fluorescent nucleotide was added. On day 10^{th} -11^{th} the wells stained and observed with the conventional fluorescence microscopy to analyze the in vitro proliferation of lymphocytes. The serum of the same donors used for the enzyme-linked immunosorbent assay (ELISA) for detecting antibody to RV.**Results:** In the study group, after10 years, 86% of subjects were still sero-positivite to rubella virus and all of them (100%) showed a significant cell proliferation with regard to antigenic stimulation by Rub IhS.**Conclusion:** The cell-immunity to rubella infection was activated timely, in individuals who vaccinated 10 years ago, thus Takahashi strain in MMR (Razi) has potency of stimulating humoral and cellular immunity to RV after a decade. This implies that the vaccine used can create maintainable safety in the target population.

Keywords: Rubella, Cellular immunity, Humoral immunity.

2298P

Cloning, expression and purification of Influenza A virus M2e antigen and Leishmania major HSP70 as a recombinant chimer protein in E.coli

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Background: The 24-amino acid ectodomain of the M2 protein (M2e) of influenza A virus represents an attractive target for developing a universal vaccine, as the sequence is highly conserved amongst human variants of this virus. This peptide is a poor immunogen, and in humans, anti-M2e antibody is not reliably induced after infection or split-virion vaccination. Hence, in recent years several researchers have been trying to improve the immunogenicity of the M2e peptide such as increasing the number of epitopes and using different adjuvant. **Methods:** In thepresent study, The sequence 3M2e protein was successfully subclonedinto pet28aup stream of hsp70(221-604). Then expression of 3M2e-HSP chimer proteinwas induced with IPTGandgenerated on small and large scales.Recombinant protein was purified through dissolvinginclusions in 8M urea buffer, absorbing to Ni-NTA resinsand eluted in Imidazole solution. Finally, urea and Imidazole were removed by sephadexG25 column.**Results:** The

recombinant chimer protein (3m2e-hsp) was successfully expressed in E. coli BL21 (DE3) andpurified. Also, Western blotting analysisshowedthat recombinant protein is specifically recognized by monoclonal antibodiesagainst the M2e.**Conclusion:** Heat shock proteins (HSPs) are some of the most conserved proteins present in all prokaryotes and eukaryotes which posses highly immunogenic effect and function as adjuvant that may play a crucial role in integrating innate and adaptive immunity. The recombinant chimer protein prepared here will be administrated in mice to evaluate the efficacy of hsp as an adjuvant.

Keywords: Influenza virus, M2e, Hsp70

2249P

Seroprevalence of measles, rubella,and hepatitis B virus in Babolmedical students.

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Background: Serological surveillance provides estimates of population-level immunity against vaccine-preventable diseases. Seroepidemiology is important for evaluating the impact of vaccination programs, as these programs change the immunity of populations, both vaccinated and unvaccinated.The aims of this study were to assess the seroprevalence of vaccine-preventable infections in Babol medical students, and to provide scientific evidence for implementation of a cost-effective immunization guideline and policy for medical school admission.**Methods:**This cross-sectional study involved 187Babol medical students (preclinical and clinical) attending the College of Medicine and Health Sciences at Babol University of Medical Sciences. Demographic characterization, data on vaccination and history of infectious diseases were collected from participants. Blood samples were collected between July 1, 2011 and May 30, 2012 for serological testing.**Results:** The prevalence of seropositivity to rubella virus was 98.9%, measles virus 50% and hepatitis B virus (HBV) 85.5%. More than ninety percent of students received Measles-Mumps-Rubella (MMR) vaccine at birth; 31.5% of students were negative for Antibody to Measles virus.**Conclusion:**This data can be used to evaluate the impact of vaccination programs and inform decisions concerning vaccine policy, including establishing “catch-up” vaccination programs to help mitigate the risk of outbreaks. About 50% of students were susceptible to measles virus and 14.5% for HBV. Students with inadequate protection should be reimmunized prior to contact with patients.Therefore, pre-matriculation screening for antibodies against this virus is highly recommended.

Keywords: Medical student, Immunization, Measles, Rubella, HBV, Babol.

2445P

Assessment of incorporating TLR9 agonist into conjugated PLGA-PEI as a vaccine delivery system for improving the immune responses

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Background: To accomplish successful vaccination, it is essential to design new strategies to produce vaccines that can effectively penetrate the cells particularly when the designed vaccine can be co-localized in an Antigen-Presenting Cell (APC) and produce more specific immune responses for antigen. Hence it is important to use vectors which can co-deliver antigen and adjuvant to APC and help increasing their absorption. Toll like receptors (TLRs) agonists are promising vaccine adjuvants which enhance and modulate innate as well as adaptive immune responses in great potentials.The use of nonviral vectors as delivery carriers for oligonucleotides and antigen, enhance vaccination responses. An effective way for co-delivery of antigen and CpG oligodeoxynucleotides (CpG ODN) as TLR9 agonist is to encapsulate both in one carrier to ensure that they both enter one cell.**Methods:**In this study, OVA protein as antigen was encapsulated in PLGA (poly-lactic-co-glycolic acid) which was covalently conjugated using amide chemistry to the surface of 10% covered polyethylenimine (PEI) followed by addition of CpG ODN which is a 22 mer oligonucleotide in order to produce a nanoparticulate vector for vaccine delivery .**Results:**The resulting complex was characterized in terms of size, zeta potential and the structure was confirmed by FT-IR spectroscopy. The vaccine delivery potentials of the resulting complex are under investigation.**Conclusion:** It was found that OVA protein encapsulated in PLGA covalently conjugated with PEI and followed by addition of CpG ODN non-covalently, could ideally be utilized as a nano-vector to sucessfully deliver vaccine into the desired cells.**Keywords**: Nonviral vaccine delivery, poly-lactic-co-glycolic acid, Polyethylenimine, TLR9 agonist

2396P

Evaluation of immune responses by LPS from Brucella abortus S19 and RB51 in mice

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Background: The application of new adjuvants with microbial origins is also underway to design of subunit vaccines promoting the immune responses to the antigenic determinants of subunit vaccines. Brucella abortus lipopolysaccharide (LPS) has less toxicity and no pyrogenic properties in comparison to other bacterial LPS. In the current study we evaluated biological and immunostimulatory properties of LPS from S19 and RB51, smooth and rough strains of Brucella abortus as an immunogenic determinant.**Methods:** S19 and RB51 LPS extracted and purified by two different modifications of the phenol water method. C57BL/6 mice were immunized with same amount of purified LPSs. The humoral immunity was evaluated by measuring the specific IgG levels and also the T-cell immune response of mice was assessed by measurement of Th1-type cytokines (IFN-γ, TNF-awo different modifications of the phenol. **Results:** Our results showed that biochemical nature of S19 LPS is different from RB51 LPS and also it induces better immune response than RB51 LPS.**Conclusion:** S19 LPS can be considered as a safe adjuvant and can be used as a component in prophylactic and therapeutic vaccines targeting infectious disease, cancer and allergies.

Keywords: Brucella abortus, S19; RB51; LPS; Adjuvant; Th1/Th2 cytokines; Immune response.

1398P

Immuno-reactivity of vaccine *Leishmania major* TSA recombinant protein formulated in Montanide

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Background: The genus *Leishmania* is obligate intracellular parasite that it is caused an infection called leishmaniasis . The availability of hundreds of adjuvants has prompted a need for identifying rational standards for the selection of adjuvant formulation based on immunological principles for human vaccines. The thiol-specific antioxidant (TSA) is a considerable protein of *Leishmania major*.The main aim of this study was to evaluate the adjuvant activity of Montanide on TSA protein as an appropriate *Leishmania major* subunit based vaccine.**Methods**:Plasmids contain TSA gene was sub-cloned into the PET28a expression vector. The expression of recombinant protein was confirmed with SDS page and western bloting . Forty-eight BALB/c mice were divided into four groups (TSA/Freund ,TSA/ Alum+BCG , TSA/ Montanide adjuvant and PBS control groups) and immunized with 20 μg of vaccine subcutaneously 3 times intervals on days 0, 14 and 28. The mice were challenged with parasite 21 days after final immunization. All immunologic parameters comprising, lymphocyte proliferation was evaluated with Brdu method. IL-4, IFN-gcytokines, Wound diameter and the parasite burden were assessed.Results: Vaccine formulated with recombinant TSA- Montanide induced lymphocytes proliferation as compared with the control groups. Significant difference was observed in diameter of the lesion in the TSA/Montanide group with other groups. Also a significant difference was evaluated in all groups in parasite burden in the spleen after 8 weeks challenge with the parasite. **Conclusion**: This study concludes that TSA vaccine formulated in Montanide stimulates immune responses and that can partially inhibit the infection. This vaccine recommends for further studies.

Keywords: TSA Recombinant protein; Montanide; Leishmaniasis; Vaccine

1894P

Potential B cell epitopes of a protein in Mycobacterium tuberculosis that can be a candid for vaccine

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Background: Tuberculosis (TB) is a leading infectious disease responsible for death and represents more than a quarter of the world's preventable deaths. ArabinofuranosyltransferaseC (aftC) involved in the biosynthesis of the arabinogalactan (AG) that is an essential component the mycobacterial cell wall. **Methods:** The focus of current study is to characterize aftC protein of *Mycobacterium tuberculosis* H37Rv by using bioinformatics tools. **Results:** The

results showed that aftC is a stable protein and belong to transferase protein family. The 3D structure was modeled using Swiss model workspace and the structure was validated that gives valuable understanding for the improvement of helpful rational strategies for experiments. The IEDB is used to predict regions of aftC that are probably to be recognized as epitopes in the context of a B response. The maximum length of peptide that likely predicted as epitope is from amino acid 75 to amino acid 99 with 25 amino acid length. **Conclusion:** Combination of this information may affect the development of new diagnostic tools, drugs, and vaccines for treatment in the endemic region.

Key word: In silico, Mycobacterium tuberculosis, vaccine

1555P

Immunity status to hepatitis bvirus in vaccinated medical students

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Background:Hepatitis B infection is a wide world disease and more than 1/3 of people in the world are HBs Ag positive. Hepatitis BVirus (HBV) causes chronic hepatitis, acute hepatitis, chronic cirrhosis and hepatocellular carcinoma in adults and children. This research was done for evaluation of immunity status to HBV in HBv vaccinated medical students. **Methods**: This descriptive cross-sectional study was done on 135 medical students of Jahrom University of medical science who had been vaccinated three times. The obtained sera were tested by enzyme linked immunosorbent assay (ELISA) method to determine Anti-HBs antibodies. Data were were analyzed by descriptive statistics and chi-square and t-test. **Results**: Out of 135 medical students, 102(75.6%) were women. In this study 9.6% subjects had low immunity to HBV and 90.4% had protective immunity to HBV. **Conclusion**: Although there was a protective immunity to HBV among majority (90.4%) of participants, monitorig and evaluation of immunity in medical student as healthcare workers (HCWs) after complete vaccinationis recommended.

Keywords: HBV, Immunity status, Vaccination, Medical students

3290P

Quality Risk Management is Crucial in the Purification Process of Hepatitis B Virus Surface Antigen (HBsAg) to Control the Large Size Aggregation Formation and Immunological responses

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Background: Recombinant Hepatitis B vaccine contains purified surface antigen of hepatitis B virus (HBsAg) adsorbed on aluminum hydroxide. A desirable antibody protection level is highly related to utilization of validated large-scale process to consistently produce soluble HBsAg, competent for assembly into virus-like particles (VLPs), to enhanced product *in*

vivo potency. **Methods:** Twenty-consequent batches of the HbsAg were evaluated. Total HBsAg was determined by RP-HPLC. To demonstrate the accuracy of the expression in different batches, the antigen was characterized by SDS-PAGE under reducing conditions, immunoblotting and electron microscopy. SEC was used to demonstrate the efficiency of self-assembly and large protein aggregates evaluation. **Results:** The purified-HBsAg of each batch was shown to be heterogeneous. The presence of a shoulder before the main peak in the SEC chromatogram, revealed the soluble aggregates presence. SDS-PAGE, immunoblotting and electron microscopy failed to perceive the heterogeneity of HBsAg.The denatured and reduced HBsAg aggregate showed dimmers presence in SDS-PAGE when considerably large amounts were loaded onto the gel. The detection of HBsAg-dimers by SDS-PAGE under reducing-conditions was shown to be directly related to the vaccine potency.There was no difference in the SDS-PAGE pattern of large size HBsAg aggregates and normal HBsAg assembly, because all of the large aggregates of HBsAg particles are denatured into the monomers by DTT in SDS-PAGE. A significant correlation was found between the percent of the shoulder before the main peak in the chromatogram and the immunogenicity of the products. **Conclusion:** Ultrafiltration is necessary in HBsAg purification for antigen desalting/concentrating solution. The particles could be aggregated into larger particles with irreversible structure change leading to unpredictable clinical results. HbsAg aggregation may be induced by a variety of physical factors, such as temperature, ionic strength, vortexing, adsorption on the surface or interface, inducing protein aggregation. Therefore, the critical factors affecting protein aggregating should be controlled and the Quality Risk Assessment is recommended to be developed in a validated procedure.

Keywords: HBsAg, Virus-like particles

2878P

Development of Antibody Binding Test (ABT) for measurement the potency of rabies vaccine

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Background: Precise determination of vaccine potency is the major consideration in vaccine quality control tests. Currently, all vaccine potency testsfor rabies vaccine are *in vivo*, and those are costly and time consuming. Furthermore, usage of large number of animals caused major criticize from severalcommunities. Hence, alteration of the *in vivo* test is one of the goals fromWorld Health Organization.Nowadays several *in vitro* methods are being developed to replace the *in vivo* method. Our approach is development of Antibody Binding Test (ABT) for *in vitro*measurement the potency of rabies vaccine. **Methods:** The defined Rabipur vaccinewith the potency of 12.7IU/ml and its dilutions was assumed as theoretical tests. Serial dilutions of thesetestswere preparedand incubated with neutralizing antibody of rabies virus (0.2 IU/ml) in a 96-well plate as quadruple. Then, liverabies virus (5000 $TCID_{50}$) was added to each dilution andagain incubated. Finally, cell suspensionof “Baby Hamster Kidney”(50000 cells/well) were added to the mixtureand incubated for 24 hours.Cells were fixed and stained

with fluorescent antibody to determine the percentage of infection. The $ED_{50}$was calculated using Reed &Muench method and the relative potency of each vaccine was measured based on reference vaccinepotency.The mentioned test were repeated for 5 times to calculate the reproducibility of the developed test. **Results**: This study demonstrated that results of ABT method are close to expected potencyof examined vaccine by gold standard in vivo test. Also the linearity of our modified antibody binding test was determined (R^2=0.98). **Conclusion:** The designed procedureis a promising approach for *in vitro* calculation of rabies vaccine potency. The intended method can be more evaluated with other vaccines (human and veterinary vaccines) to determine the accuracy of this method.

Keywords: Antibody Binding Test, Rabies vaccine

2595P

Evaluation of humoral immune response against the IRIBA vaccineincattle in Jahrom.

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Background: Brucellosis is one of the most important and most common diseases among humans and animals.Despite over a century the disease detection and vaccination of, but yet have not eradicated.Since the outbreak of the infection in humans is extremely unlikely,animal and human complications of this pollution is too costly and Researchonthis subjectis low,so requires detailed research regarding the immunogenicity of these vaccines does take place. **Methods:** After consent ranchers and completing the questionnaires at least from 30cow 10cc blood sampled.specific serum antibody against Brucella titers determined by ELISA. The animals which don't have antibody immediately injected with the vaccine and1 month were kept away from the herd then above experiments were repeated. Data analysis by using pre-test was done. **Results:** was considered.In the first stage blood samples were collectedfrom40heads of calves.1calfantibody titer above 12U/mL and 2 calf have a 8-12 U / mL anti body titer,in total, these three calves due toproduce antibodieswere excluded the study andto37othercalvesIRIBA vaccineimmediatelyinjections.In the second stage,fromeach animalblood samples were collected afteramonth.That21headsofcattle Antibody titerover 12U/mL,inonecalfantibody titerbetween 8-12 U / mL and In15calves,antibody titersbelow 8 U / mL measured. **Conclusion:** First sampleswichhave apositive titer canhavereasonssuch asdirect transmissionfrommother,dairy airpollutionand…Intiterafter vaccinationwere obtainedonly 21calvesaccounted for56% of the samples thatwere suitableantibody with60 percent ofwhichwerelistedon the vaccine boxis contrary56%is not acceptableimmunogenicity.

Keywords: Brucella, IgG, Jahrom, ELISA, immunogenicity

2985P

FimHadhesin-tFliCfusionproteinasavaccinecandidateagainsturinarytractinfection(UTI)

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Background: Urinary tract infections (UTIs) are among the most common bacterial infections worldwide. Uropathogenic *Escherichia coli* (UPEC) is the most common cause of UTIs. FimH adhesin, is one of the most important virulence factors of UPEC. The prevalence of antibiotic resistance in patients with UTI is increasing. Therefore, there is an urgent need for development of an effective and safe vaccine. We have previously designed a fusion protein consists of FimH from uropathogenic *Escherichia coli* and truncated forms of flagellin (tFliCs) from Enteroaggregative *Escherichia coli*. tFliCs (forms A and B) are potent adjuvants and according to our previous *in silico* studies, A-FimH-B fusion could effectively induce innate immune responses. In this study, the ability of this fusion protein in activating humoral and cellular immune responses was investigated. **Methods:** FimH-tFliC fusion protein was cloned, expressed, purified and quantified by Bradford protein assay. Mice were immunized, bled and sera were collected. Antibody (Total IgG, IgG1and IgG2a) and cytokine (INFɣ and IL-4) levels were evaluated by enzyme-linked immunosorbent assay (ELISA). **Results:** Antigen specific IgG response was detected after the first immunization compared to the negative control (PBS). The IgG, IgG1and IgG2a responses following stimulation with the fusion protein was significantly higher than the control groups. Significantly higher levels of IFN-γ and IL-4 production were observed. **Conclusion:** According to our results, this fusion protein could be considered as an effective vaccine against UTI. However, further *in vivo* studies are still necessary to investigate the ability of this vaccine in providing long-lasting protection.

Keywords: FimH, tFliC, Fusion protein, Urinary tract infection

2747P

Evaluation of safety of gentamicin-attenuated *Leishmania infantum* in vaccinated dogs in field trial

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Background: Dogs are main reservoir for visceral leishmaniasis for humans and control of disease in dogs reduces the human incidence. The canine model is particularly useful in evaluating vaccine candidates since successful vaccination of dogs might control the spread of disease to humans in endemic areas where the dog is the reservoir of the parasite. A canine vaccine may represent the most effective method by which to reduce the incidence of human visceral leishmanisis, (3) and it could also provide the way toward the development of vaccine for human. The attenuated parasites also closely mimic the natural course of infection and presents a full complement of *Leishmania* antigens to the host immune system. We previously reported that a cultured attenuated line of *L. infantum*, identified as *L. infantum* H-line, was selected by culturing promastigotes *in vitro* under pressure of the aminoglycoside antibiotic gentamicin. **Methods:** Promastigotes of *L. infantum* were cultivated in HOMEM supplemented with 10% FCS. *L. infantum* H-line was generated in the same medium supplemented with gentamicin at 20 µg/ml. Stationary phase promastigotes of H-line and WT were harvested after 48 subpassages. One hundred and three healthy dogs from different breeds from non

endemic between 6-18 months old were used. The dogs were vaccinated subcutaneously (s.c) with 100 µl of the suspension of stationary phase promastigotes in the foreleg of the animals. The control dogs were remained as placebo injected (s.c.) with 100 µl of PBS. We included vaccinated and control dogs individuals in each house, whenever possible, in order to equalize their degree of exposure to the risk of natural infection. **Results:** The overall seroprevalence rate for anti specific-*Leishmania* antibody at the initial screening was 39.2% (>1/100) by IFA. Five out of 55 (9.1%) dogs presented one or more clinical signs of disease while other 50 dogs were asymptomatic (90.9%). Only five dogs were positive demonstrated by all methods in which three of them showed clinical signs

3109P

Seroprevalence of Measles Antibodies in Children at School going age in KPK

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Background: Measles is a respiratory disease caused by a virus. The disease is also called Rubeola. It Grows in the cells that line the back of the throat and lungs.CDC- 20 million cases and 197,000 deaths each year. About 30% of measles cases develop one or more complications, including Pneumonia.Ear infections ,occur in about 1 in 10 measles cases and permanent loss of hearing can resul. Diarrhea reported in about 8% of cases. Maternal antibodies confer protection during first few months of life.MCV is administered to children at the age of 9 and 15 months.Vaccine coverage up to 95% may interrupt endemic transmission of measles. According to WHO/UNICEF estimates MCV coverage rate in Pakistan is 86%. To determine the IgG antibodies against measles Assess the need of second dose/ booster of vaccination. **Methods:** Sample of 500 school going children between 5-7 years of age.Schools of Peshawar, and Charsadda districts.Consent was taken from parents.5ml blood taken and serum tested for IgG antibody titer through ELISA.Cut-off level of IgG antibody determined. **Results:** Results shows gender distribution out of 500 children 260 were female and 240 male.52.25 were urban schools and 43.75 were rural schools. Measles Antibody titer was negative in13%,negative in 75% and on borderline in 12%.Booster requirement was present in 25Booster dose according to gender male 52.4% female47.60%.Booster dose in more than 6 years is 68%. **Conclusion:** About 25% children are unprotected against measles.strategies need to be developed to scale up this protection both in urban and especially in rural children with effective vaccination. Booster dose is required soon after children lose maternal protection.

Keywords: Measels, antibodies, KPK

3073P

In silico predictional linear and conformational vaccine for l6 and l7 protein in breast cancer affected by papiloma virus

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Background: The most common cancer worldwide among women is breast cancer. The initiation, promotion, and progression of this cancer result from both internal and external factors. The International Agency for Research on Cancer stated that 18-20% of cancers are linked to infection, and the list of definite, probable, and possible carcinogenic agents is growing each year. Among them, biological carcinogens play a significant role. **Methods:** Recently, the vaccine against human papillomavirus (HPV) was introduced in the national vaccination programmes of several countries worldwide. The established association between HPV and the progression of cervical neoplasia provides evidence of the expected protection of the vaccine against cervical cancer. During the last two decades several studies have also examined the possible involvement of HPV in non-genital cancers and have proposed the presence of HPV in oesophageal, laryngeal, oropharyngeal, lung, urothelial, breast and colon cancers. The possible involvement of HPV in these types of cancer would necessitate the introduction of the vaccine in both boys and girls. **Results:** In this aricle we peresent a vision of predictional linear and conformational vaccine for l6 and l7 in breast cancer. **Conclusion:** In Asia, with the considerable variation evident in both breast and cervical cancer incidence rates, as well as in cultural and other environmental factors,this cancer is so important.

2977P

Comparison between memory B cell populations induced by heat labile toxin B subunit and tetanus toxin binding domainin mice model

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Background: Heat labile toxin (LT) is major virulence factor of ETEC. Binding subunit(LTB) as a vaccine can induce a short term (six month) immunity in human.On the other hand, the binding domain of tetanus neurotoxin (THc) has a critical role in immunogenicity and long term immunity (ten years) in human. The objective of this study is flow cytometeryanalysis and measurement of memory B cells populations inmice immunized with LTBor THc. **Methods:** Recombinant LTB and THc proteins wereexpressed in optimized conditions and then purified. Afterward, mice were immunized withrLTB or THc.After six months, mice spleencells were extracted; stained with antibodies against CD19, IgD, and IgG andanalyzed by flow cytometry.

Results: SDS-PAGE gel wasconfirmed expression and proper purification of rLTB subunit and THc protein. ELISA data was shown high titer antibody level against rLTB and rTHcin mice. After six months, there was no significant difference between memory B cell population in test and control group in LTB immunized mice. In comparison, memory B cell population induced by THc was preserved in test group versus control. **Conclusion:**LTB specific memory Bcells completely removed aftersix months.It seems LTB subunit couldnot induce a long term memory B cell in mice model.Meanwhile, THc specific memory B cell was persisted after six months and THcmayhave a pivotal role in long term memory induction. Antigen nature has a potential role in memory induction.

Keywords: LTB subunit, Tetanus toxin binding domain (THc),Memory B cell, Flow cytometry

1896P

Can we determine that outer membrane protein 31 (Omp31) of *Brucella melitensis* by in silico analysis to be a candid for vaccine?

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Background: Brucellosis is a zoonosis and the infection is almost transmitted by contact with infected animals or their products. Brucellosis control programs are based on vaccination of infected animals. The duration of the human illness means that brucellosis is an important economic and a medical problem for the patient. **Methods:** The focus of current study is to characterize Omp31 protein of *Brucella melitensis* by using bioinformatics tools and identify a suitable T cell epitope, which might be efficient against *Brucella melitensis*. **Results:** The results showed that Omp31 Analysis with different bioinformatics tools revealed that this epitope was highly stable and capable to induce T cell-mediated immunity.The secondary structure was predicted that random coil is predominantly present followed by extended strand and alpha helix. The IEDB is used to predict regions of Omp31 that are probably to be recognized as the epitope could bind with at least 6 major histocompatibility complex class-II (MHC II) and 5 MHC I molecules that the epitope shared ~86.93% identity with Omp31 of all human antigenic Brucella. **Conclusion:** Determination of sequence, function, structure and predicted epitopes of Omp31 protein is important to design of inhibitor of this protein that will help to plan new drugs for theses strains. Combination of this information may be the target for epitope-based vaccine in humans against brucellosis.

Key word: *Brucella melitensis*, Omp31, In silico

2057P

In silico selection of the best immunogenic region of *Acinetobacter baumannii* FepA as a vaccine candidate

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Background: Acinetobacter baumannii is a rapidly emerging nosocomial pathogen causing infections with high mortality rates due to inadequate available treatment and remarkable capacity to acquire antimicrobial resistance. Ferric siderophore complexes are produced by most bacteria to acquire iron, a vital element. These complexes are transported across the outer membrane by receptor proteins such as FepA (ferric enterobactin protein). Antibiotics directed against these proteins assosiated with iron uptake exert a bacteriostatic or bactericidal effect against *A.* baumannii in vitro, by blocking sidrophore mediated iron uptake pathology. Identification and characterization of B-cell epitopes in target antigens is one of the key steps in epitope-driven vaccine design. Computational techniques offer a fast, scalable, and cost-effective approach for predicting B-cell epitopes. Attempt should be made to discover peptides that could mimic protein epitopes and possess the same immunogenicity as the whole protein. The present study we exploited immunoinformatic tools to select the appropriate region as effective B cell epitpes. **Methods:** Amino acid sequence of FepA was extracted from NCBI database. Three-dimensional structure was predicted by homology modeling and threading methods. Linear and conformational epitopes of FepA were determined via several saftware. Top ranked epitopes were determined. **Results:** The best immunogenic region include epitopes with high scores of immunogenicity introduced with consensus method, was selected. **Conclusion:** the selected region as the best immunogenic region, can be used as an epitope vaccine. This peptide can be able to induce protective immunity in the host and it is not necessary to use whole protein as a vaccine.

Keywords: Acinetobacter, Baumannii, FepA, B-cell epitopes, Immunogenic region, Immunoinformatics

2387P

Insilico study for designing a T-cell based polytopic HPV vaccine

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Background: Approximately %99 of cervical cancers contains HPV DNA of high-risk genotypes, with type HPV16 being the most prevalent, followed by types 18, 31, 33 and 45. Many studies have found continued expression of E6 and E7 proteins in the majority of cervical cancers. Aim of this study is designing apolytopic protein vaccine by *in-silico* study of E6 and E7 sequences of main carcinogenic HPV genotypes. **Methods**: E6 and E7 sequences of HPV retrieved from NCBI databank. *In-silico*linear and conformational epitopes for all proteins

were determined. For obtaining maximum effectiveness of final construct in stimulation of immune system, among all predicted epitopes, only epitopes with highest antigenicity score were selected. Selected epitopes were attached to each other in different patterns and evaluated for best statuses of proteasome cleavage sites. Codon usage was optimized for compatibility of designed vaccine with bacterial expression systems. **Results**: Top rated epitopes and ratio of number of epitopes to protein length for all six high risk genotypes were determined. The highest immunogenic epitopes, included eight epitopes were selected. **Conclusion**: For more accuracy of processes of prediction and designing, we used hybrid approach to predict T cell epitopes. Polytopic vaccines (based on *in-silico* approaches) cause concentration of immune responses on important epitopes and decrease adverse effects of vaccination. In the present *in-silico*study, we proposed a single candidate polytopic construct for stimulation ofCTL cells against six high-risk HPVgenotypes with final goal of designing a universal therapeutic vaccine.

Keywords: HPV, *In-silico*, Polytopic protein

2511P

CpG ODN /Alum complex as a new adjuvant to induce stranger humoral immune responses in mice immunized with whole inactivated influenza virus.

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Background: Influenza is a major viral respiratory infection of humans. Vaccination is the most effective means to prevent influenza infection. The commercial influenza whole-inactivated (WIV) vaccine has been used for a number of years. In order to increase the vaccine efficiency, aluminum hydroxide (alum) was added as an adjuvant. Alum adjuvant mainly induces a Th2 immune response while the CpG motif induces Th1. Since bothTh1 and Th2 immune responses are required to prevent and treat viral infection, CpG and alum were added to the vaccine. **Methods:** A/PR/8/1934 H1N1 (PR8) influenza viruses were grown on MDCK cells. The virus was purified by sucrose gradient and ultracentrifugation and inactivated by ultraviolet (UV). Four groups of mice were immunized with different formulations of the vaccine (WIV with or without aluminum hydroxide and CpG). Control mice were injected with phosphate buffer only. The mice were bleed before and after injection and their sera were isolated and tested for antibody using ELISA. ELISA plateswere coated with0.4µg of PR8. Appropriatedilutions of sera of individual mice were appliedto the plates, and incubated for 2 hat 37 ◦C. Subsequently, plates were washed and incubatedwith horseradish peroxidase-conjugated goat anti mouse IgG-antibodies. **Results:** Our results showed that mice immunized with alum+CpG had higher titers of antibody than those with only one adjuvant. **Conclusion:** As results, formulation of alum+CpGadjuant with WIV influenza vaccine stimulates humoral and cellular immunity more than alum with WIV influenza vaccine and CpGwith WIV influenza vaccine.

Keywords: CpG ODN /Alum complex, Influenza vaccine

2932P

Design and in silico analysis of a pentavalent chimeric protein as a candidate vaccine against 3 enteropathogenic bacteria: ETEC, EHEC, and Shigella dysentery

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Background: Enteropathogen bacteria cause health problems and deathsin both developing and developed countries. Among them E. coli and shigella species are so important.ETEC is responsible for many deaths in children under 5 and EHEC and Shigella cause hemorrhagic diarrhea that could be mortal in any ages. So, there is a great interest for the development of an effective vaccine against these pathogens. The progresses in bioinformatics have led to a new discipline: immunoinformatics. Insilico tools are now available to design and evaluate the immunogenicity of the vaccine candidates. **Methods:** We designed a chimeric protein involving: the heat labile toxin subunit B and STa toxin of ETEC, EspA and Stx B of EHEC, and the N-terminal part of IpaD of Shigella. These proteins were fused together by an appropriate linker. The phisico-chemical parameters and the secondary and tertiarystructures of the chimeric protein werecalculated. Antigenic B-cell and T-cell epitopes were predicted by appropriate softwares. Then the protein was reverse-transcribed to DNA. The multigenes DNA was then codon optimized for expression in *E. coli*Bl 21 host. And at last the RNA secondary structure and RNA stability was obtained. **Results:** The results showed a good 3D structure of the protein in which many of epitopes were exposed and the domains were separated completely. The mRNA structure was in good condition and it was stable invivo. **Conclusion:** The designed protein structurally and immunologically has almost all factors of an efficient candidate vaccine and can be tested in experimental tests.

Keywords: Immunoinformatics, Vaccine Design, In Silico Analysis

3085P

Optimization of flowcytometry for identification of memory B cells in mice model

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Background: Flow cytometry is routinely used in the diagnosis of disorders in clinic, but has many other applications in basic research. Memory B cells are formed from activated B cells that are specific to the antigen encountered during the primary immune response. These cells

are able to live for a long time, and can respond quickly following a second exposure to the same antigen. CD27 and IgD antigen represents key markers for memory B cells. **Methods**: Memory B cells phenotype using the surface markers by flow cytometry on spleen of test mice versus controls. **Results:** Memory B cells were detected by flow cytometric approach using selective markers. **Conclusion:** Memory B cells were detected by flow cytometry and the percentage of these cells in spleen cells were significantly different between control and test mice.

Keywords: Immunization, Flow cytometry, Memory B cells, Protein BONT/A-HC

3086P

Evaluation of immunization level between recombinant proteins of binding subunit of ETEC and botulinum (A) toxins

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Background: Among the bacterial agents, the most common cause of diarrheal disease is Entrotoxigenic Escherichia coli. The LTB subunit of LT toxin could induce six months immunity. Clostridium botulinum causes botulism disease. BONT/A-Hc toxin subunit could induce two years immunity against disease. It seems the immunogenicity potency of these two subunits maybe influence on the memory longevity. The aim of this study is assessment of LTB and BONT/A-Hc immunogenicity and investigation of their relations to immunological memory longevity. **Methods:** The transgenic E. coli BlDE3 with pET28a vector, containing recombinant genes LTB and BONT/A-Hc separately were used to expression of recombinant proteins. LTB and BONT/A-Hc expressed as insoluble and soluble forms respectively; and their purity were confirmed on SDS-PAGE gels. Finally, mice immunization were carried out and antibody titration of both recombinant proteins were evaluated and compared. **Results:** After immunization of Mice, a difference in antibody titer was observed between two proteins i.e. BONT/A-Hc and LTB. **Conclusion:** There was a significant difference of antibody titration between LTB and BONT/A-Hc proteins. That can represent strong adjuvanticity of LTB and almost the same time interval is limited immunogenicity.

Keywords: Antibody, Titration, Immunization, BONT/A-Hc, LTB

2934P

Immunization of mice by canola (*Brassica napus*) oilseeds-derived hepatitis C virus (HCV) core protein-A plant based HCV vaccine

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Background: The oilseeds of ¨canola¨ offer a novel and inexpensive route for expression and purification of recombinant proteins via oil-bodies. Besides, the extracts of the oilseeds may function as an adjuvant for formulation of antigenic vaccine candidates. Herein, we provide data of immunization studies for canola oilseeds-(derived) HCV core protein (HCVcp) and *E.coli*-(derived) HCVcp formulated in canola-oil extracts for development of a plant-based HCV vaccine. **Methods:** A sysnthetic codon-optimized gene corresponding to HCVcp (413 bp) was inserted into the plant expression vector pBI1400 and transformed into canola via *Agrobacterium tumefaciens.* Transgenic plants were screened and regenerated from selection media containing kanamycin and analyzed by PCR via vector and gene specific primers. Alternatively, HCVcp cloned in pIVEX2.4a was expressed in *E.coli* BL21-AI and purified by Ni-NTA chromatography. BALB/c mice were immunized by either canola-HCVcp or *E.coli*-HCVcp formulated in canola-oil extracts at days 0, 14, 28 and analyzed for various immunological responses. **Results:** Analyses by PCR and sequencing reactions confirmed the accuracy of the HCVcp harboring transgenic plants. Of total soluble protein, around 0.1% HCVcp was detected in transgenic canola seeds. Assessment of mice antibodies via ELISA, cytokines and Proliferation and CD4/CD8 ratio by flow cytometery indicated that canola-HCVcp induces immune response against HCV core protein. **Conclusion:** Canola-HCVcp has potential as both as an antigen for diagnosis and a vaccine candidate while canola oil extracts might be utilized as adjuvant to formulate *E.coli*-HCVcp.

Keywords: Plant-based vaccines, HCV core protein, Adjuvant, Oil bodies

2757P

Simulation of chimeric GMCSF-Her2-INF γ vaccine against breast cancer

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Background: Breast cancer is a leading cause of cancer-related deaths in women worldwide and there is no effective therapy for patients with invasive and metastatic breast cancer. Immunotherapy may be proved effective in treating patients with advanced breast cancer. We have designed a complex immunogen derived from the extracellular domain of HER-2/*neu*-(480–620) that represents a three-dimensional epitope (as major antigen of breast cancer),nearly whole of GMCSFsequence(18 – 144) and IFNγ sequence(24 -161). **Methods:**Related sequences of Her-2,GMCSF and IFNγ were obtained from UniProtKB/Swiss-Prot.The physico-chemical properties were analyzed using the Expasy'sProtParam software.Segments were selected based on prediction of immunogenic epitopes.Linear B-cell epitopes of construct

were estimated usingBepiPred1.0Server,and bcepred.Discotope1.2 was employed to predict discontinuous B-cell epitopes.In general,epitopes having VaxiJen cutoff values of >0.5 was selected. For T-cellepitope prediction.HLApred, MHC2Pred and Propred were employed to predict peptides from the protein binding withMHCII.The chimeric construct codons were optimized based on plant host by using EMBOSS. The mRNA secondary structure of the gene was evaluated by the mfold software. The prediction of the secondary structure of the protein was performed using the GOR-IV.The I-TASSER was employed for tertiary structure prediction.The tool AccelrysDiscoveryStudio2.5 was used to visualize the modeled3D structures.3Dstructural stability of the protein was evaluated by Swiss-PdbViewer software for energy minimization and RAMPAGEserver. AlgPred software and SDAP allergen library were used to predict the allergenicity of the protein. **Results:** All of the results suggest that the construct can be an appropriate vaccine candidate against Breast cancer. **Conclusion:** Before working in lab we should be confident about the validity of vaccine throw in silico analyses.
Keywords: Cancer, Chimeric vaccine, IFNγ, GMCS

2755P

In silico analysis of chimeric GMCSF-MUC1-Her2-INF γ vaccine against breast cancer

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Background: Breast cancer is a leading cause of cancer-related deaths in women worldwide and there is no effective therapy for patients with invasive and metastatic breast cancer. Immunotherapy may be proved effective in treating patients with advanced breast cancer. We have designed a complex immunogen derived from the extracellular domain of HER-2/*neu*-(480–620)that represents a three-dimensional epitope,7tandem repeats of MUC1(VNTR) as major antigens of breast cancer and nearly whole of GMCSFsequence(18 – 144)and IFNγ sequence(24 -161). **Methods:** Related sequences of Her-2,MUC1,GMCSF and IFNγ were obtained from UniProtKB/Swiss-Prot.The physico-chemical properties were analyzed using the Expasy'sProtParam software.Segments were selected based on prediction of immunogenic epitopes.Linear B-cell epitopes of construct were estimated using BepiPred1. 0Server, and bcepred . Discotope1.2 was employed to predict discontinuous B-cell epitopes. In general,epitopes having VaxiJen cutoff values of >0.5 was selected.For T-cellepitope prediction. HLApred, MHC2Pred and Propred were employed to predict peptides from the protein binding withMHCII.The chimeric construct codons were optimized based on plant host by usingEMBOSS.The mRNA secondary structure of the gene was evaluated by the mfold software. The prediction of the secondary structure of the protein was performed using the GOR-IV.The I-TASSER was employed for tertiary structure prediction.The tool AccelrysDiscoveryStudio2.5 was used to visualize the modeled3D structures.3Dstructural stability of the protein was evaluated by Swiss-PdbViewer software for energy minimization and RAMPAGEserver. AlgPred software and SDAP allergen library were used to predict the allergenicity of the protein. **Results:** All of the results suggest that the construct can be an appropriate vaccine candidate against Breast cancer. **Conclusion:** Before working in lab we should be confident about the validity of vaccine throw in silico analyses.
Keywords: Cancer, Chimeric vaccine, IFNγ, GMCSF

2754P

Simulation of chimeric GMCSF-MUC1-Her2 vaccine against breast cancer

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Background: Breast cancer is a leading cause of cancer-related deaths in women worldwide and there is no effective therapy for patients with invasive and metastatic breast cancer. Immunotherapy may be proved effective in treating patients with advanced breast cancer. We have designed a complex immunogen derived from the extracellular domain of human HER-2/*neu*-(480–620)that represents a three-dimensional epitope,7tandem repeats of MUC1(VNTR) as major antigens of breast cancer and nearly whole of GMCSFsequence(18 – 144). **Methods:** Related sequences of Her-2,MUC1and GMCSF were obtained from UniProtKB/Swiss-Prot. The physico-chemical properties were analyzed using the Expasy's ProtParam software. Segments were selected based on prediction of immunogenic epitopes.Linear B-cell epitopes of construct were estimated usingBepiPred1.0Server,and bcepred.Discotope1.2 was employed to predict discontinuous B-cell epitopes.In general,epitopes having VaxiJen cutoff values of >0.5 was selected.For T-cell epitope prediction. HLApred,MHC2Pred and Propred were employed to predict peptides from the protein binding withMHCII.The chimeric construct codons were optimized based on plant host by usingEMBOSS.The mRNA secondary structure of the gene was evaluated by the mfold software. The prediction of the secondary structure of the protein was performed using the GOR-IV.The I-TASSER was employed for tertiary structure prediction.The tool AccelrysDiscoveryStudio2.5 was used to visualize the modeled3D structures.3Dstructural stability of the protein was evaluated by Swiss-PdbViewer software for energy minimization andRAMPAGEserver. AlgPred software and SDAP allergen library were used to predict the allergenicity of the protein. **Results:** All of the results suggest that the construct can be an appropriate vaccine candidate against Breast cancer. **Conclusion:** Before working in lab we should be confident about the validity of vaccine throw in silico analyses.

Keywords: Cancer, Chimeric vaccine, Antigens, GMCSF

2759P

In silico analysis of chimeric GMCSF-MUC1-IL2 vaccine against breast cancer

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Background: Breast cancer is a leading cause of cancer-related deaths in women worldwide and there is no effective therapy for patients with invasive and metastatic breast cancer.Immunotherapy may be proved effective in treating patients with advanced breast cancer. We have designed a complex immunogen derived from 7tandem repeats of MUC1(VNTR)as major antigens of breast cancer and nearly whole of GMCSFsequence(18 – 144)and IL2sequence(21-153). **Methods:** Related sequences of MUC1,GMCSFand IL2 were obtained from UniProtKB/ Swiss-Prot.The physico-chemical properties were analyzed using the Expasy'sProtParam

software.Segments were selected based on prediction of immunogenic epitopes.Linear B-cell epitopes of construct were estimated using BepiPred1.0Server,and bcepred.Discotope1.2 was employed to predict discontinuous B-cell epitopes.In general,epitopes having VaxiJen cutoff values of >0.5 was selected.For T-cellepitope prediction.HLApred, MHC2Pred and Propred were employed to predict peptides from the protein binding withMHCII.The chimeric construct codons were optimized based on plant host by using EMBOSS. The mRNA secondary structure of the gene was evaluated by the mfold software. The prediction of the secondary structure of the protein was performed using the GOR-IV.The I-TASSER was employed for tertiary structure prediction.The tool AccelrysDiscoveryStudio2.5 was used to visualize the modeled3D structures.3Dstructural stability of the protein was evaluated by Swiss-PdbViewer software for energy minimization and RAMPAGEserver .AlgPred software and SDAP allergen library were used to predict the allergenicity of the protein. **Results:** All of the results suggest that the construct can be an appropriate vaccine candidate against Breast cancer. **Conclusion:** Before working in lab we should be confident about the validity of vaccine throw in silico analyses.

Keywords: Cancer, vaccine, MUC1, GMCSF, IL2

2758P

In silico analysis of chimeric Her2-INF γ vaccine against breast cancer

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Background: Breast cancer is a leading cause of cancer-related deaths in women worldwide and there is no effective therapy for patients with invasive and metastatic breast cancer. Immunotherapy may be proved effective in treating patients with advanced breast cancer. We have designed a complex immunogen derived from the extracellular domain of HER-2/*neu*-(480–620)that represents a three-dimensional epitopeand IFNγ sequence(24 -161).**Methods:** Related sequences of Her-2 and IFNγ were obtained from UniProtKB/Swiss-Prot.The physico-chemical properties were analyzed using the Expasy'sProtParam software.Segments were selected based on prediction of immunogenic epitopes.Linear B-cell epitopes of construct were estimated usingBepiPred1.0Server,and bcepred._Discotope1.2 was employed to predict discontinuous B-cell epitopes.In general, epitopes having VaxiJen cutoff values of >0.5 was selected.For T-cellepitope prediction. HLApred ,MHC2Pred and Propred were employed to predict peptides from the protein binding withMHCII.The chimeric construct codons were optimized based on plant host by usingEMBOSS.The mRNA secondary structure of the gene was evaluated by the mfold software. The prediction of the secondary structure of the protein was performed using the GOR-IV.The I-TASSER was employed for tertiary structure prediction. The tool Accelrys Discovery Studio2.5 was used to visualize the modeled3D structures. 3Dstructural stability of the protein was evaluated by Swiss-PdbViewer software for energy minimization and RAMPAGEserver. AlgPred software and SDAP allergen library were used to predict the allergenicity of the protein. **Results:** All of the results suggest that the construct can be an appropriate vaccine candidate against Breast cancer. **Conclusion:** Before working in lab we should be confident about the validity of vaccine throw in silico analyses.

Keywords: Cancer, Chimeric vaccine, IFNγ, her2

2782P

Isolation and cloning of Mycobacterium tuberculosis antigens

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Background: Mycobacterium tuberculosis is a pathogenic species. These bacteria primarily affect the lungs. One of the basic steps of the bacterial pathogenesis is the attachment of the microorganism to the host cells. Identifying adhesions involve in the early stages of the bacterial attachment and colonization is suitable targets for constructing new vaccines or designing new drugs. HBHA in Mycobacterium tuberculosis as an adhesion molecule binds to proteoglycan heparan sulfate in lung epithelial cells and plays a role in the spread of infection outside the lungs. New studies showed that combination this protein with BCG vaccine can improve effectiveness of the conventional vaccine. C-terminal domain of mtb32a (Mtb32C) can strongly motivate TCD8 cells that produce IFN- gamma have been isolated and cloning process was confirmed by PCR and digestion method. **Methods:** HBHA gene and Mtb32C fragment, were isolated from Mycobacterium tuberculosis H37Rv strain (Pasture Institute of Iran) by using two set of primers that designed by gene runner software. Amplified genes along with pCDNA 3.1 digested by specific restriction enzymes. Insertion of HBHA and Mtb32C to pCDNA 3.1 vector, was performed. The construct was introduced into Ecoli JM109 and the accuracy of the cloning was confirmed by colony-PCR and restriction enzyme analysis. **Results:** HBHA and Mtb32C showed 600bp and 384 bp fragments, respectively in PCR amplification and restriction enzyme analysis. **Conclusion:** This construct can be used for further studies such as evaluation different immunogenic properties of them.
Keywords: Mycobacterium tuberculosis; HBHA; Mtb32C;

2781P

Expression mycobactrial antigens in eukaryotice expression system

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Background: Mycobacterium tuberculosis is the major cause of human tuberculosis. According to the latest WHO reports Tuberculosis causes 8.6 million new TB cases in 2012 and 1.3 million TB deaths, therefore development new drugs, diagnostic methods and more effective vaccines to overcome this problem is very critical. In this study, expression of two highly immunogenic mycobacterial antiges (HBHA, Mtb32C) were evaluated in eukaryotic system. **Methods:** In this study, HBHA and Mtb32C, mycobacterial immunodaminant antigens, cloned into pCDNA3.1 vector. Hela cell line was used to investigate the expression of mycobacterial antigens. These cells were transfected with the calcium phosphate method and expression of a new chimeric protein was assessed by RT-PCR. **Results:** Results of RT-PCR showed expected band of 984 bp that it confirmed the expression of recombinant protein in this cell line. Conclusion: In this study, the constructed vector could produce two immunodominant mycobacterial antigens that connection of these two antigens together produces a chimeric antigen with new immunologic characteristics. Other attempts are needed to evaluate these

new immunologic characteristics in future studies.
Keywords: Mycobacterium tuberculosis; HBHA; Mtb32C; cloning

2898P

Cloning and sequencing fusion Ag85B and TB10.4 genes of *Mycobacterium tuberculosis* into eukaryotic vector

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Background: Despite the usage of BCG vaccine since 1921 until now, tuberculosis is yet remains as second of causes of mortality among infectious diseases. According to increasing resistance of Mycobacterium tuberculosis to common antibiotics, broad spectrum drugs and also coinfection HIV &*Mycobacterium tuberculosis*, conflict to disease is more challenging. Identification of effective antigens in protective immunity could be as candidate in design to new vaccines. Among these antigens were TB10.4& Ag85B respectively 10 and 30KDa which identified by T lymphocytes and induced Th1 strong response and increased production of IF-γ. In this study was used from this two antigens in order to construction of gene cassette. **Methods**: DNA of *Mycobacterium tuberculosis* was extracted by routine method. Genome of antigens were amplified by specific primers with PCR method and then was fused and after enzyme digestion, it was cloned into eukaryotic pcDNA3 vector. Recombinant plasmid was transformed into *E.coli* DH5α and after purification, was confirmed with enzyme analysis and sequencing. **Results**: The results of sequencing and digestion confirmed that cloning was done successfully. **Conclusion**: The fragmens of TB10.4 and Ag85B were fused and cloned successfully into pcDNA3 vector. In future studies could be assessed gene expression in cell line and was used from gene cassette as DNA vaccine
Keywords: TB10.4 and Ag85B, Mycobacterium tuberculosis

2890P

Molecular cloning of fusion of GP96 and *Leishmania major* Kinetoplastid membrane protein 11 in pEGFP-N1 expression vector as a DNA vaccine candidate against cutaneous Leishmaniasis

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Background: The protozoan parasites of the genus Leishmania are the causative agents of the various clinical diseases. Main subject of cutaneous leishmaniasis in Iran is Leishmania major and currently cutaneous Leishmaniasis is seen as endemic disease in many areas of Iran. Because of the importance of this disease ¸creating new drugs or vaccines for this disease is necessary. KMP-11 (Kinetoplastid membrane protein-П) exists in all species of kinetoplastid family and is fully protective and the protein that products by this gene can induce very high cellular immune response. It has been frequently reported that gp96 acts as a strong biologic adjuvant. **Methods:** KMP-11gene from *L. major* (MRHO/IR/75/ER) DNA and NT-

Gp96 from pBluescript-Gp96 plasmid containing the Xenopus Gp96 DNA amplified by PCR and the purified PCR products were cloned into the pJET1.2/blunt plasmid vector and then subcloned into pEGFP-N1 plasmid as an expression vector. The KMP-П gene was fused with GP96 (immunologic adjuvant) and this combination cloned in pEGFP-N1. **Results:** KMP-11and NT-Gp96 genes were cloned successfully into the pJET1.2/blunt cloning and pEGFP-N1expression vectors. KMP-GP96 Fusion cloned successfully into pEGFP-N1. All cloned genes confirmed by PCR and enzyme digestions. **Conclusion:** In conclusion we successfully cloned all construct including fusion of two genes in pEGFP-N1 for future use as DNA vaccine.
Keywords: KMP-11, GP96, *Leishmania major*, vaccine.

2889P

Live recombinant Leishmania tarentolae with KMP-11 – NTGP96-GFP fusion as a vac cine candidate against cutaneous Leishmaniasis

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Background: Leishmaniases are neglected tropical diseases that cause human infections varying from self-healing cutaneous lesions to mucosal diffuse cutaneous and visceral forms and because of the importance of them ,creating new vaccines are necessary .It has been reported that KMP-11 (Kinetoplastid membrane protein-П) is fully protective antigen and induces very high cellular immune response and NT-Gp96 acts as a strong biologic immunologic adjuvant. The use of the non-pathogenic *Leishmania tarentolae* as a live vaccine vector to deliver specific Leishmania antigens is a recent approach. **Methods:** KMP-11gene from *L. major* and NT-Gp96 from Xenopus Gp96 DNA amplified by PCR and the PCR products were cloned into the pJET1.2/blunt cloning vector and then subcloned into pEGFP-N1 expression vector. The KMP-11, NT-Gp96 and GFP fused in pEGFP-N1 and then subcloned into pLEXSY-neo vector. Finally this construct transferred to Leishmania tarentolae by electroporation. **Results:** KMP-11and NT-Gp96 genes were cloned successfully into the pJET1.2/blunt and pEGFP-N1 vectors and KMP- NT-Gp96-GFP Fusion cloned successfully into pLEXSY-neo vector and this construct successfully transferred to Leishmania tarentolae. **Conclusion:** we successfully cloned fusion of three genes into pLEXSY-neo vector and transferred it to Leishmania tarentolae for future use as live recombinant vaccine.
Keywords: KMP-11, GP96, Leishmania major, vaccine.

2981P

High-yielding influenza A virus in Vero cell by over expression of sialyltransferase

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Background: Influenza A viruses are the cause of annual epidemics of human disease with occasional outbreaks. **Methods:** Some cell lines such as MDCK, Vero, HEK-293, and PER-C6 has been used for production of Influenza vaccines. **results:** Influenza A virus bind to sialyloligosaccharids present on the host cell surface glycolipids or glycoproteins.Human influenza viruses bind to α2-6 Gal. while avian influenza viruses bind to α2-3 Gal. In these studies we have produced a Vero cells stably expressing Siat7e and examed its capacity to propagation of influenza A virus. Vero cells transfected by the plasmid containing Siat7e gene and Lipofectamin 2000 reagent. Transfect ion efficiencies has been showed by flow cytometry method and stably transfected cells cloned by limiting dilution.mRNA samples were isolated from parental Vero cells and from clones of Siat7e- expressing cells so over expression of Siat7e was showed by Real time PCR.Virus growth were determined by HA titer in parental Vero cells and Siat7e expressing cells. The result showed that stable Siat7e expressing Vero cells were able to supports 5 times higher than parental cells in influenza A virus propagation. **Conclusion:** So according to this study, Vero-Siat7e cells are a promising system for production of seasonal and pandemic influenza vaccine.
Keywords: Influenza A virus, MDCK, Vero, HEK-293, and PER-C6 cells, Sialyloligosaccharid, Siat7e, HA titer.

3179P

Producing of anti-*E.coli* O157:H7chimeric antigen chicken egg yolk antibody as a prophylactic agent

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Background: *E.coli* O157:H7 is one of the intestinal pathogens that cause a wide range of A/E lesions in mammalian and human. Two groups of the most important factors in pathogenicity of this bacteriumare shiga toxin and colonization factors. IgY technology has a lot of advantages that can be used in passive immunization against such pathogens. **Methods**: Fusion gene composed of *espa*, *stx2b* and *eae* genes was prepared as synthetic and transferredinto *E. coli*BL21. The recombinant expression of chimeric protein was done by IPTG and then purified with Ni-agarose column. Antigen injection to laying hens was done subcutaneously in 3 intervals (0 day, 14 and 28). Eggs were collected one week after 2nd and 3th injections. Indirect ELISA was done to determination of IgY production against the chimer. **Result**: Soluble recombinant expression of chimeric protein was performed in 18˚C and overnight incubation time. Data from indirect ELISA showed that the IgY can interaction with chimeric protein even in very diluted concentrations like 1/128000titer. **Conclusion**: Our results showed that this designed chimer has good potency to develop IgY in immunized hens.
Keywords: *E. coli*O157:H7, Chimeric protein, Chicken immunoglobulin.

3098P

Preparation and immuno biological evaluation of trivalent complex of *Haemophilus influenzae* type b polyribosylribitolphosphate with OMV of *Haemophilus influenzae* and LOS of *Haemophilus influenza* as a meningititis vaccine in animal model.

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Background: *Haemophilusinfluenzae* type b is referred as one the important cause of bacterial meningitis in children. Polysaccharide ribitol ribosyl phosphate capsule (PRP) is present in all of the Haemophilus influenzae strains and refereed as the most cause of meningitis, therefore this structure is exist in all of the Haemophilus vaccines. The adjuvant property of lipooligosacarid (LOS) and outer membrane vesicle (OMV) makes them as a potential adjuvant with microbial origin and promise for the vaccine research applications. In this regard, we in this study conjugated PRP-LOS-OMV and evaluated its function in animal model **Methods**: PRP and LOS from Haemophilus influenzae type b and OMV from Neisseria meningitidis were extracted according to the protocol and trivalent complex made up using conjugation procedure. Amount of IgM and IgG antibodies in animal was investigated by ELISA. **Results:** ELISA results revealed that the trivalent complex (PRP-LOS-OMV) could raise the highest IgM and IgG antibodies than bivalent complexes (PRP-LOS, PRP-OMV, OMV-LOS). **Conclusion:** according to the obtained experiments, trivalent complex resulted in the highest immunity in animal and so it can be potential Haemophilus vaccine candidate in further investigations

Keywords: Meningitis, Haemophilus influenzae, PRP, LOS, OMV

3271P

N-Terminal Fragment of Gp96 Enhance Cellular and Humoral Immune Responses against HCV DNA Polytope Vaccine

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Background: Hepatitis C virus causes chronic disorders in more than 50-80% of infected individuals. Up to now, there is no effective vaccine against HCV due to virus variability and immunosuppressive effect of viral proteins. A polytope DNA vaccine containing B and T-cell epitopes could be a promising vaccination strategy against HCV. Heat shock protein gp96 proved to be a potent adjuvant to improve cellular and humoral immunity. **Methods**: We designed and constructed a DNA polytope (PT) vaccine harboring HCV immunodominant CTL epitopes (HLA-A2 and H-2^d) from Core, NS3 and NS5B, a Th CD4 epitope from NS3 and a B cell epitope from E2. We fused N-terminal fragment of gp96 (NT(gp96)) to 5' or 3' end of the polytope DNA named NT(gp96)-PT and PT-NT(gp96). **Results**: CB6F1 mice were immunized with the polytope vaccines and then cellular and humoral immune responses were evaluated. Using multicolor flow cytometry, we showed that DNA immunization with fusion of gp96 to the 3' and 5' end of the PT resulted in more effective and functional T-cell response than PT alone. We de-

termined: 6.8%, 5% and 0.9% of IFNγ-positive CD8 T-cells in splenocytes of mice immunized with PT-NT(gp96), NT(gp96)-PT and PT respectively. Moreover, the HCV-specific antibodies levels were significantly higher in mice immunized with gp96 fusion constructs than with PT alone. **Conclusion**: Our results show that fusion of the N-terminal domain of gp96 to the polytope induced better cellular and humoral immune responses. Currently, we investigate the CD8 T-cell response in HLA-A2 transgenic mice and determine neutralizing E2-specific antibodies.
Keywords: HCV, vaccine, Polytope, gp96

3238P

Formation of self-assembled virus like particles 2/6/7 of rotavirus in stably transfected high-five insect cell line and their immunogenicity evaluation

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Background: Rotaviruses (RVs), as a genus of the Reoviridae family, are a common cause of severe diarrhea in children <5 years of age worldwide. Recently, RotaTeq™ and Rotarix™, as live attenuated vaccines, were developed. Concerns regarding safety and efficacy skewed researchers' focus on alternative vaccine candidates such as non-living RV vaccines among which virus like particle (VLP)-based vaccines have shown the most promising results. **Methods and Results:** An insect High-Five cell line was generated constitutively and stably expressing the VP2, VP6 and VP7 proteins of rotavirus leading to the formation of VLPsof rotavirus. The presence of VP2, VP6 and VP7 genes and their expression in stably transfected high-five cells were verified by molecular and protein analyses. Self-assembled VLP2/6/7 were observed by transmission electron microscopy. To evaluate the immunogenicity of VLPs, we assessed the humoral and cytokine responses induced by VLP2/6/7 in BALB/c mice immunized intra-peritoneally (I.P.) and intra-rectally (I.R.). Enzyme-linked immunosorbent assay (ELISA) and Relative quantitative (RQ) Real-time PCR were used to evaluate the antibodies levels in serum (IgG and IgA) and stool (IgA) and mRNA levels of IL-6, IL-10 and IFN-γ in spleen cells, respectively.Our results showed the I.P. and I.R. administration of VLPs generated by the stable double transfected High-Five cells are able to induce high level of IgG and IgA titers. Moreover, mRNA levels of IL-6 and IFN-γ were significantly elevated in mice immunized intra-peritoneally with VLP2/6/7 compared to control group. **Conclusion:** Our findings indicated that VLPs constructed via a stable insect cell line not only induce humoral immune responses but also elicit T cells responses in mice administered intra-peritoneally and intra-rectally. Interestingly, it may be useful for vaccine development.
Keywords: High-Five cell; Rotavirus; VLP; immunogenicity

3266P

Specified and unspecified CpG-ODN has different effect for inducing specific immune response against S1 subunit of Pertussis toxin

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Background: Bordetella pertussis is a gram negative bacterium that causes respiratory tract infection in human (whooping cough). Pertussis toxin is thought to play a major role in the pathogenesis of whooping cough and also believed to be a major protective antigen. S1 is the most important immunogenic part of PT. The most important type of immune response for pertussis is cellular immunity and bacterial CpG motifs are good adjuvants for stimulation of this type of immunity. The aim of this study after producing of recombinant S1 (rS1) in *E.Coli*, was comparing three different CpG motifs as adjuvants for stimulation of immune response and switching of it to Th1 system. **Methods:** The rS1, produced in *E.coli*, was purified with nickel column. Different BALB/c mice groups were immunized with rS1 and different adjuvants (CFA, DNA fragments of B.pertussis, DNA fragments of *E.coli* and synthetic CpG motifs). **Results and Conclusion:** Subcutaneous administration of the rS1 and different adjuvants significantly induced immune response in mice which were associated with Anti-rS1 total IgG and IgG2a isotype antibodies production as well as IFN-γ cytokines which proposed Th1 immune responses. Increasing total IgG and IgG isotypes titers was comparable between CFA and different CpG motifs. DNA fragments of *B.Pertussis* could induce IFN-γ secretion better than other adjuvant. This study showed when rS1 is formulated with CpG moifs an stimulate specific immune response(specially cellular), but DNA fragments of specific bacteria is better than unspecific Bacterial DNA fragments, synthetic CpG motifs and CFA for inducing of specific Th1 responses.

Keywords: Pertussis Toxin, *B.pertussis*, S1, CpG motifs, DNA fragments

3307P

Interference of Rev1 vaccine against brucellosis with diagnosis tests

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Background: The gram negative bacteria of the genus brucella are interacellular parasites that cause brucellosis, a world-wide distributed disease that represents a serious problem for animal and human health. Vaccination is critical to control and eradicate ruminant brucellosis. The attenuated Brucella melitensis Rev1 vaccine, used against brucellosis infection, interferes with serological diagnosis tests, may induce abortions in pregnant animals, and may infect humans. Rev1 vaccin induce antibodies against the O chain of the smooth lipopolysacharide which is the main antigen in serological diagnosis, therefore, differentiation between the vaccinated animals and the infected by B.melitensis is difficult. **Methods:** Brucella melitensis 16M and the vaccine strain Rev1 was obtained. Indirect ELISA and western blotting were methods that used for this purpose. **Results:** in order to overcome these drawbacks, we should consider

other structurs of this organism to design the vaccine. The properties of the wadC mutant provide the proof of concept for this new approach.The smooth lipopolysacharide consists of three sections: lipidA, core oligosaccharide and O chain. In order to more efficacy of vaccine, we should attention to lipidA as a target.Also vjbR gene can candidate for differentiation between infection and vaccination. Studies have shown to evaluate the induction of protective immunity against brucellosis by vaccination with a combination of naloxone and alum.
Keywords: brucella immunity, Rev1, live attenuated vaccine.

3333P
Investigation of antibody production against recombinant protein (StxB IpaD) of *Shigelladysenteriae* type 1 in mice

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Background: Shigellosis is acute intestinal infection caused by Shiga toxin and Shiga-like toxin of Shigella and enterohemorrhagic Escherichia coli (EHEC) and Escherichia entrotoxinogenic (ETEC) and has high prevalence rates in the world. StxB is a part of Shiga toxin and has the property of immunogenicity. **Methods**: Fusin gene (STxB-IpaD) was cloned in pET28a (+) vector and then transferred into the E. coli BL21 DE3 bacteria and was confirmed by culturing this bacteria on selective medium and with PCR method. Recombinant expression of protein was done by IPTG and analyzed on SDS-PAGE. The nasal prescription of STxB-IpaD performed in mice for four intervals. Antibody production against chimeric antigen was analyzed indirect ELISA. **Results**: The recombinant protein was isolated and purified by nickel column. The Nasal administration of STxB-IpaD protein performed in mice without any adjuvant, four consecutive times and antibody titer was assessed with ELISA.IgG antibody titers were observed in the nasal condition. **Conclusion**: The results of this study indicated that properties of STxB protein adjuvant and absorption of STxB-IpaD antigen by epithelial cells of mice and it's nasal vaccine candidate were appropriated.
Keywords: Shigelladysenteriae, Shiga toxin, StxB,Cloning, Protein expression, Immunization

2648P
Molecular characterization of the *SG1B* of *Anopheles stephensi* as a transmission blocking vaccin (TBV) target in EMR/WHO endemic malaria region

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Background: Malaria is an important infectious disease in the world which it causes one million deaths in each year, especially in child and poor countries. Furthermore, because malaria drugs are expensive, their preparation by poor countries has many difficulties. Therefore, developing a safe, effective and protective vaccine is a priority of WHO malaria eradication program. A transmission blocking vaccines (TBV) is a vaccine that prevents or hinders the sexual development of parasite within the mosquito or prevent of transmission malaria parasite from mosquitoes to human. One of the mosquito based TBVs is SG1B which

its blocking caused severe inhibition of Plasmodium parasites transmission. **Methods:** For SG1B characterization, cloning primers were designed based on SG1B sequence in database. Total RNA was extracted from the *Anopheles stephensi* midgut and RT-PCR was performed. DNA was extracted from the *Anopheles stephensi* as well. PCR was performed on DNA and cDNA and PCR products were sequenced. **Results:** In this study, the sequence of *SG1B* gene of *An. stephensi* was sequenced and its comparison with its related mRNA was performed for the first time. This study showed that the *SG1B* gene doesn't have intron sequence. **Conclusion:** We characterize and sequenced the *SG1B* of *Anopheles stephensi* for the first time in this study. Its comparison with its related protein in *An. gambiae* showed high similarity in nucleic and protein sequence. According to our findings and previous reports, SG1B can be introduces as a TBV target in regions that An. stephensi is the main malaria vector.
Keywords: Malaria, SG1B, Transmission Blocking Vaccine (TBV), *Anopheles stephensi*

2585P

Evaluation of adjuvant activity of *Pseudomonas aeruginosa* flagellin and /or exotoxin A

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Background: Flagellin and exotoxin A of *P.aeruginosa* have been known to possess in vivo adjuvant activity. We have evaluated flagellin and/or exotoxin A ability to function as a vaccine adjuvant in mice. **Methods:** We constructed purified a exotoxin A(I,II)-flagellin(N-terminal) fusion protein by recombinant method. This fusion protein was used to immunize BALB/c mice by subcutaneously injection in two groups (6 mice per group). In first group, 20µg of fusion protein was injected with complete Freund's adjuvant on days 0 and 21, 42 with incomplete Freund's adjuvant and day 72 without adjuvant. In second group, 20µg of fusion protein was injected on days 0, 21, 42 and 72 without adjuvant. In third group, equal volume PBS was injected as control group. 1 week after the last injection, sera were collected from mice in all groups and antibody production was evaluated by ELISA method. The immunized mice were challenged with approximate 2X LD50 of clinical strain of *P. aeruginosa*. **Results:** ELISA results demonstrated that vaccination with the fusion protein produced significant amount of specific IgG antibodies in both with (OD_{450}=2.96, P=0.0001) and without (OD_{450}=0.37) immunized groups comparison with the control (OD_{450}=0.078, P=0.0001). But, the antibody titers in fusion protein with adjuvant vaccinated group were very higher than fusion protein without adjuvant vaccinated groups. The mice immunized with fusion protein and adjuvant were afforded significant protection against challenge with clinical strain of *p.aeruginosa*, whereas fusion protein without adjuvant vaccinated groups was partially effective in survival rate. **Conclusion:** *P. aeruginosa* flagellin and /or exotoxin A in exotoxin A(I,II)-flagellin(N-terminal) fusion protein have not adjuvant activity.
Keywords: *P. aeruginosa*, Adjuvant, Flagellin, Exotoxin A, Vaccine

2682P

Vaccination of mice with recombinant heat shock protein confers protection against *Brucella melitensis* infection

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Background: Brucella melitensis infection is still a major health problem for human. In spite of tremendous efforts to develop an effective vaccine, the proposed live vaccines are still less than ideal because of some drawbacks including induction of abortion and antibiotic resistance. In order to study the potential of heat shock protein (HSP) for development of a *Brucella* subunit vaccine, we evaluated the immunogenicity and protective efficacy of recombinant HSP (rHSP) protein from *Brucella melitensis* in BALB/c mice. **Methods:** The HSP gene was cloned in pDEST42 and resulting recombinant protein was used as subunit vaccine. Antigen-specific antibody responses and cell proliferation, production of TH1/TH2 cytokines, bacterial load and protection efficacy of the vaccine were evaluated in reference to the *Rev.1* live vaccine. **Results:** rHSP elicited mixed TH1-TH2 immune responses with higher titers of specific IgG1 than IgG2a. In lymphocyte transformation assay, splenocytes of immunized mice exhibited a strong recall proliferative response with high amounts of IFN-γ, IL-12, IL-10 and IL-6 and very low levels of IL-5 and IL-4 production. rHSP significantly reduced bacterial load and conferred a significant degree of protection against *B. melitensis* challenge compared to control mice ($P<0.001$). **Conclusion:** These results suggest that rHSP may be a suitable candidate for the development of subunit vaccine against brucellosis.

Keywords: Brocellosis, Subunit vaccine, Heat shock protein, Cytokine, Protection

3255P

Evaluation of Immunogenic properties recombinant hemagglutinin influenza A virus expressed in MDCK cell line

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Background: The recent pandemic swine H1N1 influenza (2009) outbreak demonstrated that egg-based vaccine manufacturing does not adequately respond to pandemic strains. Recent study has established an alternative for subunit vaccine by the use of the recombinant hemagglutinin protein (rHA) that can be produced in large scale in reasonable time **Methods**: In this study a recombinant hemagglutinin gene of influenza A virus was designed and expressed in MDCK cell which could be secreted out of cells. Immunized mice with this protein induced both humoral and cellular response against influenza A virus. .**Result:** The immunized mice showed increased immunological indicators such as IFN γ and IL-2, IL-12, IL-4 and induced

suitable CTL response. **Conclusion**: These findings suggest that rHA expression in MDCK cell may provide a new approach for developing a novel vaccine that may protect not only specifically against a now circulating strains, but is expected to protect broadly against new virus strains possessing common epitopes with conserved sequences. The rHA0 protein is a highly purified single protein that might enhance tolerance against the antigen and allows administration of higher doses and produce stronger immunological response and protection against the mentioned virus.

Keywords: Influenza vaccine, hemagglutinin, Immunological indicators, CTL response.

2999P

Improvement of the rabies inactivated vaccine potency through DNA boosting

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Background: Rabies virus is a medically important neurotropic virus. Limited access to healthcare facilities and the high costs and complex schedules of rabies biologics often hamper human rabies prophylaxis in the developing countries. Recent advances in recombinant DNA technology have made it as a good candidate of vaccination. Rabies virus glycoprotein (RVG) has been the major target for new vaccine development. The rabies virus surface glycoprotein responsible for induction of virus neutralizing antibodies could provide complete protection against RV challenge. In this study recombinant rabies virus glycoprotein was used as a potent DNA vaccine. **Methods:** Glycoprotein gene has been synthesized and cloned into pBluescript vector and then sub cloned into eukaryotic expression vector. After verification of the cloning, the recombinant plasmid was transfected into BSR cell line. The plasmid construct was injected to MNRI mice twice and Prime-Boost vaccination strategy was done by prime dose of plasmid construct and the second dose of PVRV or vice versa. **Results:** The authenticity of the recombinant plasmid had been confirmed by a quick check method, restriction endonuclease digestion analysis and after transfection into eukaryotic cells; SDS-PAGE and Western blot analysis were also performed. This construct which is able to produce rabies virus glycoprotein, was used as a DNA vaccine. Rabies virus neutralizing antibody (RVNA) titers of the serum samples were determined by RFFIT. **Conclusion:** This study demonstrated that the construction of eukaryotic expression plasmid for rabies virus glycoprotein is possible. In comparison, serum neutralizing antibody response in a low dose of PVNA primed DNA boosted group is similar to that of PVRV.

Keywords: Rabies virus glycoprotein, DNA vaccine, Prime boost immunization

2503P

Immunogenic properties of four consensus envelope domain III proteins of dengue virus

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Background: Dengue virus is an emerging mosquito-borne pathogen that causes a range of illnesses including dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. Dengue infections with high percentage of mortality are prevalent in tropical and subtropical areas of the world. There is no approved vaccine or drug treatment against dengue virus infections. Dengue envelope protein (E protein) is an important antigen for vaccine development. The dengue E protein consists of three structurally distinct domains (I, II, and III), which the envelope domain III (EDIII) is the most important part for induction of protective immunity against dengue virus. Here we have designed and expressed four consensus EDIII proteins for all four serotypes of dengue virus and evaluated the immunogenicity of these proteins in mice. **Methods:** Megalign and Optimizer software were used for sequence alignment and gene sequence optimization, respectively. Stable protein expression was carried out in *E. coli*. The Western blotting and SDS-PAGE methods were used for protein identification; and ELISA, FRNT, and cytokine assays were used to evaluate immunogenicity of the proteins. **Results:** Four consensus envelope domain III proteins were designed, expressed and characterized. The immunized mice with recombinant EDIIIs developed high titer of neutralizing antibody responses against the corresponding serotype of dengue virus. According to the result of cytokine assays, both of Th1- and Th2-type immune responses were induced following immunization. **Conclusion**: We showed that the recombinant EDIIIs can induce humoral and cellular immune responses in mice and can be applicable to produce a dengue vaccine candidate.

Keywords: Immunogenic, Dengue virus, Envelope protein

2411P

Production and enhancement of native-like form of *Plasmodium vivax* apical membrane antigen-1 in *E. coli* expression system for *P. vivax* vaccine development

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Background: In recent years, production of high amount with correctly conformation of antigen in minimum time and affordable manner appears to be essential in vaccine strategy. This study was performed to improve the production level of the apical membrane antigen-1 (AMA-1) as a promising malaria blood stage vaccine candidate, in *E.coli* M15 for vaccine development program. **Methods:** Cloning and expression of PvAMA-1 ectodomain was done in pQE30 expression vector. The expression level of rPvAMA-1 was measured by densitometer. Purification was performed using Ni-NTA agarose under denaturing conditions. The SDS-PAGE and western blotting was performed in both reducing and non reducing condition to show the purity and correct folding of the recombinant proteins. To evaluation whether the polyclonal antibodies raised to rPvAMA-1 could recognize the native protein on the merozoites, the pooled anti-sera collected from immunized mice with PvAMA-1 antigen were analyzed by IFAT. **Results:** The expression level of recombinant PvAMA-1

was 65 mg/liter. SDS-PAGE and Western blot analysis showed that the reduced and non-reduced recombinant PvAMA-1 migrated at different sizes on SDS-PAGE, suggesting that disulfide bounds have been formed in order to formation the correct folding of the antigen. Furthermore, anti-rPvAMA-1 antibodies produced in mice recognized the native protein expressed by *Plasmodium vivax* on the late schizonts. **Conclusion:** In this study, we have shown that expression improvement of PvAMA-1 can be obtained in a prokaryotic expression system as a native-like aglycosylated form. This recombinant antigen has induced antibody responses that could recognize native AMA-1 antigens on parasite surface.
Keywords: *Plasmodium vivax*, Apical Mmbrane Antigen-1, Vaccine, native-like protein

2924P

The chimeric protein as vaccine candidate against Enterotoxigenic Escherichia coli (ETEC) and Entero hemorrhagic Escherichia coli (EHEC), an in silico approach

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Background: Diarrheal diseases remain major global health problems due to bacterial infections. Diarrheagenic Escherichia *coli* is regarded as the most common bacterial cause of diarrhea. Among bacteria, EHEC and ETEC cause the largest number of diarrheal cases. EHEC strains are characterized by the ability to form A/E lesions, and the production of shiga like toxins. ETEC adheres to intestinal epithelial cells via colonization factors (CFs) and producing labile or stable enterotoxins. **Methods:** The genes of intimin, B subunit from labile and shiga like toxins and major subunit part in CFA/I were selected for this study. The secondary structure of hypothetical protein was predicted by GORIV secondary structure prediction method. For 3D structure prediction, I-TASSER ab initio online software,was used. The predicted structure was validated by Ramachandran plot in PROCHECK software. Epitope prediction was done with web server IEDB. The presence of possible allergenic sites was analyzed by AlgPred .Finally the synthetic gene was codon optimized for expression in plant host. **Results:** The optimized chimeric gene sequence had a codon adaptation index of 0.85. ΔG of the best-predicted structure by RNA molecules was −610.20 kcal/mol and the first nucleotides at 5′ did not have a long stable hairpin or pseudoknot. Expasy's ProtParam classifies this protein as stable. GOR analysis results showed three helix peaks corresponding to the linker fragments. The Ramachandran plot analysis revealed that 77% of amino acid residues were incorporated in the favored regions. This protein was not detected as potential allergen, in analysis by AlgPred tool. The antigenic analysis show that the epitopes of the chimeric protein could induce both B-cell and T-cell mediated immune responses. **Conclusion:** Our data indicated that in silico analyses could act as a powerful instrument in reverse vaccinology.
Keywords: Bacterial infections, EHEC, Allergen, Shiga toxins, Colonization factors

2545P

Evaluation of recombinant antigen Rop1 efficacy in diagnosis of Toxoplasma gondii infection

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Background: Toxoplasma gondii is a ubiquitous obligate intracellular parasite and producing proteins are strong antigens that can start strong immune reactions. One of these kinds of antigens is rhoptry protein 1 (ROP1) that is discharged from rhoptry cell-organ. These entire attribute for ROP1 makes it a competitor for protein vaccine and recombining vaccine against toxoplasmosis. A main objective of the current study was the Cloning and expression ROP1 of Toxoplasma gondii in a cloning vector for later studies. **Methods**: Genomic DNA of Toxoplasma gondii was removed and used for amplifying of ROP1 gene as a template. Then PCR product was cloned into the EcoR1 and BamH1 sites of cloning vector, pUET1, and transformed into Escherichia coli BL21 plysS strain and sub cloned from pTROP1 into the HindIII and EcoRI sites of the pcDNA3 to produce recombining eukaryotic declaration vector pcROP1. The cloned ROP1 was verified by PCR, limitation enzymes (HindIII and BglI) digestion and nucleotide sequencing. Then we have evaluated the diagnostic utility of Toxoplasma gondii recombinant ROP1 in immunoglobulin G (IgG) and IgM recombinant enzyme-linked immunosorbent assays. **Results and Conclusion**: A fragment about 757 bp was separated for more nucleotide sequence analysis of the ROP1 cloned in pUET1vector revealed high homology (96%) with RH strain Gene Bank Accession No. M71274. This expressed ROP1 to make recombinant vaccine against toxoplasmosis will be useful in the future study. Our results suggest that the combination Ag Rop1 in an IgM Rec-ELISA can replace the tachyzoite antigen in IgG and IgM serologic tests.

Keywords: *Toxoplasma gondii*, ROP1, Expression, Diagnosis

2991P

In Silico analysis, expression and purification of a novel *Pseudomonas aeruginosa* PilQ-PilA fusion protein as vaccine candidate

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Background: *Pseudomonas aeruginosa* as opportunistic pathogen cause infection in immunocompromised individuals but there is no yet protective vaccine against. The Bacterial fimberia and OMPs was highly regarded as vaccine candidate. In the current study

bioinformatic analysis, expression and purification of pilA binding site and PilQ as a fusion protein was evaluated for potential vaccine. **Methods**: Bioinformatic analysis of PilQ-PilA was performed using various online softwarse. The pET-28a (+) - *pilQ-pilA* construct was synthesized by Canadian company Biomatic and transformed into the BL21 (DE3) E. coli cells. Transformation was confirmed by PCR. Following induction by IPTG, the bacteria harvest was collected and then sonicited. The fusion recombinant protein was purified and isolated by Nickel affinity chromatography and finally was confirmed by SDS-PAGE and Western blot analysis. **Results**: The extensive bioinformatics analysis showed the good water solubility, low hydrophobicity, no TM and well exposed regions, high levels of Random coil and extended strand conformation , 14 antigenic determinants, 15 liner B-cell epitopes, good scattering of aromatic, charged and polar amino acids about our candidate. The PCR confirmed the accuracy of the construct and transformation. The SDS-Page and western blot results showed the revealed the expression of target recombinant protein. The concentration of the protein was measured by the Bradford method as 0.6 mg/ml. **Conclusion**: According to our In silico and biochemical analysis on the pilA-PilQ recombinant protein, it could be considered as vaccine candidate for prevention of Pseudomonas aeruginosa infections. However further studies on animal model is recommended.

Keyword: *Pseudomonas aeruginosa*, PilQ, PilA, vaccine

Veterinary Immunology

Oral Presentations:

2417O

Brucella abortus Outer Membrane Protein 19 Loaded N-trimethyl Chitosan Confers Protection against B. abortus, B. melitensis and B. suis in Mice

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Background: Brucellosis or malt fever is one of the most important zoonotic diseases in animals and humans. The results of brucellosis include abortion, reduction of milk production and infertility in infected animals and transmission probability to human. The disease caused by Brucella spp as facultative intracellular bacteria. As Brucella infections occur mainly through mucosal surfaces, the development of mucosal administered vaccines could be good strategy for the control of brucellosis. Previous studies have shown that Omp19 can result in protection against B. abortus, B. melitensis and B. suis in mice. Since recombinant proteins as subunit vaccines have low immunogenicity, use of impressive antigen delivery system is critical. It has been shown N-trimethyl chitosan (N-TMC) nanoparticles are promising delivery system for oral vaccination. Based on these findings, TMC nanoparticles loaded with Omp19 was administrated in Balb/c mice through intraperitoneal and oral routes.**Methods:** Synthesis of N-TMC polymer, evaluation of chemical structure and degree of quaternisation (DQ), cloning, expression and purification of recombinant Omp19, nanoparticle preparation, characterization of antigen-loaded TMC Nanoparticles, mice immunization, ELISA, splenocyte cultures and lymphocyte proliferation and protection assay were performed.**Results:** NMR analysis showed N-TMC with a DQ of 18% has been synthesized. Mean size distribution and zeta potential of nanoparticles were 200-300 nm and 26.1± 1.4, respectively. Mice orally administered with Omp19 nanovaccine showed higher antibodies and cytokine titers and protection level than mice intraperitoneally administered.**Conclusions:** Our results suggest TMC loaded with Omp19 can be a promissing candidate vaccine against different Brucella spp.

Keywords: Brucellosis, TMC, Oral Vaccine, Nanoparticle.

2522O

Construction of human polyclonal recombinant Fab library, using peripheral blood of snake bitten victims

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BackgroundHuman poisoning from snake bitten is a serious threat in many subtropical and tropical countries among in Iran. The best acceptable systemically treatment of envenomated humans is antivenoms. Most of antivenoms generally produced in horses, have aseries ofeconomic and medicalproblems and need more improvements. **Methods** In this study a combinatorialhumanimmunoglobulingenelibraryagainst Iranian poisonoussnake venoms wasconstructed. For preparing the total RNA, peripheral blood lymphocytes of twopatientswhorecoveredfrom snake biting was used. RT-PCR was used to amplify the heavy (Fd) and light chains. Afterward PCR products were inserted successively into the cloning vector pComb3x to construct the human combinatorial library of polyclonal Fab antibodies. **Results**The amplified fragments of Fd and κ gained by RT-PCR were about 650 bp. Fd and κ PCR products were subsequently inserted into the vector pComb3x, resulting in a insertion rate of 60% and also the volume of bacterial transformed by recombinant vector, reached 3×10^5. **Conclusion**According tothe results,the fidelity of procedure and transformation rate is acceptable. Procedures and pitfalls will be discussed in detail.

Keywords: Fab, Combinatorial library, snake, Pcomb3x, immunoglobulin

1906O

Construction of human polyclonal recombinant Fab library, using peripheral blood of snake bitten victims

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Background: Human poisoning from snake bitten is a serious threat in many subtropical and tropical countries among in Iran. The best acceptable systemically treatment of envenomated humans is antivenoms. Most of antivenoms generally produced in horses, have a series of economic and medical problems and need more improvements. **Methods:** In this study a combinatorial human immunoglobulin gene library against Iranian poisonous snake venoms was constructed. For preparing the total RNA, peripheral blood lymphocytes of two patients who recovered from snake biting was used. RT-PCR was used to amplify the heavy (Fd) and light chains. Afterward PCR products were inserted successively into the cloning vector pComb3x to construct the human combinatorial library of polyclonal Fab antibodies. **Results:** The amplified fragments of Fd and κ gained by RT-PCR were about 650 bp. Fd and κ PCR products were subsequently inserted into the vector pComb3x, resulting in a insertion rate of 60% and also the volume of bacterial transformed by recombinant vector, reached 3×10^5. **Conclusion:** According to the results, the fidelity of procedure and transformation rate is acceptable. Procedures and pitfalls will be discussed in detail.

Keywords: Fab, Combinatorial library, snake, Pcomb3x, immunoglobulin

1929O
CIITA gene characterization in chicken (Gallus gallus)

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Background: Class II transactivator (CIITA) is a member of a nod like receptor family of cytosolic proteins that regulate expression of various genes in the immune system**.** There have been considerable progresses toward understanding its role as an activator of MHC II genes in human, but there are a few knowledge about this genein other animals such as chicken. **Method:** For obtaining the coding region sequence, three sets of overlapping chicken CIITA gene specific primer were designed according to the related sequences provided by the Ensemble database. The effect of immune system stimulation on the CIITA gene expression were accessed by Semi-quantitative RT-PCR. **Results:** A partial 5´ and 3´ mRNA sequence including 1688 base pairs were obtained and aligned with different species. The **comparison** of Nucleotide and **amino acidsequence of** the CIITA segment among chicken, human and mouse showed strong **similarities**in amino acid composition**,** secondary structure and phosphorylation sites**.** CIITA mRNA amounts were increased in WBC, kidney, liver, thymus and spleen of chickens following stimulation with *Brucella* antigen**. Conclusion:** This investigation may indicate that CIITA molecule has an important role in the chicken immune responses as well as human and other animals.

Keywords: Chicken, CIITA gene expression

1884O
The effects of Excretory-Secretory(ES) antigens of hydatid cyst on the expression level of ovine Toll-like receptor 2 and 4(TLR2 and TLR4) in peripheral blood mononuclear cells (PBMCs)

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Background: Hydatid cyst develops in many organs, mostly in liver and lungs. Immune responses against *hydatidosis* comprises of various mechanisms of innate and adaptive immunity. In recent years the importance of innate immune responses and in particular, pattern recognition receptors (PRRs) has been recognized as an essential mechanisms for development of an effective immune response. PRRs are the main sensors of pathogen and danger signals in innate immunity. Toll like receptors (TLRs) are the most studied and best characterized PPRs which are responsible for sensing pathogen associated molecular patterns (PAMPs). The role of TLRs in the molecular mechanisms underlying the pathogenesis and immunity in helminths infection has not clearly been defined. Therefore in the present study, we aimed at dissecting the role of hydatid cyst derived antigens on TLRs expression in immune cells of young lambs. **Methods:** In this study we focused on the expression levels of two important ovine TLRs transcripts, namely TLR2 and TLR4 in a culture of ovine lymphocytes exposed

to different concentrations of Excretory-Secretory(ES) antigens of hydatid cysts in a time and dose dependent manner. Blood samples were taken from healthy young lambs and after isolation of peripheral blood mononuclear cells (PBMC), the cells were cultured with different concentrations(50µg/ml &100µg/ml) of ES antigens (antigens were extracted and concentrated according to the standard protocols) in different time points. Then, total RNA was isolated from the cell pellets and cDNA was synthesized using Oligo dT primers. Afterwards, the primer pairs for TLR2, TLR4 as target genes and GAPDH as housekeeping and calibrator gene were optimized in a gradient RT-PCR experiment. Subsequently, qPCR analysis was set up to quantify and compare the relative expression levels of TLR2 and TLR4 transcripts in PBMC of antigen treated versus control (untreated) samples.**Result:** Statistical analysis showed an up-regulation of TLR2 and TLR4, in treated compared to untreated control group. **Conclusion:** the results presented in this study, can shed more lights to the insight mechanisms behind the molecular immuopathogenesis of hydatidosis which might eventually pave the way for designing novel and more effective therapeutic as well as preventive strategies.
Keywords:*Echinococcosis*, PBMCs, TLRs, Real-time quantitative PCR.

2543O

Purification of a major protein of hydatid cyst fluid by chromatography in Sephadex G100 and evaluation of it's immune response in Balb/C mice

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Background:Hydatid cyst fluid of *echinococcus granulosus* consist different proteins. In this study, we attempted to purify a major hydatid cyst fluid protein and evaluation of immune response in mice model.**Method**: Hydatid cyst fluid was prepared from infected tissue of sheep and goat from North West region of Iran. All proteins in HCF were precipitated by ammonium sulfate and dialyzed with phosphate buffer and then run to sephadex G100 media. Bulb\C mice strain was immunized three times with the emulsified protein in Freund's adjuvant. Two weeks after last immunization, splenocytes were cultured to RPMI media in presence of protein for 72 hours. **Results** :The levels of IFN-γ, IL10 and IL4 in splenocytes of immunized mice with HCF major protein were evaluated by sandwich Quantikine Elisa. Results showed significant increase in levels of cytokines IFN-γ, IL10 in immunized mice compared to control groups. **Conclusion**: These results suggest that this protein have key role in Th1 and Th2 response of host. More study need to evaluation of this protein as a vaccine candidate against echinococcus granulosus.
Keywords: hydatid cyct fluid, Cytokine, IFN-γ, Il10, Il4

1435O

Production of monoclonal antibody (Mab) against recombinant glycoprotein erns and NS3 protein of bovin viral diarrhea virus (NADL strain)

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Background: Bovine viral diarrhea (BVD) is an economically important cattle disease. Animals persistently infected with BVDV are considered as the major source of spreading infection within and among herds. Therefore, detection and elimination of these animals is essential. Usually there are no pathognomonic clinical signs of BVDV infection. Diagnostic investigations therefore rely on serological detection and virus isolation. Erns and NS3 as immunogenic proteins of BVDV are conserved among different isolates. Therefore, these proteins are candidate antigens for developing ELISA for serological studies or identification of PI animals. **Methods:** In this research a segment of BVDV genome coding for Erns was cloned into expression vector pMalc2x, under the control of the strong *lac* promoter. The recombinant MBP-Ernsprotein was expressed in *E.coli* BL-21and analyzed by SDS-PAGE and western blotting. Also MBP-NS3 was produced contemporary in lab. The recombinant proteins purified andused as antigen in Mab production. After immunizing Balb/c mice, the mouse showing highest titer of antibodies was selected for fusion. The cells in fusion mix were resuspended in HAT medium and distributed in 96-well plates. Then culture supernatants of clones were screened by indirect ELISA. **Results:** The positive clones after 3 times cloning were selected and the reactivity of the Mabs with recombinant and natural antigens was established by Western blotting.**Conclusion:**Based on our results, it appears that Erns and NS3 recombinant antigens and the specific Mabs produced against them may be suitable for developing BVDV laboratory diagnostic assays.

Keywords: BVD, Monoclonal antibody, Recombinant antigen,Erns, NS3

Poster Presentations:

1436P

Association ofchicken Major Histocompatibility Complex with antibody response to vaccine

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Background: Major Histocompatibility Complex play a central role in regulating and controlling the immune responses to infectious diseases. Due to the MHC polymorphism, individual differences in response to different vaccines were observed in chicken. Studying the association of chicken MHC with immune responses to vaccines will help to control diseases and vaccination success. The aims of the present study were evaluating the MHC polymorphism and its association to secondary antibody response against Gumboro, Newcastle and Influenza vaccines in Khorasan indigenous chickens.**Methods:** LEI0258 microsatellite marker and

fragment analysis method was used for MHC genotyping. Antibody titers were measured by ELISA against IBD and Haemaglutination Inhibition against ND and AI vaccines. Population genetic analysis and deviation from Hardy-Weinberg equilibrium were estimated using SAS/ Genetics software. Statistical analysis was performed using SPSS software and univariate regression analysis and least square test were used to determine the coefficient effect of alleles. **Results:** In Khorasan chickens thirteen LEI0258 microsatellite alleles were identified that indicates a high MHC genetic diversity in this population. The allele 361 bp had the highest and the allele 350 bp had the lowest frequency respectively. In evaluating the association of MHC with immune responses, 311 and 313 bp alleles were significantly associated with elevated immune responses to Newcastle vaccine, while allele 266 bp was associated with lower IBDV antibody titers ($P<0.05$). **Conclusion:** According to the important role of MHC in controlling disease resistance or susceptibility and quality of immune responses, these results could be used for selection and improving the populations under selective breeding.

Keywords: Major Histocompatibility Complex, LEI0258 microsatellite, Gumboro, Newcastle, Influenza

2695P

Effect of cottonseed on chicken embryonic liver antioxidant status

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Background: Oxidative stress mechanisms are involved in the embryotoxicity. The objective of this study was to assess hepatotoxicity of cottonseed in chicken embryos. **Methods:** Fertile eggs were randomly divided into 4 groups; three experimental and one control groups, (N=25, for each group). 0.1, 1 and 10 mg concentration (with free gossypol 0.25 ppm, 2.5 ppm and 25 ppm respectively) on day 4 , and incubated for 48 h at 37°C with a relative humidity of 63%. The experiment was terminated on day 20 of incubation. Then, the embryos were decapitated and livers of embryos were collected for biochemical analysis. The total antioxidant capacity, malondialdehyde (MDA), glutathione (GSH), carotenoid and total protein levels were measured by spectrophotometer. **Result:** The result of the present study indicated that the levels of total antioxidant in the livers of embryos exposed to cottonseed were higher than control, as well as the levels of MDA compared to control group ($p<0.01$). The levels of GSH, total carotenoids and total protein were lower in all groups exposed to cottonseed than control group ($p<0.001$). In addition, protein concentration in cottonseed group was higher than of other groups ($p<0.05$). **Conclusion:** So far, cottonseed may lead to induce toxic response of oxidative system in liver throughout embryonic period.

Keywords: Cottonseed; Chicken Embryos; Developmental Toxicity

1560P

Enhancment of some innate immune response in Bluga regarding to intraparitoneal injection of *Aloe vera* methanolic extract

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Background: Our research is directed in an alternative, promising area. Herbs can act as immunostimulants, conferring early activation to the non-specific defense mechanisms of fish and elevating the specific immune response. **Method:**Two injection treatment, in addition to control (no herbs), were used to determine the effect of *Aloe vera* on non-specific immune response of *Huso huso* which is known as highly endangered species listed under IUCN.These contained injection of 100 mg (A100) and 200 mg (A200) *Aloe vera* methanolic extract/kg body weight of bluga. Fish were assessed for serum Lysozyme and alternative complement activity 3 weeks after intraparitoneal injection. Data has statistically analysed using SPSS software version 16.**Result:** The results of this study showed that Aloevera methanolic extract obviously enhanced Lysozyme activity in both treatment A100 (159.12±6.2 ug/ml) and A200 (146.03± 4.9 ug/ml) comparing to control group (114.31±5.9 ug/ml)($p<0.05$). Alternative complement activity of serum (ACH50) was also improved significantly by intraparitoneal injection of A100 (344.9±28 unit/ml) and A200 (313.26±27 unit/ml) compared to control (280.7±15 unit/ml) ($p<0.05$).**Conclusion:**Thus, it can be concluded that Aloevera methanolic extracts acted as immunostimulants and appeared to improve the immune status in Huso huso.

Keywords: Huso huso, Lysozyme, ACH50, innate immunity

1559P

Study on lysozyme and alternative complement activity in wild fish species (Pygmy Perch and western minnows) native to Western Australia

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Background: Lysozyme and ACH50 activity was studied in three species of fish (2 wild species and cultured Carasius auratus) native to western Australia.**Materials and Method:** 25 Goldfish (*C. auratus*) having average weight (5-6 g), 25 Western Minnow (3-2g) and 25 Pygmy perch (1-2g) were obtained from a local fish farm located in WA , Australia, in early September 2013. The mucus samples were collected from the acclimatized healthy Pygmy perch, Western minnow and gold fish. The mucus was carefully scraped form the anterior to posterior on dorsal body using a sterile spatula. Mucus was not collected in the ventral side to avoid anal and sperm contamination. The collected mucus samples were thoroughly mixed with equal quantity of sterilized physiological saline (0.85% Nacl) and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and stored at -70°C for measuring ACH50 and lysozyme activities of mucus. Alternative complement**:** The assay was performed on 96-well microtiter plates described by Ferriani et al (1999) with slight modification. Lysozyme assessmaent: Fifty micro-liter of serum was mixed with One hundred seventy five micro-liter of 0.3mg/ml suspension of Micrococcus lysodicticus (SIGMA) in phosphate buffer (0.05 M, pH 5.8) in a microassay plate. Absorbance was recorded at 450 nm

using a spectrophotometer (Beckman Coulter, DTX 880 multimode Detector). **Results:** Our results indicate that Pygmy perch (23.61±15.64) and gold fish (15.76±7.75) had significantly highier ACH50 than Minnows (2.47±1.69). Lysozyme activity in Gold fish (247.94± 167.08) had the highest amount compared to 2 other species ($p<0.05$). In minnow and pygmy perch it was respectively (97.23±20.36) and (30.37±17.39).

Keywords: Lysozyme, ACH50, innate immunity, Fish

2544P

Evaluation of cytokine level in mice C57Bl/6 immunized with synthetic peptide EgP-29$_{aa134-142}$ from *Echinococcus granulosus*.

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Background:*Echinococcus granulosus* is the causative agent of cystic hydatid disease, a worldwide zoonotic infection that affects humans and livestock.The two most abundant antigens are *E. granulosus* antigen 5 and antigen B. P-29, a 29-kDa antigen from *E. granulosus*, is a metacestode-specific component. The immunologic cross reactivity between P-29 and a major diagnostic antigen ofE. granulosus (Ag5) indicated that P-29 might be another useful antigen of E. granulosus to be used in diagnosis or in multi epitope vaccines to prevent secondary echinococcosis.In this study, the effects of a synthetic peptide (include Linear T-Cell epitope) on the expression levels of cytokines IL4, IL10 and IFNγ in the splenocytes of immunized mice were evaluated.**Method**:In this study Inbred C57BL/6mice wasimmunized subcutaneously with 10 ug ofEgP-29 synthetic peptide emulsified in Freund's adjuvant (Complete/incomplete) three times with two weeks interval. Forty days after last immunization spleen tissues were extracted and splenocytes were cultured in RPMI media in presence of antigen for 72 hours. Supernatants were collected and used for cytokine assay by Quantikine kit (R&D). **Results**:Sandwich ELISA results were analysed by SPSS software and showed no significant differences in levels of three cytokines (IL4, IL10 and IFNγ) between immunized and control groups. **Conclusion**:In this study the linear T-Cell epitope which is considered in synthetic peptide could not induce immune response in splenocyte of C57Bl/6 mice. In our opinion, immunogenicity of P29 might be related to conformational epitopes.

Keywords: Echinococcus granulosus, synthetic peptide antigen, EgP-29$_{aa134-142}$, cytokine assay

1691P

Effect of natural antibiotic alternatives as a pragmatic approach on Salmonella enteritidis shedding in broiler chickens

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Gorgan, Iran

Background: The extensive use of antibiotics in animal farms has led to an imbalance of the beneficial intestinal flora and the appearance of resistant bacteria. Acidifiers and herbal plants have been used extensively in recent years and apparently have the ability to reduce *Salmonella*shedding in feces. Then, the aim of the present study was to investigate the effect of *Euphorbia hirta* and organic acid on *Salmonella enteritidis* shedding in broiler chickens. **Methods:** A total of 120 day-old male broiler chicks, free of *Salmonella* contamination, were inoculated by 1 mL *S. enteritidis* (1.5×10^8 cfu/bird) on d 3. Dietary treatments were: 1) Basal diet (Control), 2) Basal diet + 2 g/Kg organic acid (OA) and 3) Basal diet + 7.5 g/Kg *E. hirta* (EH7.5). In order to check *S. enteritidis* shedding after inoculation, cloacal swabs were taken from the inoculated chicken on d 2, 10, 17, 23 and 28 post inoculation. Swabs were directly enriched in RV at 41°C for 24 hrs, mixed thoroughly and plated on XLT-4 agar for 24 hrs at 37°C to screen positive *S. enteritidis* samples. **Results:** All chicks infected on d 3 were positive for *S. enteritidis* at 2 days after challenge. Cloacal shedding of *S. enteritidis* was affected by treatments at 10 d after challenge. *S. enteritidis* shedding in EH7.5 and OA were significantly reduced compared with the control group. There were no significant differences between EH7.5 and OA groups.**Conclusion:** In conclusion, the study indicated antimicrobial capacity and *in vivo* protective role of organic acids and Euphorbia hirta against *S. enteritidis* shedding. Further, more studies are needed to evaluate the beneficial effect of acidifiers and phytogenics component in the diet of broiler chickens.

Keywords: Antibiotic, Acidifiers, Salmonella, Euphorbia hirta, Broiler

3077P

Evaluation crude extract of *Trichophytone verrucosum*on CMI in experimental model

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Background: TricophytoneVerrucosum*(T. V)* related to numerous skin infectionin both men and animal. It is widely scattered in the include tinea of cows. We studied the effect of crude extract of *T. V* variate album PTCC 5056 on cell mediate immunity (CMI). **Method:**The main aims of this survey were evaluation of immunomodulatory effects of crude extract cytoplasm of *TV* on immune response system, on delayed hypersensitivity (DHT) and on Th1,2 lymphocyte by Methylthiazol Tetrazolium test (MT.T). Album variate PTCC 5056. cultured on the sabouraud،s dextrose agar and broth media , after 45 days the cells were proliferated and harvested then centrifuged. Standard protein was measured by Bradford system in watery phase. The supernatant was collected as crude extract of protein. SDS-PAGE test wascarried to determine molecular weight and protein bands. The extract of *T V* cytoplasm assumed as antigen that can stimulate cell mediate immune system (CMI)in Balb/c mice. Three groups of mice were injected with crudeextract protein by peritoneal (I.P .) and subcutaneous (SC). Spleen cells of mice were removed, cultured, and sensitized with sheep red blood cells (RBCs) for *In vivo* culture, MTT and DHT Cytokines, IL4, IFNγ were assayed by ELISA

test. It is concluded that as IFN γ is increased, IL4 was not changed as crude extract o*f TV* cytoplasm is assumed a modulator by increasingTh1 response.**Result:** Results suggested that crude extract is a good alternative against dermatophytosis infection.
Keywords: Dermatophytosis, immunomodulatory , Tricophytone Verrucosum

3132P

Molecular genotype identification of the major histocompatibility complex in Urmia indigenous chickens

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Background: Preserving indigenous and local breeds is an important issue for the conservation of domestic animal genetic diversity. Major Histocompatibility Complex (MHC) has a strong association to disease resistance/susceptibility, production and reproduction traits in chicken. Therefore, identifying its polymorphism in indigenous populations could be used in resource conservation and genetic improvement of disease-resistant traits populations. The aim of this study was Molecular genotype identification of the major histocompatibility complex in Urmia indigenous chickens.**Methods:** 67 specimens from Urmia indigenous chickens were analyzed. MHC genotypes were determined using PCR-based fragment analysis of LEI0258 microsatellite. This microsatellite is located on microchromosome 16 and well associated with serologically defined MHC haplotypes. Pop gene software was used for population genetic analysis and deviation from Hardy-Weinberg equilibrium (HWE) was also estimated using likelihood ratio test.**Results:** In Urmia indigenous chickens 20 LEI0258 microsatellite alleles (182-487 bp) and 45 genotypes were identified that indicates a high MHC genetic diversity in this population. The allele 273 bp had the highest (22.39%) and the alleles 420 and 443 bp had the lowest (0.75%) frequency respectively. Good genotype frequency fit to the Hardy–Weinberg equilibrium was observed in this population (p=0.153)**Conclusion:** The information obtained from this study indicate high MHC genetic diversity in Urmia indigenous chicken that would be useful for genetic resource conservation and improving the populations under selective breeding.
Keywords: Major Histocompatibility Complex, LEI0258 microsatellite, Urmia indigenouschickens

2935P

A report of leukosis sacoma in the livers of some broiler turkey

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Now a days the broiler turkey breeding is increasing and there are a lots of turkey broiler farms in Isfahan region, Turkeys are sensitive to some disease like chickens which are included Bacterial ,Viral and Fungal diseases. World wild reports shows that neoplastic diseases (MD and L/S) increased during 10 last years, Retroviruses knowned as causes agent

of L/S and are resistant to disinfectant and can transmit maternally as vertical transmission , Immunosuppressant activity, vaccianl failure, increasing mortality and condemnation in slaughter house are the results of leukosis/ sarcoma group in the affected birds and Marek,s disease in chickens. Current report goes to a slaughter house investigation of some 160 days old turkey livers with neoblastic tumor lesions , In macroscopic examination a large uni or bilateral focal and/or diffused fleshy whitish colored masses occupied the livers ,Also pail anemic cachectic carcasses were visible .In microscopic examination some samples were prepared for histopathology ,In histopathology an extravascular lymphoblastic neoplasia was reported ,condemnation in the broiler turkeys. As the One of the most In current report an outbreak of leukosis sarcoma.

Keywords: Leucosis ,Liver ,Turkey

2948P

Improving the infectious disease treatment and vaccination resultsusing garlic(Allium sativum) powder in broilers

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Background: Poultry industry is the most financial system in Iran after the oil, its importance for meat and protein production for food insurance and jobs creation is increasing currently, unfortunately some of infectious disease included viral and bacterial besides the environmental changes and bad quality of farms and diets, affected the poultry farms special in broilers, on the other hand using of antibiotics shows some side effects such as bacterial resistance and increasing the costs of production, meanwhile some times it is necessary to use more than one antibiotics as mass treatment simultaneously, some bacteria like as E.coli,Motile salmonella, Pseuodbacteria and Kelebciella isolated from broilers may be resistant to Tetracyclines, Doxycycline ,Erthromycin , Florfenicol, Lincomycin ,and Colistin ;**Method:**Regarding to antibacterial activity of Garlic (Allium sativum) against G+ and G- bacteria, usage of garlic powder proposed for some farms infected by superior bacteria next to the antibiotic,so garlic powder were added 1% in the diets for 2 to 3 days around infection and just garlic powder around vaccination time. **Result:**Our observations in the farms used the garlic powders with or without other antibiotics, bacterial infection were controlled and the efficiency ratio were better than the others, in the slaughter houses the carcasses were better and the omitted ones were less than the others Meanwhile the vaccination result interestingly were increased.

Keywords: Garlic, Broiler, Infection, immune ,stimulation

2937P

AI and ND investigation in some pigeons of Isfahan by serology

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Background: Pigeons breeding is a job for different attitudes included the pigeon player, pigeons exhibitors and some time used as food. Pigeons fly around the homes and poultry farms ,so they may have an important role in disease distribution particularly the important

viral diseases like AI and ND. Reports show that lentogenic /mesogenic /vellogenic NDV as much as NHPAI,LPAI and HPAI (H9N2,H5N1 and H7N9) could be isolated from pigeons around the world. In current study ND and AI (H9N2 and H5N1) were investigated in some pigeons of Isfahan city .**Method:**The sterile blood sampling were done in 5 pigeon farms in 5 points of the city and about 300 blood samples prepared for serum examination, so samples were transported to labs and using the HI test (standardized with HA using pigeon RBC) anti ND titer and anti AI titer (H9N2 and H5N1) were examined .**Result:**Regarding to the results the all of the sera were positive for ND and AI(H9N2),The CV in ND titers was 80.6% with min titer of 0 (in 17 sera)to max titer of 9(in 16 sera), the mean titer in ND were 6.3In AI measurements, the anti H5N1 titers were 0 but for H9N2 the CV was 96.7% and the min titer were 1(20 sera),the max titer were10 (16 sera) and mean titer was 7.4.

Keywords: AI,ND, Pigeon, Serology ,Isfahan

3256P

Expression of Toll-like receptors 2 and 9 in cells of dog jejunum and colon naturally infected with *Leishmania infantum*

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Background: Infection with parasite protozoa is a long-term health issue in tropical and subtropical regions throughout the world. The Toll-like receptor (TLR) signaling pathway is one of the first-responding defense systems against *Leishmania.* The aim of this study was to investigate the expression of TLR2 and TLR9 in jejunum and colon and its correlation with CD11c, CD11b, and CD14 receptors used as markers for dendritic cells and macrophages. **Methods**: thirty five dogs infected with Leishmania infantum were used in this study. Cytometry was carried out in lamina propria cells from jejunum and colon using markers for TLR2, TLR9, CD11b, CD11c and CD14.**Results:** Cellular inflammatory exudate was diffuse in the mucosa and submucosa, predominately comprising mononuclear cells: plasma cells, macrophages, and lymphocytes. Despite the parasite load, microscopy showed no erosion was evident in the epithelial mucosa layers. The colon harbored more parasites than the jejunum. Flow cytometry revealed higher frequency of TLR2+ and CD11c+ dendritic cells in the colon than in the jejunum. Conversely, TLR9-expressing cells were more frequent in jejunum. Moreover, frequency of macrophages (CD11b+ and CD14+) expressing simultaneity TLR9 were lower in the colon than in jejunum, while CD11c+ cells predominated in the colon. Despite of the negative ELISA serum results, IL-10 and TNF-α were higher in jejunum than colon of infected animals. However, IL-4 was higher in colon than jejunum of infected animals. A higher expression these cytokines were demonstrated in infected dogs compared to uninfected dogs.**Conclusions**: There was no correlation between clinical signs and pathological changes and immunological and parasitological findings in the gastrointestinal tract in canine visceral leishmaniasis. However, jejunum showed a lower parasite load with increased frequency and expression of CD11b, TLR9, CD14/CD11b/TLR9 receptors and IL-10 and TNF-α cytokines. Conversely, the colon showed a higher parasite load along with increased frequency and expression of TLR2, CD11c receptors, and IL-4 cytokine. Thus, *Leishmania infantum* is able to interfere in jejunum increased expression of TLR2, TLR9, CD11b, CD14, CD14/CD11b/

TLR9 receptors, IL-10, and TNF-α; and in colon increased expression of CD11c, TLR2, TLR9, CD11b, CD14 e, CD14/CD11b/TLR9 receptors, IL-10, and TNF-α.

Keywords: Toll-like receptor 2, Toll-like receptor 9 **,** Leishmania infantum

Keywords: Canine visceral leishmaniasis, Jejunum and colon, Toll-like receptors 2 and 9, Parasite burden,

1693P

Cecal colonization response of *Salmonella enteritidis* challenged broiler chickens to phytogenic and organic acid supplementation

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Background: More recently a new group of feed additives was introduced in poultry farms based on the antibacterial in vitro effects such as organic acids and phytogenic on gram-negative bacteria. Then, the aim of the present paper was to determine the effect *of* Euphorbia hirta and organic acid on gut colonization to Salmonella enteritidis challenged broiler chickens. **Material and Methods:** A total of 120 day-old male broiler chicks, free of *Salmonella* contamination, were inoculated by 1 mL *S.* enteritidis (1.5×10^8 cfu/bird) on d 3. Dietary treatments were: 1) Basal diet (Control), 2) Basal diet + 2 g/Kg organic acid (OA) and 3) Basal diet + 7.5 g/Kg *E.* hirta (EH7.5). The chicks (8 challenged birds/treatment) were killed by cervical dislocation on d 10^{th}, 17^{th} and 31^{th} of the experiment. After opening the abdominal cavity of each bird aseptically, the ceca from each bird were collected for *S.* enteritidisenumeration. **Results:** Seven days after challenge, the number of *S.* enteritidiswas significantly higher in the control group than the other groups. A significant decreased in *S.* enteritidis enumeration from birds receiving OA and EH7.5 diets were observed. At 14 days after challenge, a significant higher number of *S.* enteritidiscolony-forming units per gram was observed in the control treatment. The rate of ceca enumeration dropped significantly after 14 days in OA compared to the EH7.5treatments. Irrespective of treatment, on 31 days of age *S.* enteritidiswas not detected.**Conclusion:** In conclusion, this study showed that based on *S.* enteritidiscecal enumeration, OA supplemented diet appeared to be more effective than EH7.5 on controlling Salmonelosis in broiler chickens.

Keywords:*:*Acidifiers, Salmonella, Euphorbia hirta, Broiler.

2874P

Effect of probiotic in high or low nutrient density diet on immuneresponse of broiler chickens

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Background: Use of antibiotics as growth promoters is no longer acceptable and therefore it is forbidden in EU countries.There aresome evidencesshownthat use of probiotics in poultry human diets increase the resistance to some disease and reduce the stress. **Methods:** This study was conducted for evaluating the effects of probiotic in high or low nutrient density diet on immune response and heterophil to lymphocyte (H/L) ratio in broiler chickens.Birds were randomlyallottedtooneof4 treatmentsina2 ×2factorialarrangement(5replicateswith20broilersperreplicate)withtwolevelsofnutrientdensity(highorlownutrientdensity) and two levels of probiotic (0and 1.5%)(65×10^8available spores/g of *Bacillus subtilis* endospores). Dietary treatments were offered *ad libitum* to chickens from 1 to 42 days of age. On days of 28 and 35, forty birds from each replicate were injected intravenously (brachialvein) with 0.4mlof0.5%sheepredbloodcells (SRBC). Antibody production against SRBC was measured after 7 days of injection. Titers were expressed as the log2 of the reciprocal of the highest dilution in which there was agglutination (Wegmannand Smithes, 1996). Heterophil and lymphocyte were measured by the count of those cells in the blood. In 42nd days of age blood smears were stained using giemsa stain and hetrophils and lymphocytes and white blood cells were counted to a total of 100 cells. SRBCs used for inoculation and antibody titration were obtained from the same donor sheep. **Results and Conclusion:** Results shown that nutrient density has significant effect on heterophil and H/L ratio ($P<0.05$), as indices stressof broiler chickens was decrease but no significant effect on other blood cells. Probiotic significantly decrease heterohil and H/L ratio, but increase lymphocyte in broiler chickens ($P<0.05$). Neither nutrient density nor probiotic have significant effect on antibody titer against SRBC ($P>0.05$). result are shown high nutrient density increased the IG total and IGm in broiler chickens ($P<0.05$). Based on the results of this experiment, supplementing of probiotic or benefits live bacteria to high as well as low nutrient density diets improve immune potency in broiler chicken.

Keywords: Probiotic, Nutrient density, Immune system, Broiler chicken

2863P

Effect of avian infectious bronchitis virus serotype 4/91 on acute-phase proteins (haptoglobin, serum amyloid A, and C-reactive protein) in experimentally infected broilers

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Background: Measurement of acute phase proteins has important in diagnosis of animal diseases. In present study effects of avian infectious bronchitis virus (IBV) infection on acute phase response and acute phase proteins (APPs) were determined. **Method:** Thirty 1-day-old commercial broiler chicks were reared in experimental room and at the age of 21 days, birds were challenged intranasally with 0.2 ml of allantoic fluid virus suspension (titre $10^{6.5}$ EID50 per 0.1ml). Serum samples were obtained prior to challenge and at days 1, 2, and 5 post inoculation, then Haptoglobin (Hp), serum amyloid A (SAA), and C- reactive protein

(CRP) concentrations were measured. **Results and Conclusion:** According to the results, all investigated APPs increased significantly after infection with IBV, with mean maximum concentration from 24 h to 48 h. No correlation was observed between plasma APPs in the chickens prior and post inoculation of bronchitis virus. Hp was most sensitive factor to change in the exposed birds.

Keywords: Acute phase proteins, Chicks, Infectious bronchitis

3167P

Immune responses to oral and IM administration of M2e-Hsp70 construct

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Background: The eukaryotic expression plasmid of M2e-Hsp70is a candidate M2e-based DNA vaccine. In order to evaluate the immunization potential of this construct, Specific Pathogen Free chickens were immunized either intramuscularly or orally with this plasmid. **Materials & Methods:**Aro A mutant of Salmonella typhimurium was used as carrier for oral delivery of plasmids. The plasmids were transformed into *S.* typhimuriumby electroporation. Six groups (Eight chickens per group) of SPF chickens were orally or intramuscularly administered with PBS, pcDNA M2e-Hsp70C-terminal or control vector of pcDNA Hsp70C-terminal. Intramuscular injection was done in the breast muscle without any adjuvant and oral administration was handled by the use of oral dispenser. After 3 rounds of immunization, humoral and cellular responses were tested by ELISAand Lymphocyte ProliferationAssay, respectively.**Results and conclusion:**Our results indicate that both humoral and cellular immune responses are conferred against M2e-Hsp70 plasmid in either of the intramuscular or oral routs of administration; however, these responses are significantly higher in intramuscular injection in contrast to oral administration. When it comes to mass vaccination of commercial chicken flocks oral administration is preferred due to the ease of application as well as its capability of eliciting mucosal, humoral and cellular immune responses; so measurements should be taken to improve the immunization potency of our orally delivered DNA vaccine.

Keywords: Avian influenza virus; M2e-Hsp70; Salmonella typhimurium

3043P

Study on efficacy of Iranian-madevaccinesin comparison with importedvaccines against Newcastle disease

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Background:Newcastle disease (ND) is an important viral disease in poultry industry globally, negatively affecting trade and poultry production in both developing and developed countries. Control and prevention of ND is based on immunization, both live-virus and

inactivated vaccines, which their efficacy is measured by HI assay. Vaccines used in Iran's poultry farms are sourced from both, Iranian and foreign manufacturer companies. One of the controversial issues regarding the use of vaccines made by Iranian manufacturers is their less efficacy in compression with imported vaccines. In this study we compared the potency of an Iranian ND vaccine with a foreign vaccine.**Method and results**:A few more than 500thousandlaying hens (L.S.L strain)kept in twelve houses inone of the most important poultry farms of Iran, were divided into two groups and vaccinated orally with LaSota strain live ND vaccine. Hens from group one (5 houses), were immunized using a foreign vaccine (LohmannGB Co.) and others from group 2 (7 houses), were received an Iranian vaccine (Razi Inst.). Allbirdsobservedin this studyinterms ofage,nutrition, healthand hygieneconditionswereidenticalandweremanagedundera same condition. During thestudy periodand before, any unusual changes inproductionand mortality curves and specific clinical signs were not observed.28 days after vaccination, Blood samples were collectedfrom each groups (46 samples from group 1 and 67 from group 2) and examined using HI assay and The result was analyzed with Mann-Whitney test using the SPSS 16.0 software. The analyze showed that there wasn't significant difference between means of titer of HI antibody of group 1 and 2 (P>0.05). **Conclusion**:The considerable result of this study which was conducted in a full mechanized farm, demonstrates that on the condition of good managing and proper vaccine administration, vaccines produced by Iranian manufacturers, in contrast with imported vaccines, are effective to induce an acceptable level of HI antibody. Therefore, to determinereasons ofvaccination failure and the incidenceof ND invaccinatedflocks, it should be more concentrated on other potential factors causing vaccination failure rather than vaccine efficacy.

Keywords: Newcastle disease, Vaccine, efficacy, laying hens

2415P

Effects of betamethasone on apoptosis of germ cells and primary spermatocytes in male mice

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Background: In recent years, due to the growth and increased use of drugs to control the disease, the researchers have paid attention to the Side effects of drugs, on various body systems. In this regard, Betamethasone is one of the most widely consumed drugs used in veterinary and medicine which has many physiological effects. This study was designed to investigate the possible effects of Betamethasone on reproductive system in male mice.**Methods:** Fifty matured male mice were divided into five groups including control, placebo and three treatment groups. Placebo group received normal saline only and treatments were Betamethasone (0.1, 0.5 and 1 mg/kg) which were injected in peritoneum every other day for a period of twenty days. After 20 days, the mice were anesthetized and testes for preparation tissue sections stained with hematoxylin-eosin were dissected. Germ cell and primary spermatocytes were counted. The obtained data were analyzed using Duncan's multiple ranges test by SPSS program. **Results:** Histological studies showed that doses of 0.1, 0.5 and 1 mg/kg Betamethasone, significantly decreased the number of germ cell and primary spermatocytes compared with the control (P<0/05).**Conclusion:** Since Betamethasone is a synthetic Glucocorticoidand causes the immune system be suppressed, cellular immunity is also reduced, so that, the cells become

more sensitive to environmental factors and stress. Moreover, the level of apoptosis will be increased. Therefore, Betamethasone has a negative effect on male reproduction and fertility.
Keywords: Betamethasone, Germ cell, Primary spermatocyte, Mice

2814P

A single radial haemolysis test for rapid detection antibodies , antigens and titration of viruses

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Background: For rapid detection antibodies or antigens and titration animal vaccines SRHT was developed .This technique in comparison with other serological tests as ELISA ,CIE, ,SN, AGPT is rapid ,simple , reproducible , time efficient and inexpensive .Plates of test result could be kept for a long time at 4 °C.The resultsof SRHT showed only 10-20% differences with other sensitive tests as golden test. **Method:**the procedure of this test is summarized as preparation of soluble antigens of virus and purified according to Isloor and Negi .10% suspension was homogenized and its supernatant was purified by centrifuged at 23000 rpm for two hours supernatant was removed. And packed cell were collected in tris buffer and treated with poly ethylene glycol (PEG,) next step was preparation of hyper immune sera against soluble antigens of virus in adult rabbits by repeated injection on 0,2,3 and 7 weeks and ten days after last injection sera of rabbits were collected . Fresh serum of guinea pig was used as complement and 1% dilution of agarose in distilled water was made up in a plate and then after cooling the mixture of coating RBCs with SAgs , complement were added at once in to plate , wells were punched in plate and filled with serum and stored in moist condition for 2-3 hours to allow diffusion.**Results:** formation of lyses zones around each well showed antibodies against virus and if cycles made larger ,showed more lyses of RBCs . this test is applied for detection of antigens against monoclonal antibodies and titration of animal viruses by preparation different concentration of soluble antigens of virus .
Keywords: SRHT, RBC, Ag,Ab

2793P

Changing face of development sheep and goat pox virus vaccines to a single vaccine for mass production

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Background: Pox disease is classified in group a diseases of the OIE. It is an acute to chronic disease of sheep and goats, highly contagious and characterized by generalized pox lesions throughout the skin and mucous membranes. These diseases are not serologically distinguishable because of antigenic homology among them. Goat pox virus is highly host specific infecting only goats, but host specificity varies from isolate to isolate, Various strains of sheep and goat pox virus (SGPV) cause disease only in sheep, others only in goats, and some in both sheep and goats.**Method**: Preparation of a safe and immunogenic single

vaccine against sheep and goat pox disease via using two different types of cells (primary lamb kidney and vero cell line) with 0240 pox strain that was isolated from sheep in an outbreak is described. As following **Virus strain** provided (o240),Preparation of cell culture-Inoculation 0240 strain pox virus, Virus titration and minimum infecting dose, Vaccine preparation,Safety and potency test,Stability of 0240 single vaccine**. Results:** The prepared vaccine, was administered subcutaneously and induced complete protection against experimental sheep and goat pox challenge virus. For the final evaluation, the efficacy of the prepared vaccine was used in the field trial under the supervision of veterinary clinicians. This vaccine was proved to be more effectiveness in compared with routine vaccines now being produced in Razi institute. **Conclusion:**This paper describes the development of a single vaccine against capripox virus with titre $10^{-6.7}$ TCID50/ml for both sheep and goats, by employed 0240 strain that was isolated from an outbreak of sheep pox disease.

Keywords: Single vaccine-sheep pox virus-lamb kidney cell

2791P

Serological evidence abortifacients in a dairy herd with History of abortion in industrial breeding farms livestock

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Background: Abortion is common among dairy herds cows . However, except for Brucella *abortus*, little is known about other prevalent abortifacients. Therefore, seroepidemiological study was conducted in a dairy herd with a history of abortion located in breeding cattles farms. **Method**: Blood samples (5 ml) were taken from each animal (n = 90) in the herd. Seropositivity to infectious bovine rhinotracheitis (IBR), *B. abortus*, blue tongue virus (BTV), bovine viral diarrhea virus (BVDV) among livestock**. Results:** seropositivity was most frequent for IBR (69.8 and 70.3%, P>0.05) followed by *B. abortus* (32.6 and 42.6%, P>0.05), BVDV (9.3 and 6.4%, P>0.05) and BTV (4.7 and 6.4%, P>0.05), whereas,. With respect to age, there was a significant difference P<0.05) in seropositivity to *B. abortus*, BTV, and to multiple infectious agents in buffaloes. Additionally, a history of prior abortion was found to be significantly associated with current abortion in buffaloes and cows (P<0.001). While several significant associations between seropositivity to various agents and abortion were initially found, further analyses showed no significant associations in cows or buffaloes. **Conclusion:**This studyis concluded that seropositivity to the studied infectious agents was not significantly associated with abortion when accounting for other covariates, while prior abortion was found to be significantly associated with current abortion in both cows and buffaloes. However, owing to the small preliminary nature of the study, small sample size, and small number of abortion events, further studies are needed to ascertain the validity of these results

Keywords: Cattle , Infectious agents, abortion

2870P

Increased risk of horse sensitization among horse riders in Southwestern Iran

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Background The presence of large amounts of horse allergens in the work place may induce allergic sensitization in horse riders. The aim of this study was to investigate the frequency of sensitization to horse allergens and clinicalsymptoms in horse riders.**Methods:**A total of 42 horse riders and 50 controls were examined using skin prick tests to a panel of horse and common animal allergens. The clinical symptoms of individuals were recorded with a self-completed questionnaire and pulmonary function tests were done by spirometry.**Results:**The rate of sensitization to horse allergens was 31% by skin prick test in horse riders whereas horse sensitizationwas not seen in the control group.Occupational allergy symptoms were reported by 19 horse riders, 15 (79%) of whom had allergic rhinitis, 9 (47%) allergic conjunctivitis, 6 (31.5%) asthma and 5 (26.3%) skin symptoms.Two horse riders with no history of clinical symptoms showed positive skin reactions to horse allergens. **Conclusion:**Our findings reinforce the importance of allergen exposure that affects allergic sensitization. High risk of occupational sensitization among horse riders need to improve conditions at the workplace to reduce the load of airborne horse allergens.

Keywords: horse rider; horse allergen; lung function; horse

2911P

Evaluation of Rev-1 vaccine efficacy in lamb and goat in Semnan province

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Background: Brucellosis is one of the most important infectious diseases in animals and is considered to be the most serious zoonotic disease for humans. *B.melitensis* is primarily responsible for brucellosis in sheep and goats. It occurs in small ruminants in the Mediterranean and Middle Eastern countries, particularly Iran. Eradication of this infection is based on serological identification and slaughter of positive animals, so economically it is much expensive. Accordingly, the vaccine base control of disease is desirable. Rev-1 vaccine is one of the best and most valuable existing vaccines to prevent and control goat and sheep brucellosis suggested by the international organizations. The aim of this study was evaluation of antibodies titration against Rev-1 vaccine in sheep and goat in Semnan by Rose Bengal plate test and Wright- 2ME.**Methods:** 283 blood samples, 14 days after vaccination, were collected from 36 sheep and goat herds in Semnan province. Rose Bengal plate test, Wright and 2ME agglutination test were carried out according to kit instruction (IDEXX, Institut Pourquier, FRANCE). SAT titers ≥1:40 were indicative of Brucella antibodies.**Results:** among 383samples, 255(66%) samples showed the presence of agglutination in Rose Bengal plate test. 67 samples were analyzed with Wright and 2ME. Results showed in 27 samples (40%) antibody titer caused by vaccine.**Conclusion:** althoughresearches indicate that Rev-1 vaccine induce acceptable protection in sheep and goat, but it is not in agree with results of this study. Incorrectly vaccine administration or conditions of vaccine storage may to cause thisresults.

Results of this study showed some animal were infectious before vaccine administration. Because of importance of this zoonotic disease, more studies need to evaluate the vaccine efficacy in Iran to apply beneficial policy to control of this infection in animals and human.
Keywords: brucellosis, efficacy, vaccine, Rev-1

3153P

Humoral immunity in broilers challenged with *Eimeria*prior to and following anticoccidial vaccination, by means of ELISA

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Background: Despite the use of prophylactic chemotherapy and vaccination, coccidiosis is still one of the most devastating diseases in poultry industry. This study was designed to evaluate the antibody response induced by a live attenuatedanticoccidial vaccine (LivacoxQ) in chickens,before and after challenge, by means of ELISA. **METHODS:** One hundred and twenty one-day-old male Ross308 chicks were randomly divided into 4 groups of 30 birds. In 4th day of age, half the birds were orally vaccinated; the challenged groups received the infective dose at 14th day of age via oral administration. Weight gain, lesion score and oocyst count in 21st day old birds were recorded and humoral immunity was assessed by means of ELISA on serum samples taken from 7 and 21day-old birds. **Results**: The average of optical density (OD) showed significant difference between vaccinated (0.553) and unvaccinated (0.686) birds ($p \leq 0.05$),three days post vaccination. In 21 day-old birds, the OD of unvaccinated-unchallengedgroups (0.331) differed significantly with vaccinated-unchallenged (0.663) birds. The average of lesion score in vaccinated-challenged birds (2.22) showed significant dissimilarity with unvaccinated-challenged groups. No difference and correlation were observed comparing average of weight gain and oocyst count with serum optical density among treatment and control groups. **Conclusion**: The results indicated that ELISA can be used for evaluating immunity uniformity of a flock after vaccination. Besides inducing antibody responses comparable to challenge with wild oocysts, vaccination with live attenuated coccidiosis vaccines may have inhibitory effects in intestinal lesion scores which are responsible for pathogenesis and economic loss during coccidialinfections.
Keywords. Anticoccidial vaccine,Coccidiosis,Humoral immunity, Poultry *Eimeria*

3253P

Evaluation of two commercial quanlitive and semi quantitative ELISA methods for diagnosis of acute BOVINE leptospirosis

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Background: leptospirosis is most widespread zoonosis in the world.especially in tropical and temperate region.leptospirosis caused by spirochetes of the genus leptospira that is fastidious bacterium.isolation of these bacteria by culture is difficult and time consuming. The microscopic agglutination test(MAT) is most reliable assay but generally requires paired sera for detection of seroconversion and is considered too complex for routine use.**Objective**: we performed this study to final a simple and reliable method for diagnosis of acute bovine leptospirosis,ELISA is another common method for diagnosis of leptospirosis we have developed and evaluated an in-house indirect elisa with antigen extracts of endemic isolates.**Material and method**:we examined 37 single sera of bovine who were suspected of leptospirosois by a commercial none quantities and in-house semi quantative ELISA assays and compared their results with MAT.**Result**: On testing different samples of sera from infected and non-infected animals,all specimens with titer ≥ 1:100 against a pathogenic serovar in MAT were regarded as confirmed leptospirosis.all specimens wich were positive in any IgM_ELISA assays were compared with the results of MAT.in our study sensitivity,specifity and accuracy of in-house ELISA were 80% , 87% ,83% consequently but were 42% ,75%,56% for commercial IgM_ELISA assay consequently.**Conclusion:** the results of our study show that in-house semi quantative IgM ELISA was more specific and aensitive than commercial qualitative IgM ELISA
Keywords; Leptospirosis,BOVINE, ELISA, MAT

1687P

The study of common carp (*Cyprinus carpio*) mucus and serum proteases by gelatin zymography

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Background:The innate immune system of fish isconsidered the first line of defense against a broadspectrum of pathogens. Being a component of innateimmunity and lying at the interface between fish andthe aqueous environment, skin mucus plays a frontierrole in protecting fish from infections. In the presentstudy some proteases as potential innate immune factors for skin mucus and serum of common carp,*Cyprinus carpio*, were investigated.**Methods:** Proteases were profiled and characterized by performinggelatin zymography on 10% resolving polyacrylamidegels containing 0.1% (w/v) gelatin in thepresence of 0.1% SDS. The protease classes were determined through theapplication of specific methaloprotease inhibitor, 5 mM EDTA (ethylenediaminetetraacetic acid), during the incubation step in zymography. **Results:**Gelatin zymography revealed that metalloproteases were the major mucus and serum proteases inthis fish. several protease bandsof varied molecular weights in skin mucus and serum of fishwere detected. In general, there weresome protease bands of medium molecular weights between 27 and 40 kDa andhigh molecular weights between 100 and 200 kDa. **Conclusion:**A marked difference wasobserved in the protease profile of mucus and serum.
Keywords: Innate immunity, Common carp,Fish skin mucus, Protease , Gelatin zymography

1712P

Effect of in ovo injection of Mentofin® (a herbal product) on antibody response of broiler chickens against ND vaccine virus

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Background: Mentofin® is a herbal productcomprised mainly of essential oils, which has been demonstrated that is suitable in preventing respiratory problems, improving performance, andstimulating the immune system when it was used indrinking water of poultry. This study was conducted to determine the effect of in ovo injection of Mentofin® on humoral immunity of broilers from 1st to 3th week of age. **Method:**Fortyfertilized eggs were collected from a commercial breeder flock. The eggs were divided into two experimental groups; controlT1 (vaccine injection) and group T2 (vaccine + Mentofin® injection). All of groups were incubated at normal temperature and humidity. In ovo injection was done in group T2 via chorioallantoicmembrane at day 9 of incubation. T1 day-old chicks were vaccinated with CEVAC® VITABRON L contains Newcastle Disease virus (NDV) and Infectious Bronchitis virus (IBV) via eye drop. Hatched chicks were reared in battery brooders and provided standard diet and other managemental condition. At the age of 21 days, blood samples were collected from all chicks and sera were tested by hemagglutination inhibition (HI) assay. **Results and Conclusion:**This study showed that in ovo injection of Mentofin® has a positive effect on poultry humoral immune system and Mentofin® treated broilers showed higher consistent antibody titer as compared to untreated broilers, but not significant (P>0.05).

Keywords: Mentofin, NDV HI antibody titer, In ovo, Broilers.

2690P

A survey on association between IBD maternal antibody titer and mortality rate of the broiler flocks in Isfahan province

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Background: Infectious bursal disease virus (IBDV) is the etiological agent of “Gumboro diesease”.IBDV is a member of the family Birnaviridae, genus Avibirna-virus.The causal virus is extremely lymphocidal with an affinity for immature B cells resulting in bursal atrophy approximately four days after infectionand it decreases the level of immunity in poultry. Conventional IBD is controlled by vaccination of mother flocks for transferring maternal antibody to chicks. In this study the association between IBD maternal antibody titers in one day old chicks with the mortality rate in during of training period in poultry flocks of Isfahan province was surveyed.**Methods:** blood samples were collected from 32 broiler chicken flocks from one day old chicks (10 chicks in every flock) in Isfahan province. Serum obtained by centrifugation of samples and subjected to Elisa test (kit of FLOCKSCREEN IBD) according to protocol of the maker company. Optical Densities (OD) taken by Elisa Reader calculated with

the specific software of the kit. Data were analyzed in software environment R (version 3.0.2) with software MCMglm by elliptical method. Because the mortality rate didn't have the normal distribution we assumed that data had poisson distribution. **Results:** The results showed there was a significant association between the mortality rate and IBD maternal antibody titer (PV=0.04) **Conclusion:** Higher titer of IBD maternal antibody protects chicks from IBDV as immunosuppressive agent especially in chicks during early days and plays important roles to reduce mortality rate in raring period of broiler chickens.

Keywords: IBD, Maternal antibody, Elisa test, Mortality rate

1814P

In vivo immunomodulating activity of aqueous extract of Astragalus verus Olivier in NMRI mice as an remedy in Kurdish ethno medicine

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Background: Astragalus verus Olivier, has been used as an immunopromoting remedy in Kurdish ethno medicine and aim of present survey was to determine of the effects of immunodulatory activities of eqeous extract of A. verus in a mice model.**Methods**: animals injected with the aqueous extract (5, 10 and $20_{mg/kg}$) of Astragalus verus Olivier, intraperitonaly, and Haemagglutinating antibody titers of serum, as a factor of humoral mediated immunity were investigated at days 0 and 14. Also, Footpad swelling test was used to determine delayed type hypersensitivity (DTH) for cell mediated immunity in animals that received extract via gavage.**Results**: It was found that the aqueous extracts (5, 10, $20_{mg/kg}$) of Astragalus verus Olivier did not appear to have haemagglutinating activity on SRBC, indicating that this extract have not Lectin-like activity. Moreover, the DTH reaction promotes on 14 days after administration of $10_{mg/kg}$ ($p<0.05$) of Astragalus verus Olivier, showing the CMI response of the extract.**Conclusion**: In this study, by attention to the above lines, we could conclude that Astragalus verus Olivier has the potential in vivo immunomodulating activities and these new findings support our pervious in vitro studies.

Keywords: Astragalus verus Olivier, Delayed type hypersensitivity, Haemagglutinating antibody, Kurdish ethnomedicine

1994P

Innate ocular immunity against street strain of rabies virus in mice

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Background: The most common mode of rabies virus transmission is through a bite wound or mucous membranes (i.e., eyes, nose, and mouth) expose to infected saliva of the rabid animal. Accidental exposure to rabies virus might be occurred during work in laboratories or during examination and surgical operations on animals in veterinarians in contact with saliva

or other infectious fluids splash into their eyes. Previous records shown that some viruses include HIV and HBV may enter via intact conjunctiva and cornea. So we decided to examine the possibility of ocular rabies pathogenesis in mice as an animal model. Our results will determine if Street Rabies Virus (SRV) is able to infect the CNS of mice via the ocular route. **Methods:** Fifty NMRI mice were selected and divided into 2 groups. SRV was isolated from brain of a wolf and passaged once interacerebrally in mice. A 10% suspension was prepared by homogenizing the brains of mice in diluents and was done titeration to obtain LD_{50}. The LD_{50} was calculated $10^{-4.7}$. 10 µl of 10% suspension was instilled into eyes of mice in group 1. 10 µl of Normal Saline was instilled into eyes of mice in group 2. And all of mice were kept 3 months under observation.**Results**: In during 3 months of observation, non of mice showed clinical symptoms of rabies and all of them were healthy and alive.**Conclusion:** The results of this study indicated the intact conjunctiva and cornea doesn't allow to the retrograde spread of the virus in mice as an animal model. This study showed innate ocular immunity can prevent entrance of street rabies virus in experimental model.

Keywords: Street Strain, Rabies, Eye, Mice

1994P

Innate ocular immunity against street strain of rabies virus in mice

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[1]University of Shahrekord, Shahrekord, Iran,[2]Department of pathobiology, faculty of veterinary medicine, University ofShahrekord, Sharekord, Iran, [3]Student of veterinary medicine, university of Shahrekord, Shahrekord, Iran

Background: The most common mode of rabies virus transmission is through a bite wound or mucous membranes (i.e., eyes, nose, and mouth) expose to infected saliva of the rabid animal. Accidental exposure to rabies virus might be occurred during work in laboratories or during examination and surgical operations on animals in veterinarians in contact with saliva or other infectious fluids splash into their eyes. Previous records shown that some viruses include HIV and HBV may enter via intact conjunctiva and cornea. So we decided to examine the possibility of ocular rabies pathogenesis in mice as an animal model. Our results will determine if Street Rabies Virus (SRV) is able to infect the CNS of mice via the ocular route. **Methods:** Fifty NMRI mice were selected and divided into 2 groups. SRV was isolated from brain of a wolf and passaged once interacerebrally in mice. A 10% suspension was prepared by homogenizing the brains of mice in diluents and was done titeration to obtain LD_{50}. The LD_{50} was calculated $10^{-4.7}$. 10 µl of 10% suspension was instilled into eyes of mice in group 1. 10 µl of Normal Saline was instilled into eyes of mice in group 2. And all of mice were kept 3 months under observation.**Results**: In during 3 months of observation, non of mice showed clinical symptoms of rabies and all of them were healthy and alive.**Conclusion:** The results of this study indicated the intact conjunctiva and cornea doesn't allow to the retrograde spread of the virus in mice as an animal model. This study showed innate ocular immunity can prevent entrance of street rabies virus in experimental model.

Keywords: Street Strain, Rabies, Eye, Mice

1769P

Developing an indirect ELISA test using whole tachyzoite for sero-diagnosis of *Neospora caninum* in water buffalo from north-west of Iran

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Background: *Neospora caninum* is an obligate intracellular parasite introduced in 1988. It can infect water buffalo (*Bubalus bubalis*) inducing abortion and causing reproductive failure. Developing simple methods with high accuracy for detecting the infection has always been a goal for researchers. **Methods:** To assess the prevalence of *N. caninum* antibodies in buffalo, a new ELISA-based method was established using the whole parasite. 83 serum samples were collected from buffaloes slaughtered in Urmia abattoir. Plates were coated with 2×10^6 whole tachyzoites and incubated at 25˚C for 3 days. After washing and blocking with relevant buffers, serum samples as well as negative and positive controls were applied. HRP conjugated anti-bovine IgG and tetramethylbenzidine substrate were used to detect the bound antibodies. Optical density (OD) values were measured and the antibody level was determined by calculating the ratio of sample/positive control (s/p) ODs and those with the ratio more than 0.50, were accounted as positive.**Results:** A total of 83 serum samples were examined for presence of anti *N. caninum* IgG. 16 samples (19.27%) were positive from which 3 were male and 13 were female. 33 (39.75%) showed a significant level of antibody where their s/p ratio were below 0.50. No significant level of antibody was observed in 34(40.96%) samples. **Conclusion:** Whole *N. caninum* tachyzoites can be used as antigen for detection of the parasite in an ELISA-based assay. There is a high level of infection in water buffalos, which indicates a necessity for application of control strategies in this merit livestock.

Keywords: ELISA, *Neospora caninum*, water buffalo, tachyzoite, Iran

3078P

Evaluating the crude extract of *Trichophytone verrocosum* on cell mediated immunity in experimental model

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Background: *Tricophytone Verrocosum* is related to numerous skin infectionsin both men and animals. It is widely scattered in the tinea of cows. We studied the effect of crude extract of *Tricophytone Verrocosum*variate album on cell mediated immunity . The main aims of this study were to evaluatio of immunomodulatory effects of crude extract cytoplasm of *Tricophytone Verrucosum*on immune response system.The **Methods:** objective of this study was to evaluated the crude extract of *Trichophytone verrocosum* on cell mediated immunity in balb/c mice .To this end variaty Album was cultured on the sabouraud،s dextrose agar and sabouraud،s dextrose broth media.The rate of chloramphenicol was used 0.05gr/ml and cyclohexamide was 0.5 gr/ml. After 40 days, 10gr of mycelium were proliferated, harvested and centrifuged. Standard protein was measured by Bradford. The supernatant was collected

as crude extract of protein. The extract of *Tricophytone Verrucosum* cytoplasm was assumed as antigen that can stimulate cell mediate immune system in Balb/c mice. Three groups of mice were injected with crudeextract protein by peritoneal and subcutaneous and spleen cells of mice were removed, cultured, and sensitized with sheep red blood cells for In vivo culture, Methylthiazol Tetrazolium test and delayed type hypersensitivity Cytokines, IL4, IFNγ were assayed by ELISA test. So we resulted that as IFN γ is increased, IL4 was not changed as crude extract of*Tricophytone Verrocosum* cytoplasm is assumed a modulator by increasingTh1 response**. Results:**results suggested that crude extract is a good alternative against dermatophytosis infection.

Keywords: Dermatophytosis, immunomodulatory , *Tricophytone Verrocosum* delayed type hypersensitivity (DTH)،subcutaneous

1886P

The effects of somatic antigens of Ostertagia-circumcincta on the expression level of ovine Toll-like receptor 2 and 4(TLR2 and TLR4) in Peripheral blood mononuclear cells (PBMCs)

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Background: Immune responses against Ostertagiosis comprises of various mechanisms of innate and adaptive immunity. In recent years the importance of innate immune responses and in particular, pattern recognition receptors (PRRs) has been recognized as an essential mechanisms for development of an effective immune response. PRRs are the main sensors of pathogen and danger signals in innate immunity. Toll like receptors (TLRs) are the most studied and best characterized PPRs which are responsible for sensing pathogen associated molecular patterns(PAMPs). The role of TLRs in the molecular mechanisms underlying the pathogenesis and immunity in helminthes infection and in particular Ostertagiosis has not been clearly defined. Therefore in this study, we aimed at dissecting the role of somatic antigens of Ostertagia-circumcinta on TLRs expression in immune cells of young lambs. **Methods:** In the present study we aimed on the expression levels of two important ovine TLRs transcripts, namely TLR2 and TLR4 in a culture of ovine lymphocytes exposed to different concentrations of Ostertagia-circumcincta somatic antigens in a time point experiments. Blood samples were taken from healthy young lambs. After isolation of peripheral blood mononuclear cells (PBMC), the cells were cultured with different concentrations (50µg/ml & 100µg/ml) of antigens (antigens were extracted and concentrated according to the standard protocols), in different time points. Then, total RNA was isolated from the cell pellets and cDNA was synthesized using Oligo-dT primers. Afterwards, the primer pairs for TLR2, TLR4 as target genes and GAPDH as housekeeping and calibrator gene were optimized in a gradient RT-PCR experiment. Subsequently, qPCR analysis was set up to quantify and compare the relative expression levels of TLR2 and TLR4 transcripts in PBMC of antigen treated versus control(untreated) samples. **Result:** Gene expression analysis showed up-regulation of TLR2 and TLR4 transcripts in treated as compared to the untreated control group which might indicate the presence of Immunostimulatory components in Ostertagia-circumcincta somatic antigens.

Conclusion: The results presented in this study, can shed more lights to the insight mechanisms behind the molecular immuopathogenesis of ostertagiosis which might eventually pave the way for designing novel and more effective therapeutic as well as preventive strategies.
Keywords: Ostertagiosis, PBMCs, TLRs, Real-time quantitative PCR.

2005P

C2005oadministration Of Iranian Native Probiotic, *Bacillus Subtilis*, As Supplement And Recombinant Chicken IL-2 As An Adjuvant On Increasing Immune Response In Broiler Chickens Vaccinated With Commercial And Recombinant Infectious Bursal Disease Vaccine

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Background: The aim ofthisstudy was to investigatethe effects of Bacillus subtilis GQ618019 as probiotic bacterium, and chicken interleukinII as an adjuvant on the inductionof antibodyresponsesinchickensvaccinatedwith recombinant proteins VPXand commercial vaccine (c.v) of infectious bursal disease virus (IBDV).**Methods**: one-day-old chickens were divided as fallow: 1: (vaccine (v)$^{-}$, probiotic (p)$^{-}$), 2: (v^{+}, p^{-}), 3: (p + c.v), 4: (p+ VPX), 5: (p + VPX +IL-2), 6: (VPX), 7: (VPX+IL-2). Probiotic was orally gavagedfrom day 7 to 42. Thechickswere injected withVPXandinterleukin II intramuscularly on days17 and 26. All chickens were orally challenged with of(IBDV). Totalantibodies titer was assayed with ELISAtest, each week..**Results:** primary titers of antibody was similar in all groupsandsignificantly were decreased until day25($p < 0.05$). Inthatday decliningof antibody titer ingroup 2 wassignificantlyhigher than negative control group. In group1, decreasing of antibodies titer was continued until day32whilein othergroups,the titers were increased at the same time. At theend of day32 in group thatreceivedprobiotic,titers of antibodyincomparedto theother groups were increasedsignificantly. Injected viruses caused 100% mortality in negative control group meanwhile only 10% mortality was observed in group 6. **Conclusion**: The use of probiotic can stimulate immune system against IBDVand increase thetiters of antibody and there is synergy between probiotic and interleukin II.
Keywords: probiotic, IL-2, bursa of fabricius virus, immune system

2602P

Humoral immunity response assessment in chickens with coccidiosis before and after treating by Salinomycin

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Background: The effect of coccidiostats on oocyst shedding, intestinal lesions, feed conversion ratio and body weight gain has been investigated in many researches. In this paper, we assess the relation between antibody titer and each of these factors, as well.**Method:**This study aims to examine and measure humoral immunity response in broiler chickens with mixed eimeria infection, before and after treating by salinomycin. 40 Ross 308 were divided into 5 groups including 2 infected ones which had been fed with drug, 2 infected groups without treatment, and one as negative control. One of the infected groups was selected from Hamedan and another from Mazandaran. The antibody titer was assessed through ELISA method.**Results:** Body weight gain among the infected, treated groups was more significant than others which were infected, but not treated ($p<0.05$). Lesion scoring among the primary groups is significantly lower than the latter ones. In the case of treated chickens of the category 2, OPG (oocyst per gram) had a more significant difference ($P\leq 0/05$) than non-treated ones of the same category. In category 1, however, there was no significant difference. For the factor of OD, a significant difference was observed for the negative control group compared to the other four groups ($P\leq 0/05$).The results show more virulence in category 2 compared to category 1.**Conclusion:** Salinomycin has relative impacts on the investigated factors. Intestinal lesions show that there is no significant relation between protective immunity and antibody titers.

Keywords: Eimeria, Humoral Immunity, Salinomycin

2404P

Efficacy of combination of Amitraz plus G2 adjuvant for the treatment of generalized canine demodicosis

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Background: In this case report, the efficacy of G2 adjuvant plus amitraz sponge-on formulation against severe generalized canine demodicosis is described.**Method:**A six month-old male Spitz dog was presented with diffused alopecia, pruritus, crusted skin lesions, poor general condition and a history of unsuccessful ivermectin/lindane combined treatment. The dog was treated with G2 adjuvant as a TH-1 stimulator, amitraz together with antimicrobial and anti-inflammatory agents. Amitraz was topically used at one week intervals for 2 months. **Results:**Clinical examination on 12th days of treatment showed the bacterial infection of skin disappeared, general clinical conditions improved and skin lesions recovered without any further signs of pruritus. The number of adult mites was noticeably decreased and they were approximately too rare at 30 days post treatment and two consecutive skin scrapings resulted negative for mites at end of cure.**Conclusions:**These results indicate that G2 adjuvant as immune activator plus amitraz associated with the antibiotic therapy is highly effective for treating severe generalized demodicosis and could also be effective toward controlling other opportunistic parasitic infections.

Keywords: Demodex, Generalized demodicosis, Dog, G2 adjuvant

2324P

Association ofMajor Histocompatibility Complex with Humoral and Cell mediated immune responses in chicken

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Background: Major Histocompatibility Complex has long been considered the most important region in genome with respect to infectious diseases, autoimmunity and transplantation. Association of MHC with a wide range of immune and non-immune traits makes it a valuable predictive factor for disease pathogenesis and outcome. The aims of present study were investigating the MHC variability and its association with humoral and cell mediated immune responses in Ross 308 broiler chicken. **Methods:**The MHC haplotypes were determined by PCR-based fragment analysis of LEI0258 microsatellite.LEI0258 microsatellite is physically located within the MHC region and associated with serologically MHC haplotypes. The humoral immune responses were assessed by measuring the serum antibody titer against ND, IBD and AI vaccines. Lymphocyte proliferation assay using MTT was also performed for evaluatingcell mediated immune response. Statistical analysis was performed using SPSS software and univariate regression analysis to determine the coefficient effect of alleles. **Results:**In 107 Ross 308 broiler chickensseven LEI0258 microsatellite alleles and nineteen genotypes were identified that indicates a moderate MHC variation in this population. The allele 207 bp had the highest (32.21%) and 265 bp (3.37%) had the lowest frequency, respectively. In evaluating the association of MHC with immune responses,alleles194, 298, 361, 385 and 443 bp were associated with higher IBDV antibody titers, and allele 265 bp was significantly associated with elevated immune responses to Newcastle vaccine($P<0.05$).**Conclusion:**Due to the significant role of MHC in disease resistance or susceptibility and quality of immune responses, these results could be used in marker-assisted selection (MAS) and genetic breeding programs regarding to improvement ofbroiler populations.

Keywords:MHC, Humoral immune responses, Cell mediatedimmune responses, Ross broiler chicken.

1593P

Chicken MHC variability in Iranian indigenous breeds

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Background: Major histocompability complex (MHC) genes play important roles in the immune response, resistance or susceptibility to disease, autoimmunity, and life history strategy. With increasing interest in breedingandraising commercial chickens, information about MHC genes could be used for increasing immune competent and resistant animals in the population. Microsatellite LEI0258, as a genetic marker for chicken MHC haplotypes, can provide valuable data for breeding programs.**Method:**MHC genotypes were investigated using LEI0258 microsatellite marker. Variability of LEI0258 was determined in 693 chickens of 8 indigenous chicken populations (Arian 58, Marandy 42, Khorasan 314, Ross 27, Leghorn 29, Mazandaran 46, Uromie 67, and Esfahan 79). Diversity values were calculated according to Nei′s genetic distance using UPGMA method and NEIGHBOR procedure.**Result:** Collectively,

162 different alleles and 264 different genotypes could be found. The observed level of heterozygosity was %77.63. Alleles 367 and 321 bp (%11), and Genotypes 321/367, 261/261, 321/321 had the highest frequency. Our results indicate that LEI0258 diversity in Marandy and Esfehan populations were higher than other populations. With respect to dendrograms, Iranian indigenous breeds were clustered in completely different evolutionary brunch from Ross commercial broilers.**Conclusion:** This study has revealed pivotal information about indigenous chicken populations and their importance as animal genetic resources

Keyword: MajorHistocompatibility Complex, LEI0258 microsatellite,Indigenous chickens

1746P

Development and ELISA-based detection of anti-M2e IgY antibodies to oral and IM administration of M2e-Hsp70 construct

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Background: The use of IgYs in a variety of methods in different areas of research, diagnostics, medical application and biotechnology should be considered widely. Development of antibodies against extra cellular domain of influenza M2 (M2e) protein in egg yolk of laying hens. **Methods:** A Fusion construct harboring C-terminal of bovine heat shock protein 70 (Hsp70) and influenza M2e coding genes was injected to laying hens. Serum and egg yolk antibodies were screened for the presence of anti-M2e antibodies by indirect enzyme-linked immunosorbent assay (ELISA). **Results:** Anti-M2e antibodies were detected in egg yolks and sera of injected hens from 13 and 7 days post injection (PI), with the peak titer detected on 41 and 35 days PI, respectively. **Conclusions:** Anti-M2e IgY titers could be an index for expression potential of pcDNA3.1-M2e-HspC-terminal construct in laying hens. This construct could be considered as a promising tool in production of anti-M2e polyclonal, monospecificIgY antibodies. Such anti-M2e antibodies could be exploited for influenza diagnostic and therapeutic measures.

Keyword: M2e , Hsp70 , ELISA , IgY, avian influenza

2422P

Normal haematological values and morphology of blood cells in white-chested hedgehogs (*Erinaceus concolor*) from Tabriz, northwestern Iran

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Background: *Erinaceus concolor* and *Erinaceus europaeus* are the most dominant hedgehog species in Western Europe and northwestern Iran. National wild-life conservation strategies and growing demand for keeping these small mammals as companion animals are increasing the necessity for expanding the basic knowledge about the normal conditions to ease the diagnosis of pathological situations.**Method:**20 healthy free-living hedgehogs (12 males and 8 females, 1-3 years old) were gathered from Tabriz suburbs and stayed captive during the study period. To reduce the confounder factors, monthly faecal examination was done to detect endoparasitic infestation and skin was examined for exoparasites. Peripheral blood sampling (1 cc) was done through lateral saphenous vein at the beginning of each season. Thin blood smears were prepared and stained with Wright-Giemsa dye to study the morphology of white and red cells.

Blood samples analyzed using Sysmex® XE-5000 automated hematology analyzer. Statistical analyses of data were performed using SPSS 21.0.**Results**:Microscopic characteristics of each type of cells described thoroughly in addition to shape and diameter of cytoplasm and nuclei. Normal ranges for hematological values (HCT, RBC, WBC, platelet and differential count) measured and determined for males and females separately. Total lymphocytes and MCV of females was slightly higher than males.**Conclusions:**The hematologic findings of blood samples in different seasons demonstrated many similarities to dogs and cats, with some differences. The outcome of this study may assist veterinarians and wild-life medicine professionals in interpreting the CBC tests of Iranian hedgehog population.

Keywords: Erinaceus concolor,hematological values, RBC, WBC, morphology

1623P

Polymorphism identification of BoLA-DRB3.2 gene in Iranian Holstein cattle

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Background: Bovine major histocompatibility complex (BoLA) is a multiallelic and polymorphic genetic region located on chromosome 23 and organized in to class I, II, and III genes. There are at least three BoLA-DRB loci, but only DRB3 gene is functional and due to its extremely wide polymorphism has been extensively studied in cattle. The BoLA-DRB3.2 alleles potentially are found to have association with infectious diseases. **Method:** Total of 60 DNA samples of Iranian Holstein cattle were studied. Nested-PCR and direct sequencing were performed for DNA amplification and allelic genotyping. **Results:** we identified 19 BoLA-DRB3.2 alleles in comparison to reference sequences in NCBI. The most frequent alleles were DRB3.2*0101, DRB3.2*1101, DRB3.2*1501, DRB3.2*1201, DRB3.2*2703 with 26.6%, 14.1%, 9.16%, 8.33%, 7.5% respectively, and the least were DRB3.2*1901with 0.83% and 1.66%, for both DRB3.2*3501 and DRB3.2*0801. **Conclusion:** Based on previous studies, carried out on Iranian Holstein cattle, our results could confirm the six most frequently detected alleles, (PCR-RFLP alleles *8, *11, *16, *22, *23 and *24 corresponding to DRB3.2*1201, DRB3.2*0902, DRB3.2*1501, DRB3.2*1101, DRB3.2*2703 and DRB3.2*0101, respectively). These results have important implications for future vaccine design in cattle.

Keywords: Bovine Leukocyte Antigens (BoLA), DRB3.2 polymorphism, direct sequencing, Iranian Holstein cattle.

Young Researchers' Session

Oral Presentations:

2394 O

A novel method for cost- effective and on step purification of recombinant therapeutic proteins

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Background: Intein (INT) is the internal parts of the protein which can be separated from the immature protein during protein splicing process by a self-catalytic mechanism. This sequence requires no specific enzyme or cofactor for separation. This protein sequence and their characteristic of self-cleavage by thiol induction, temperature and pH changes are used for protein purification. The advantage of this method compared to the other protein purification methods is that it doesn't require any protease enzyme and protease removal steps that make this method important economically. In this study a recombinant vaccine was fused with INT in molecular form and its expression and purification was evaluated. **Methods**: In this study, a recombinant vaccine that was cloned in pET28-a in the previous studies, was cloned in pTXB1 vector containing INT tag and chitin binding domain (CBD) by specific primers and restriction enzymes. Then the resulting construct (pTXB1-V) was transformed to the ER_{2566} expression strain and cloning accuracy was confirmed by electrophoresis and western blotting. Finally on step purification carried out by thiol induction. **Results**: Data was shown that the amount of fusion protein expression (V-INT-CBD) is above 25% of the total cell proteins, that after the optimization this amount increases to more than 50%. On the other hand after thiol induction, purified protein (above 95%) obtained. **Conclusion**: This technology was setup in Iran for the first time, Finally V vaccine was purified with 16 mg/liter that compared to other protein had been purified with this system was significant.

Keywords: Intein, Expression, Purification, Recombinant protein.

2584 O

The SDF-1 3'A Genetic Variation Is Correlated with Elevated Intra-tumor Tissue and Circulating Concentration of CXCL12 in Glial Tumors

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Background: Immunological factors are important in pathogenesis of various malignancies, including neural cancers. The CXC chemokine CXCL12 is involved in the immune responses.

Therefore, the aim of the present study was to investigate the association between tumor tissue and circulating concentrations of CXCL12 as well as its genetic variation at position +801 known as(the SDF-1 3'A), in Iranian patients suffering from malignant glial tumors. **Methods:** In this study, stereotactic tumor biopsy specimens in parallel with peripheral blood samples were collected from 123 patients and 189 healthy controls. The serum level of CXCL12 was measured by ELISA and tumor tissues were subjected to Western blotting for intra-tumor CXCL12 detection; we also employed PCRRFLP to detect the SDF-1 3'A polymorphism. Demographic data were collected by a researcher-designed questionnaire. **Results:** These results demonstrated a significant difference between the A/A, A/G, and G/G genotype and A and G alleles of polymorphisms at position +801 of CXCL12. We also indicated elevated levels of CXCL12 in circulation and tumor tissue obtained from in patients suffering from malignant glial tumors. **Conclusion:** Based upon the results of this investigation, we propose that CXCL12 and its SDF-1 3'A polymorphism play a fundamental part in the pathogenesis of malignant glial tumors. It is also noteworthy that CXCL12 could probably be utilized as a beneficial biological marker in the diagnosis of these tumors.

Keywords: Anaplastic astrocytoma, Glioblastomamultiforme, Chemokine, Polymorphism, CXCL12

2521 O

Activation of Signal Transducer and Activator of Transcription-3 (STAT-3) in gastric cancer stem like cell; an in vitro model

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Background: Recently, it is proposed that cancer stem cells are responsible for the initiation of tumors. Gastric cancer is widely associated with chronic inflammation. The proinflammatory microenvironment and inflammatory factors like IL-6 and VEGF may disrupt stem/progenitor cell proliferation and differentiation.STAT-3 signaling pathway is involved in inflammation, activate in response to a number of ligands, including cytokines (e.g., IL-6) and growth factors (e.g., EGF). Here, the STAT-3 signaling pathway was evaluated in the cancer stem like cells derived from MKN-45 cell line using spheroid body formation. **Methods:** MKN-45 cells were cultured in serum free media containing EGF, bFGF andB27 to form spheroid. In spheroids; flowcytometric analysis performed to evaluate cell surface expression of some CSCs markers, expression of Nanog, Oct-4, Sox-2, Klf-4 and c-Myc as stemness genes and STAT-3 was determined by real-time PCR and MTT assay showed drug resistancy to DTX. Finally, the expression and localization of STAT-3 tested by western blotting and immunofuorescence staining. Parental cells were used as control. **Results:** Gastro-spheres showed higher expression of CD44, CD24, CD71, resistancy to drug, and overexpression of stemness genes than parental cells ($P<0.05$).Immunostaining and western blot demonstrated STAT-3 in both spheroids and parents, but translocation to the nucleus is observed in spheroids. Besides, level of STAT-3 mRNA in sheroids was higher than parents. **Conclusion:** Nuclear location of STAT-3,which is indicative of phosphorylation and activation, in spheroids concluded that STAT-3 may have role in regulation of stemness properties of gastric CSCs as recently mentioned in glioblastoma.

Keywords: Gastric Cancer, Inflammation, Cancer Stem Cells, STAT-3.

2819 O

Enhancement of Fibroblast Proliferation,Vascularization and Collagen Synthesis in theHealing Process of Third-Degree Burn Wounds by Topical Arnebiaeuchroma, a Herbal Medicine

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Background: The present study was conducted to evaluate the wound healing effect of Arnebia euchroma (AE) extract, which is traditionally used in some Indian, Chinese, and Iranian tribes, on histomorphometrical parameters involved in the healing process of third-degree burn wounds by using stereological analyses. **Methods:** In an experimental study, 48female Sprague-Dawley rats, each with a standard third-degree burn wound on the posterior surface of the neck, were divided into four groups; AE10 and AE20 groups were treated with carboxymethylcellulose (CMC) gels which contained AE hydroalcoholic extract at the concentration of 10% and 20%, respectively; the untreated burned (UB) group, which received no treatment; and the gel-base treated group. Wound closure rate, fibroblast proliferation, volume density of collagen bundles, length density, and mean diameter of the vessels were measured. **Results:** Wound closure rate, fibroblast population, volume density of collagen bundles, and length density of vessels were significantly improved by AE10 and AE20 in comparison with the gel-base and UB groups (P value <0.05).**Conclusion:** Although previous investigations on the different aspects of the wound healing effects of AE and the results of this study exhibited the positive effects of topical Arnebiaeuchroma on third-degree burn wound, introducing AEas an alternative wound healing agent requires more investigations on its efficacy on human, safety, and possible adverse effects.

Keywords: Arnebiaeuchroma, Stereology, Vascularization, Fibroblast proliferation, Wound closure rate, Collagen synthesis

Poster Presentations:

2725P

Establishment of murine model for breast cancer study

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Background: Most studies in tumor immunology depend upon the appropriate animal models. Indeed, successful targeted immunotherapy requires cells expressing a well-established tumor marker. 4T1 mammary carcinoma is a transplantable tumor cell line derived from Balb/c mice, which is highly tumorigenic and invasive that induce tumor with the very similar kinetics of human breast cancer in terms of tumor growth and metastasis. But the lack of a known tumor maker in this cell line restricts its application in breast cancer study. Here we introduce a new cell line derived from 4T1 which is genetically modified to stably express Alphalactalbumin (ALACTA) and Enhanced Green Florescent Protein (EGFP) protein fusion. **Methods:** 4T1 cell line was stably transfected with PEGFPN1 plasmid vector containing (ALACTA) and EGFP fusion gene. Expression of this fusion was evaluated by flow cytometery (for EGFP) and western blot analysis. In order to assess their capacity to induce tumor, transfected cell line were subcutaneously injected into Balb/c mice and tumorigenic propensity was investigated. **Results:** Transfected 4T1 cell line expressed (ALACTA) and EGFP fusion gene at protein level with purity of >95% over 40 passage. By western blot the expression of ALACTA protein in transfected 4T1 cell line was proved. Interestingly, transfected cell line retained their tumorigenic capacity in transplantation experiments. **Conclusion:** Here we introduce a new cell line will greatly facilitate the study of breast cancer in mice model because of the green properties of transfected cell line and expression of ALACTA protein which can be a new target for breast cancer prophylactic and therapeutic treatment approaches.

Keywords: Alphalactalbumin, Breast cancer, Mice model, EGFP

3101P

Lactobacillus acidophilus La5, Bifidobacterium BB12 and lactobacillus casei DN001 modulate gene expression of subset specific transcription factors and cytokines in peripheral blood mononuclear cells of obese and overweight people

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Background: Probiotics are believed to have interaction with immune cells through sustained effects on gene expression of different cytokines and transcription factors. **Methods**: The present randomized doubled-blind controlled clinical trial was performed recruiting 75 individuals with BMI25-35, who were randomly assigned to the following three groups: Group 1 (n=25) who consumed regular yogurt as part of a low calorie diet [RLCD], group 2 (n=25) who received probiotic yogurt with a LCD [PLCD] and group 3(n=25) who consumed probiotic yogurt without LCD [PWLCD] for 8 wk. Participants in PLCD and PWLCD groups received 200 g/day yogurt containing Lactobacillus acidophilus La5, Bifidobacterium Bb12 and lactobacillus casei DN001 10^8 cfu/gr. The expression of the FOXP3, T-bet, GATA3, TNF-α, IFN-γ, TGF-β and ROR-γt in PBMCs genes were assessed, before and after intervention. **Results:** In three groups, ROR-γt expression was reduced ($p=0.007$) and FOXP3 was increased ($p<0.001$). The expression of TNFα, TGFβ, and GATA3 genes didn't change among all groups after intervention. Interestingly, the expression of T-bet gene which was significantly decreased in PLCD and PWLCD groups ($p<0.001$), whereas gene expression of IFN-γ decreased in all three groups. **Conclusion:** Our results suggest that weight loss diet and probiotic yogurt had synergistic effects on T-cell subset specific gene expression in peripheral blood mononuclear cells among overweight and obese individuals.

Keywords: Obesity, Probiotic, Gene Expression, Low Calorie Diet, T lymphocyte

2877 P

The effect of a gluten-free diet on the quality of life of patients with celiac disease

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Background: Although pathologic changes of celiac disease resolve on a gluten-free diet, how a gluten-free diet affects the quality of life for patients with celiac disease has not yet well been studied. We examined the effect of a gluten-free diet on the quality of life of patients with celiac disease. **Methods:** Thirty patients with screen-detected celiac disease were identified by performing endomysial antibody testing on healthy first-degree relatives of patients with known celiac disease. Twenty-seven consecutive patients with symptom-detected celiac disease served as a comparison group. Quality of life measured with the Psychological General Well-Being Questionnaire (PGWB).**Results:** At baseline, the group without celiac disease and the group with screen-detected disease had similar scores for PGWB; After 1 year of following the diet, quality of life for patients with screen-detected disease significantly improved (mean PGWB score increased from 104 to 112; $P<0.01$). Similar increase was noted in patients with symptom-detected disease (mean PGWB score increased from 90 to 102; $P<0.01$).**Conclusion:** Gluten-free diet was associated with improved quality of life for patients with symptom-detected celiac disease and screen-detected celiac disease.

Keywords: Gluten-free diet, Quality of life, Celiac

2987 P

Correlation between inflammatory marker "s-CRP" and Anti-streptolysin O levels in a base screening study

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Background: Measurement of antibodies to the extracellular antigens produced by group A streptococci, antistreptolysin O (ASO) is often performed to confirm a clinical diagnosis of a previous group A streptococcal infection, especially in patients suspected of having a non suppurative sequel to this infection. Many factors are involved in achieving the desired results in interpretation of the test. These appears to be side factors that may need to be considered in this test. One of them is the inflammatory condition in which diagnostic markers can be useful for identification, such as CRP marker. We studied the correlation between antistreptolysin O levelsand serum inflammation marker s-CRP in a base screening study. **Methods:** In this study, the serum antistreptolysin O concentration were measured in the base screening population study which included 196 citizens from zanjan city. Among these population, 11 subjects had serum ASO level greater than 220 IU. The ASO measurement was carried out by using ASO latex kit in medical diagnostic laboratory of zanjan. Population subjects who had high levels were separated, and their serum CRP marker levels were measured. Along with them, the age variable was also evaluated. The results were analyzed using correlation coefficient test, the non-parametric statistical and Spearman test in SPSS 18 software. **Results:** The results were evaluated and the analysis of our data indicated that one of the nonspecific markers of systemic inflammation, CRP, had no significant correlation with the change in ASO levels in high concentrations, in ASO subjects of the population ($p > 0.006$). However differences were close to the edge. Despite the insignificant correlation between ASO and CRP, there was a correlation between theage variable and each of both variables ($p < 0.005$). In this study, the value of P (P value) less than 0.05 (<0.05) is considered statistically significant. **Conclusion:** According to these findings, the ASO level and serum CRP level run parallel to each other and a change in one gives no indication about the other. It can thus be concluded that the measurement of ASO level cannot be used to verify the level of inflammatory CRP marker.

Keywords: Correlation, ASO latex test, Inflammatory marker s-CRP, Antistreptolysin O

2881 P

Vaccination with a combination of propranolol and heated 4T1 cells elicits a beneficial response against mouse model of breast cancer

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Background: Induction of Th1 responses against tumor antigens may be as a useful strategy to control malignancy. In this respect, previous studies have shown that beta-adrenocreceptor

antagonists can promote a cellular immune response. This survey was done to investigate the efficacy of a new vaccine against breast cancer made by mixing heated 4T1 cells and propranolol, as an adjuvant.**Methods:**4T1 cells were used to induce breast cancer in Balb/c mice. After tumor growth, the mice were twice with one week interval vaccinated by a mixture of 10^5 heated 4T1 pluspropranolol (3mg/Kg). **Results:** In mice vaccinated with heated tumor cells and propranolol, decreased tumor growth rate and increased survival were seen. In addition, in these mice, the production of IL-17 and IFN-γ in splenocytes was increased and conversely, the production of IL-4 was decreased. Moreover, the frequency of $FoxP3^+$Treg cells in splenocytes of vaccinated mice was significantly decreased. **Conclusions:** Combined heated 4T1 cells and propranolol promote beneficial outcome in mouse model of breast cancer.
Keywords: 4T1, Propranolol, Breast cancer, Tumor vaccine.

1977 P

The Effect of miRNA-143 molecules on overexpression of PYCARD molecules in Jurkat cells

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Background: Micro-RNAs are regulator protected 22-noleotide series which play important roles in cell functions such as apoptosis. Apoptosis process is essential in tissue homeostasis. It would be a great success in cancer treatment if forcing cell to do apoptosis. This study examined the effect of miRNA-143 on producing PYCARD molecule (apoptosis inducing molecule).**Methods:** I n this laboratory study miRNA-143, scramble and phosphate buffer saline were transfected to the Jurkat cells (leukemia T lymphocyte), separately, and mRNA level of PYCARD molecule were measured by "Real-Time PCR" technique. Apoptosis changes were examined by FITC-anxin method. **Results**: In 30% of miRNA-143 transfected Jurkat cells apoptosis accrued. Also in miRNA-143 transfected cells producing of PYCARD were higher than scramble or PBS transfected cells. **Conclusion**: According to the results study it may be concluded that miRNA-143 is effective on increasing PYCARD production in Jurkat cells. So it seems miRNA-143 can be used in cancer treatment by induction of apoptosis.
Keywords: miRNA-143 , PYCARD, Jurkatcells

2847 P

Dendritic cells pulsed with heated tumor cells and heated yeast form of *C. albicans*cause beneficial outcome in mouse model of breast cancer

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Background: In recent years, dendritic cell (DCs) based immunotherapy has received increased interest in the treatment of specific malignancies including breast cancer. This study reports on the beneficial immunotherapy with DCs pulsed with heated tumor cells and yeastform of *C. albicans*. **Methods:** Highly metastatic 4T1 cells were used to induce breast cancer in Balb/c

mice. Dendritic cells (DCs) were isolated from the spleen of normal Balb/c mice by enzymatic digestion and nycodenz centrifugation gradient. Isolated DCs were pulsed with heated tumor cells and heated yeastform of *C. albicans*. About 10^6 pulsed DCs were injected twice with one week interval around the tumors. Tumor growth rate, survival rate and cytokine production of spleen cells were evaluated in the studied groups. **Results:** In mice vaccinated with DCs pulsed with heated tumor cells and yeast form of C. albicans, decreased tumor growth rate and increased survival were seen. In addition, in these mice, the production of IL-17 and IFN-γ in splenocytes was increased and conversely, the production of interleukin-10 was decreased. **Conclusions:** DCs pulsed with heated tumor cells and heated *yeast form of C. albicans*cause beneficial outcome in mouse model of breast cancer.

Keywords: Dendritic cells, 4T1,C. *albicans*,Breast cancer.

3314 P

The association of IL 8 polymorphismwith tuberculosis susceptibility in the Kermanshah population.

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Background: Interleukin-8 (IL-8), a CXC chemokine has an important role in the human inflammatory and leukocyte recruitment to areas of granuloma formation in tuberculosis process and become a major focus for TB researchers worldwide. In this study, the association between rs4073 polymorphism and TB susceptibility was analyzed. **Methods:** In order to evaluate the effects of the polymorphism in the IL-8 gene on tuberculosis susceptibility in a kermanshah population, polymorphism rs4073, were genotyped in 130 tuberclosis patients and 80 healthy controls by using RFLP-PCR. **Result:** The genotype and allele frequency of rs4073were significantly different between TB patient and control individuals (P-value<0.05). **Conclusion:** Our results support an association between the polymorphism rs4073 of the IL-8gene and tuberculosis susceptibility in the Kermanshah population. These data suggest that polymorphism in the IL-8 play an important role in tuberculosis susceptibility in the Kermanshah TB patient.

Keywords:IL8, Tuberculosis, Polymorphism

3245 P

The association of T cell membrane protein 3 (*Tim-3*) polymorphism with Rheumatoid Arthritis in the Isfahan population.

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Background: Rheumatoid arthritis (RA) is an autoimmune disease that caused by the complex interaction between multiple genetic as well as environmental factors. T cell immunoglobulin and mucin domain-3 (Tim-3) is a cell surface molecule expressed on differentiated Th1 cells and interacts with TIM-3 ligand to regulate Th1 responses. Genetic investigations that are associate

the Tim locus and specific Tim-3 polymorphisms with various immune-mediated diseases including Multiple sclerosis (MS), rheumatoid arthritis (RA). The aims of this study were to examine whether genetic variation in the exon region of TIM3 influenced risk for rheumatoid arthritis. **Methods:** This case-control study included 127 rheumatoid arthritis patients and 143 healthy subjects as control group. Genomic DNA was extracted from investigated subjects and Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine polymorphism of tim-3 (+4259G>T). PCR products were chosen at random for sequencing. **Result:** The genotype and allele frequency of +4259G>T polymorphism were significantly different between RA patient and control individuals (P-value<0.05). We did not observe any correlation among polymorphism with other various factor like gender, age, family history, correlation with another autoimmune disease, and other demographic features. **Conclusion:** Our results support an association between the +4259G>T polymorphism of the TIM-3 gene and RA disease. These data suggest that polymorphism in the TIM3 exon region play an important role in susceptibility to RA and it might be a therapeutic target gene for other autoimmune disease.

Keywords: TIM 3, Rheumatoid Arthritis, polymorphism

2198 P

Rational vaccine designing base on gp120 glycoprotein of Iranian Human Immunodeficiency virus 1(HIV-1) belongs to CRF_35AD subtype

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Background: In Iran, the first case of HIV was seen in 1996. Besides injecting drug users (IDUs), the prevalence of HIV among the general population in Iran growing alarmingly. In this study we attempted to design effective vaccine relied on 35AD subtype by wide array of immunoinformatic approaches.**Methods:** Briefly, steps that flowed in present study were; retrieving gp120sequences of CRF_35AD subtype, finding the mutational/conserve regions and then selecting subjected sequence. In following, wide array of immunoinformatic software and severs applied for prediction/mapping of conformational and linear B cell epitopes. For conformational epitope by respect to obtaining reliable and effective consensus immunogenic epitope, we used of ElliPro, DiscoTope 2.0, SEPPA, CBTOPE,BCEP and B-pred servers and for achieving linear epitopes combinational physic-chemical method besides machine learning approaches (BCPREDS, BepiPred 1.0, SVMTriP and Bepipred). After selecting consensus highly immunogenic and reliable B cell epitope regions, they were fused together. We continues process of designing by Visualization of tertiary structure of polytopic construct, evaluation of its primary structure,post translational modification and next steps of DNA vaccine designing. **Results:** Using different wide array of computational analyses and immunoinformatics tools, 4 unique and reliable B cell antigenic regions for gp120 protein were identified. The gp120 B cell epitopes located the different regions of this protein. Checking's of polytopic construct, revealed its reliability and efficacy for in-vitro producing and utilizing. **Conclusion:** To our knowledge this is first report of comprehensive immunoinfomatic analysis of gp120

glycoprotein of Iranian HIV-1 CRF_35AD subtype toward developing a protective and therapeutic vaccine.

Keywords: Immunodeficiency virus 1(HIV-1), CRF_35AD subtype, gp120, Vaccine

2586 P

Maternal stress in pregnancy based on Holmes-Rahequestionnaire and umbilical cord IgE

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Background: pregnancy is a stressful period in woman life and environmental changes can effect on its qualification. Maternal stress is a risk factor on pregnancy that may affect the fetal immune system and predispose newborn to allergy. **Methods**: In this descriptive-analytic study, 290 pregnant women in third trimester were questioned about stress events in pregnancy by Holmes-Rahe Stress Score Questionnaire and also umbilical cord and maternal serum IgE levels were determined. **Results**: We found that 50.3% of pregnant women had mild, 30.7% had moderate and 19% had high stress during the first and second trimester. Umbilical cord IgE(UcIgE) was high in 24%, 54% and 70.9% of neonates from women with mild, moderate and severe stress respectively(P=0.001). UcIgE was significantly more in neonates of mothers with higher serum IgE. (50.5% vs 36.2%) (P =0.018).**Conclusion**. Findings of this study indicated that high stress during pregnancy which determined by Holmes-Rahe questionnaire had a significant correlation with UcIgE.

Keywords : Holmes-Rahe questionnaire, Pregnancy stress, Umbilical cord IgE(UcIgE)

2251 O

Construction and expression of a new vaccine candidateagainst urinary tract infections

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Background: Urinary tract infection (UTI) is one of the most common infections in the world. Uropathogenic *Escherichia coli* (UPEC) are the most common causeof UTI and *Proteus mirabilis* (P.M) is a frequent cause of complicated UTI especially in catheterized patients. Type1 pili by having the adhesion FimHand MR/P pili by having the adhesion MrpHare the important virulence factors of UPEC and P.M strains, respectively. To date, any ideal vaccine against UTI has not been approved for human use and there is a need to test new target

antigens to develop an ideal vaccine against UTI. In this study, we constructed and expressed recombinant fusionprotein FimH.MrpH of UPEC and P.M strains. Assaying of the immune responses of the new vaccine candidate in vivo and in vitro is in progress. **Methods**: UPEC and P.M were isolated from the UTI patients. The *fimH* and *mrpH* genes were amplified by PCR. After sequencing, construction of fimH.mrpH hybrid was performed by overlap PCR with fusion primers. The *fimH*, *mrpH* and fimH. mrpH cloned in pET28a vector and expressed. The confirmation of the expressed proteins was done by SDS-PAGE and Western blot. Purification of the proteins and endotoxin removal of them was done by using the Triton X114 in Ni-NTA column and then LPS levels were checked by LAL test. Mice were immunized with the proteins in separate groups and evaluation of antibody responses and cytokines assay is under way. **Results**: The *fimH* and *mrpH* genes were amplified in all of the UPEC and P.M isolates tested. The *fimH* and *mrpH*sequences showed significant homology with the sequences in Genbank.We generated a fusion protein consisting of the fimH protein linked to the N-terminal of mrpHthat sequencing of the fusion showed its precise construction. SDS-PAGE and Western blot confirmed the expression of the proteins and the LPS value of the proteins after LPS removal was <0.02EU/ml. **Conclusion**: Urinary tract infections (UTIs) are the second most common infectious disease. UPEC and Proteus mirabilis are two important agents of UTI in the world. With an increasing number of UTIs being caused by antibiotic resistant strains, there is a need for alternatives such as vaccination. Some of the virulence factors of UPEC and P.Mhave been tested as vaccine targets that have limited success. Fusion protein strategies offer a significant advantage in the incorporation of multiple antigens of different pathogens in one molecule that can induce significant immune responses against the pathogens. Thus, the fusion protein fimH.mrpH could be an ideal vaccine candidate against UTI in simultaneous protection against UPEC and P.M infections.

Keywords: Urinary tract infection, *Escherichia coli*, *Proteus mirabilis*, fimH, mrpH

2961P

Molecular Dynamics Simulation Studies on stabilizers used in immunoglobulin products

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Background: Protein instability is common problem in clinical trial. For instance IVIG preparations are used in the treatment of patients which offers improved convenience in preparation but lack sufficient stability to allow room temperature storage. Polyols such as trehalose or sucrose have been known to protect proteins against loss of activity and prevent the partial or even total degradation of biomolecules. Despite various experimental and theoretical works, the detailed molecular mechanism at the origin of polyols protective ability still not well understood. MD simulations can provide valuable information about the different stages of peptide–solvent interactions. **Methods:** All simulations were performed using the gromacs software package, version 4.5.4. The Gromos9653a6 parameter set was used as force field. The starting conformation of the peptide was obtained from the protein databank (PDB) structure 1LCI. The peptide was solvated with SPC water, a mixture of sucrose, a mixture of

trehalose. **Results:** Our results showed there is main difference in water and polyols direct interaction with protein. The water molecules mainly interact with protein side chains while polyols are more involved with protein backbone. Also, we have identified variations in the secondary structure of the protein during the simulations. In pure water some part of alpha helical conformation of protein is deteriorates with time whereas in sucrose solution protein secondary structure is maintained throughout the 100 ns duration of simulations. **Conclusions:** In conclusion, according to the results presented in this study it has been suggested that polyols with effect on special part of protein and make favorable environment can prevent protein thermal unfolding.

Keywords: Polyols, Protein stability, Stabilizers, Molecular Dynamics Simulation

2869 P

The Effect of Family-centered Empowerment modeltrainingusingmultimedia on thequality of life In Asthmatic Children Of Bushehr

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Background: Asthma is the most common chronic disease of childhood. It is a chronic illness that influence the quality of life of all family members and it makes many problems which education is essential to meet them. Thus, the purpose of this study is to investigate effect of Family-centered Empowerment model training using multimedia on the quality of life in asthmatic children Of Bushehr. **Methods**: This study is a randomized clinical trial. The study population included all asthmatic children under age of 12. First, 50 patients were selected convenience & then randomly divided into two control and intervention groups. Research tools included demographic information questionnaire and general Peds QoL and asthma Peds QoL questionnaire of child. Reliability and validity of questionnaires were evaluated and family-centered empowerment model was performed by Multimedia Education on intervention group and the questionnaires were completed before and after the intervention. Findings analysed by using spss 18 software and statistical tests (chi-square) and T-test. **Results**: The independent t-test results indicated significant difference between control and intervention groups in terms of general quality of life and quality of life of asthmatic children mean score after intervention. **Conclusion**: According to our findings, implemention of this model by Multimedia Education increases the general quality of life of asthmatic children and asthma-related quality of life in the treatment & disease dimensions . Therefore, we suggest implementation of this model to improve outcomes in other chronic diseases of childhood by Multimedia education.

Keywords: Empowerment, Quality of life, Education, Multimedia, Asthma, Children

2132 P

Investigation of the effect of eight weeks aerobic exercise training on plasma interlukine-10(IL-10) and Nitric Oxide(NO),fatigue and disability score in women with relapsing remitting multiple sclerosis patients.

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the brain and spinal cord in which leads to damage of myelin and axons. It is the most common neurological disorder in young adults, and Fatigue is one of the most common symptoms of MS. Pro-inflammatory cytokines of the Th1 type play a key role in the pathogenesis of MS. In contrast, anti-inflammatory cytokines of the Th2 type have been associated with remissions and recovery from disease. Also pro-inflammatory cytokines may up-regulate free radicals such as nitric oxide (NO) whereas anti-inflammatory cytokines such as interleukin-10(IL-10) may cause reduction in free radicals and improving in the disease. Therefore, in this study we examined the effect of an eight-weeks aerobic exercise training program on the level of cytokine IL-10 and NO in the plasma, fatigue and disability score(EDSS) in women with relapsing remitting multiple sclerosis patients. **Methods:** Women with relapsing remitting multiple sclerosis(N=27) with average age of30.11±6.97 , weight of 64.55±10.80 kg, high of 156.41±19.52 cm, body mass index(BMI) of 24.87±4.12, and EDSS of 1.61±0.53 were randomly assigned to control (N=12) and experimental groups (N=15). Aerobic exercise was designed for 24 sessions during 8 weeks. Each session was started with 3 min warm-up and was followed by 30 min stationary bicycle at 60% to 80% of targeting heart rate. Serum IL-10 and NO concentrations were determined before and after exercise at *weeks 0*, *4*, and *8*. IL-10 were analyzed using ELISA kits, NO were analyzed using NO assay kit with Griss method, fatigue and EDSS respectively were assessed using fatigue severity scale(FSS) and Kurtzke Expanded Disability Status Scale. Independent T-test and repeated measurement test were used for analysis of data in significant level of P= 0.05.**Results:** The results showed IL-10 increased significantly in exercise group compared to control group. The NO decreased after 8 weeks aerobic exercise training in exercise group comparison with control group but it is not significant. The fatigue severity and disability score was significantly decreased in exercise group compared to control group. **Conclusion:** By considering the role of anti-inflammatory cytokine IL-10 in remissions of MS, it seems regular exercise training using increase of IL-10 production leads to reduction in fatigue severity and disability score in these patients. Thus, physical training as a therapeutic approach can benefit in improving multiple sclerosis.

Keywords: Multiple Sclerosis, Erobic exercise,Interlukin-10(IL-10),Nitric Oxide(NO),Fatigue

2195 P

Effects of miR-155 on expression of PYCARD and RIPK2 genes involved in apoptosis

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Background: MicroRNAs (miRNAs) are involved in several processes including cell development, proliferation and apoptosis. PYCARD (apoptosis-associated speck-like protein containing a CARD) and RIPK2 (receptor-interacting serine-threonine kinase 2) as pro-apoptotic molecules, promote caspase-mediated apoptosis. The aim of this study was to determine the roles of miR-155 in induction of apoptosis in Jurkat cell line, as a leukemia cell, and also its effects on the mRNA levels of PYCARD and RIPK2.**Methods:** Jurkat cells were divided into three groups: i) cells transfected with miR-155, ii) cells transfected with the control scrambled sequence, and iii) cells transfected with PBS as control. After transfections, apoptosis rates, survivability and mRNA expression levels of apoptosis-related genes, PYCARD and RIPK2, were analyzed by Annexin-V-FLUOS, MTT, and Real-Time PCR techniques respectively. **Results:** The results showed that 30% of Jurkat cells presented apoptotic features. The mRNA levels of PYCARD gene were significantly increased in miR-155-transfected cells (miR-155-transfected cells: 3396.89 ± 50, scrambled sequence-transfected cells: 1 ± 0.2, PBS-transfected cells: 0.9 ± 0.1), while there was no significant effect on RIPK2 mRNA levels (miR-155-transfected cells: 0.3 ± 0.2, scrambled sequence-transfected cells: 1 ± 0.3, PBS-transfected cells: 0.9 ± 0.2)**. Conclusion:** This study revealed that miR-155 can affect expression of genes involved in apoptosis. We found that miR-155 increased PYCARD mRNA levels. Therefore, it appears that miR-155 may induce apoptosis via up-regulation of PYCARD in cancer cells, hence it seems that miR-155 may be considered as a novel therapeutic option to treat cancer.

Keywords: miR-155, PYCARD, RIPK2, Apoptosis, Jurkat cells.

2212 P

Down-regulation of MDR-1 involved in Pioglitazone-induced apoptosis in human breast adenocarcinoma cells

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Background: Multidrug resistance in human cells is associated with decreased intracellular drug accumulation. MDR-1 asan energy-dependent efflux pump transporter is responsible for resistance to different cytotoxic drugs. In this study, we evaluated the effect of pioglitazone, a peroxisome proliferator-activated receptor gamma (PPARγ) agonist, on the proliferation, apoptosis and MDR-1 expression/function of human breast adenocarcinoma cells, Mcf-7. **Methods:** Mcf-7 cells was cultured in RPMI-1640 (FBS%10) and exposed to a range of concentrations [from 1-100 µM] of the pioglitazone. MTT assay was performed to examine cell proliferation; Apoptosis was detected by flowCytometry; MDR-1 transcription was determined by RT-PCR; the changes in MDR-1 protein expression investigated by Immunoblotting.

Moreover, the effect of the pioglitazoneon MDR-1 functional activity was also studied by the rhodamine 123 uptake assay. **Results:** Pioglitazone inhibited growth and induced apoptosis of HepG2 cells in a dose-dependent manner. Pioglitazone not only considerably reduced MDR-1 mRNA/protein expression in all treatment compared to the control but also slightly inhibited MDR-1 functional activity. **Conclusion:** Our finding showed that pioglitazone could inhibit MDR-1 expression and function in a dose-dependent manner. These results suggest that pioglitazone could be a novel and potent MDR-1 reversal agent and may be a potential adjunctive agent for different diseases and specially tumor chemotherapy.
Keywords: MDR-1, Pioglitazone, Mcf-7 cells.

2314 P
The role of miR-155 on expression of TNFRSF21 and TNFRSF25 genes

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Background: It has been demonstrated that microRNAs (miRNAs) play a role in the regulation of apoptosis. MiR-155 has dual roles in induction of apoptosis. Tumor necrosis factor receptor superfamily member 21 (TNFRSF21) and TNFRSF25, also known as death receptor 6 (DR6) and DR3, respectively, may activate NF-kappa-B and JNK to promote apoptosis. In this study we investigated mRNA levels of these genes in Jurkat cells after transfection with miR-155.**Methods:** Jurkat cells were transfected with miR-155, scrambled sequence and PBS. Apoptosis and mRNA levels of TNFRSF21 and TNFRSF25 genes were evaluated by Annexin, MTT, and Real-Time PCR techniques, respectively. **Results:** This study indicated that 25% of miR-155 transfected cells had apoptotic features and mRNA levels of TNFRSF25 gene were significantly decreased in the miR-155 transfected Jurkat cell line (0.003 ± 0.02) compared to the scrambled sequence (1 ± 0.1) or PBS (0.9 ± 0.3) transfected Jurkat cells as controls. TNFRSF21 mRNA levels in miR-155, scrambled sequence, and PBS transfected cells were 0.24 ± 0.2, 1 ± 0.1, and 1 ± 0.2 which were not significant. **Conclusion:** According to our results, it may be concluded that miR-155 can lead to apoptosis via downregulation of TNFRSF25 mRNA level. Thus, it seems that miR-155 can prevent apoptosis via this receptor.
Keywords: miR-155, Apoptosis, TNFRSF21, TNFRSF25.

2465 P
Application of nanoemulsion technology to increase the intestinal absorption of sirolimus from soft gelatin capsules

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Background: Sirolimus, an immunosuppressant used for prevention of transplant rejection, exhibits low oral bioavailability. As a substrate of P-glycoprotein counter transport in the intestine, sirolimus is a good candidate for being formulated in nanoemulsion systems containing solubility and absorption enhancer ingredients. This study aimed to the application

of nanoemulsion technology for increasing intestinal permeability of sirolimus. **Methods:** Response surface methodology was run to evaluate the effect of different formulation components on the responses (emulsification ability, Droplet size, Polydispersity index and Zeta potential of the nanoemulsion) and to statistically optimize the formulation variables. In vitro release and intestinal absorption of optimized nanoemulsions were evaluated and compared. **Results:** According to the results of applied statistical experimental design, the amount of oil and surfactant in nanoemulsions could significantly ($p < 0.05$) affect the droplet size and nanoemulsification efficiency. Response surface method was successfully optimized formulation components. Droplet sizes of optimized nanoemulsions were less than 50 nm and their emulsification efficiencies were more than 90%. Optimized formulations revealed significant increase in drug release (85% in 15 min). Intestinal transport of sirolimus was significantly ($p < 0.05$) increased from nanoemulsions in comparison to drug solution. In the rat model, the intestinal permeability of sirolimus from nanoemulsion containing Capryol PGMC and Cremophor RH 40 as P-glycoprotein inhibiting excipients was 4.15 fold higher than that of sirolimus solution. **Conclusion:** These results suggested that nanoemulsions are potential vehicles for delivering sirolimus filled in soft gelatin capsules and could promisingly improve solubility and intestinal absorption of this immunosuppressant drug.
Keywords: Sirolimus, Nanoemulsion, Immunosuppressant, Intestinal absorption.

2589 P

Effects of Low-dose Morphine on Nitric Oxide Concentration and Angiogenesis in Two-kidney One Clip Hypertensive Rats

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Background: We investigated the effects of low-dose morphine on nitric oxide (NO) and angiogenesis in two-kidney one clip hypertensive (2K1C) rats. **Methods:** Male rats were divided into two groups: sham-clip operated and 2K1C. Each group subdivided into saline and morphine (3 mg/kg i.p. 8 weeks) groups. Blood pressure, heart rate, plasma renin activity (PRA), NO concentration and murine matrigel angiogenesis were evaluated. **Results:** Morphine had no effects on blood pressures and HR in sham normotensive rats but attenuated diastolic blood pressure (DBP) ($P< 0.01$) and mean arterial pressure (MAP) ($P< 0.01$) in 2K1C compared with saline. PRA level was significantly higher in 2K1C compared with sham groups ($P< 0.01$) but morphine decreased it in 2K1C compared with saline ($P< 0.01$). After clipping, NO in 2K1C hypertensive rats was decreased ($P< 0.01$) and morphine increased it compared with saline ($P< 0.01$). Morphine promoted angiogenesis in both sham ($P< 0.01$) and 2K1C ($P< 0.0001$) groups. **Conclusion:** Low-dose morphine stimulated angiogenesis in two-kidney one clip hypertensive rats probably via NO pathways.
Keywords: Anaplastic astrocytoma, Glioblastoma multiforme, Chemokine, Polymorphism, CXCL12

2588 P

Relationship between cord blood IgE level and stress during pregnancy

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Background: Allergic diseases, including common diseases in children are one of the common causes of disability in many societies. In recent years, increased incidences of these diseases in many countries have been reported. Allergies in children have several risk factors, including maternal stress during pregnancy. **Methods:** In this descriptive-analytic study, 290 pregnant were referred to two general and private hospitals in Yazd. During 2010, a non-randomized simple sampling was done. Anxiety levels in pregnant was determined by Pregnancy Anxiety Questionnaire (PRAQ). Cord blood and maternal blood IgE (Immunoglobulin E) levels were determined by ELISA method. Data were statistically analyzed. **Results & Conclusion:** Our findings showed that the average anxiety score in mothers whose babies IgE were above 0.35 IU/ml was more than mothers whose babies IgE were below 0.35 IU/ml. There was a significant relationship between cord blood IgE level and Pregnancy anxiety score ($P<0.001$). There was no significant relationship between the variables of gender, gestational age and birth weight with cord blood IgE levels ($P> 0.05$). It seems that both anxiety and fear during pregnancy are risk factors of allergic diseases in children.

Keywords: Pregnancy anxiety, Cord blood IgE, Allergic Diseases

2366 P

Effect of taurine on febrile episodes in acute lymphoblastic leukemia.

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Background: The purpose of our study was to evaluate the effect of oral taurine on the incidence of febrile episodes during chemotherapy in young adults with acute lymphoblastic leukemia.

Methods*:* Forty young adult (aged over 16y) with ALL, at the beginning of maintenance course of their chemotherapy, were eligible for this study. The study population was randomized in a double blind manner to receive either taurine or placebo. Life quality and side effects including febrile episodes were assessed using questionnaire. Data was analyzed using SPSS. **Results:** Of total participants, 43.8% were female and 56.3 % were male. The mean age was 19.16±1.95 years (ranges: 16-23 years). The majority (50%) were high school students. During the study, levels of Hb, Hct, RBCs and platelets were slightly increased in

the case group compared to control patients however the data were not significant. The results indicated that the levels of WBCs are significantly (P<0.05) increased in taurine treated group. There was no elevation in blasts count. A total of 70 febrile episodes were observed during study, febrile episodes were significantly (P<0.05) lower in taurine patients in comparison to the control ones. **Conclusions:** The overall incidence of febrile episodes and infectious complications in ALL patients receiving taurine was lower than the placebo group. Taurine's ability to increase WBC count may resulted in lower febrile episodes. Future studies with large number of patients should be implemented to evaluate our results.

Keywords: Acute lymphoblastic leukemia,Taurine, Febrile episode.

2383 P

Serum levels of interleukin-17 in patients with or without acute coronary syndrome; a cross-sectional study in Babol, northern of Iran

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Background: The role of interleukin-17 (IL-17) in patients with acute coronary syndrome (ACS) is controversial. The aim of the present study was to assess the serum level of IL-17 in patients with AMI and UA in Babol, northern Iran in 2012.**Methods:** Sixty patients with ACS (thirty with AMI, 30 with UA) and 30 healthy blood donors of Babol city, Iran were recruited in this cross-sectional study. Simple sampling method was used for patient selection. The inclusion criteria were the recent (<48 hour) diagnosis of acute MI or UA. The exclusion criteria were: diagnosis of AMI or UA >48 hours, recent history of infection or use of antibiotics. The serum IL-17 level was measured using ELISA kit (Bioscience, Texas, USA). The lowest concentration of IL-17 that the kit could detect was 0.8 pg/ml. Chi-square, and kruskal-wallis tests were used for comparison of IL-17 level between participants. P-value<0.05 was considered significant. **Results:** Ten participants were excluded due to contaminated blood samples. Fifty (62.5%) were male and 30 (37.5%) were female. Four(13.3%) patients of AMI group and one(3.3%) of UA group had detectable levels of IL-17 (P=0.353) but no one in blood donors (P=0.114). There were also no significant association between the presence of detectable level of IL-17 and history of DM, HTN, HLP, smoking (P>0.05).**Conclusion:** Our study did not find any significant differences in the serum level of IL-17 in individuals with or without ACS and therefore did not support the theory in which IL-17 has deleterious role in atherosclerosis.

Keywords: Acute coronary syndrome, Blood, Interleukin-17

2735 P

Evaluating of Cell death induced by Novel Synthetic Chromene Derivatives on peripheral blood mononuclear and Hela cells

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Background: Chromenes are a group of Benzopyrones, which their anti-tumor activities have been demonstrated. With this background, we studied the anti-cancer effect of some new chromenes derivatives on Hela cells in comparison with immune peripheral blood mononuclear cells (PBMCs).**Methods:** This study investigated the effect of new synthetic derivatives on blood-isolated PBMCs from healthy people and the growth inhibition of Hela cells. Cytotoxicity and viability were determined by MTT and ELISA assays. The half maximal inhibitory concentration (IC50) was calculated for each compound. **Results:** Data indicated that some of the compounds elicit potent inhibitory effects on growth of Hela cell line, with no inhibitory effect on PBMCs. **Conclusion:** It is the first study, which examined the anti-tumor activity in novel synthetic chromenes. More studies are needed in order to validate these data and the therapeutic potential of these compounds.

Keywords: Synthetic chromene derivatives, Anti-tumor, Hela, PBMC.

3042 P

Single nucleotide polymorphisms on Leukemia Inhibitory Factor (LIF) gene and its plasma level in Relapsing Remitting Multiple Sclerosis (RRMS) patients

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Background: Leukemia inhibitory factor (LIF) is an interleukin 6 class cytokine and is observed in MS plaques. Macrophages and T cells secrete LIF and have been shown to confer oligodendrocyte protection in the CNS. The positive effect on oligodendrocytes appears to be mediated through LIF induced activation of suppressors of cytokine signaling (SOCS). In addition, LIF has opposite effects to the pro-inflammatory agent INFγ and has been demonstrated to block the cytotoxic effects of INFγ on oligodendrocytes. **Methods:** In a case-control study, genomic DNA from 125 patients and 111 healthy subjects were extracted and HRM-Real Time PCR examined the presence of rs737812. The plasma level evaluation of LIF was also examined utilizing enzyme-linked immunosorbent assay (ELISA).**Results:** Our data suggests a significant difference between the frequency of the SNP and plasma level in RRMS patients and the healthy subjects (*P*-value<0.05).**Conclusion:** Statistical analysis demonstrated the relationship between OPN genetic variant and its plasma level in RRMS patients in comparison

with the healthy subjects. This finding shows the capital role of OPN in MS progression and it might pivotal candidate for therapeutic goals.
Keywords: Single nucleotide polymorphism (SNP), Leukemia inhibitory factor (LIF), Relapsing-remitting multiple sclerosis (RRMS)

2639 P

Thymectomy as a promising treatment for patients with Multiple Sclerosis (MS) and Myasthenia Gravis (MG)

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Background: Multiple sclerosis the most common chronic autoimmune neuromuscular disease with prevalence rates that has increased in Iran especially in Isfahan population. In MS, there is a coordinated attack of innate and adaptive immune cell against central nervous system (CNS).Concurrence of autoimmune disorders is prevalent, i.e concurrence of MS and MG. MS could be associated with MG although they have different target organs. The aim of this study is to assess effect of thymectomyas a proper treatment for both MS and MG. **Methods:** We studied patients who were referred to MS clinic with the diagnosis of definite MS and MG made by a neurologist according to Isfahan MS Clinics, Isfahan, Iran (2010-2013).Concurrence of MS and MG was seen in 5 of them.Age, sex, family and medical history, general neurologic symptoms and physical examination in all patients were recorded. We analyzed the clinical, laboratory, and Brain MRI findings of patients with MG and MS in an attempt to identify parameters.**R**esults**:** Out of 3920 potentially relevant studies, we surveyed 5 (0.1%) patients who had both MS and MG. One of them had SPMS and the others had RRMS. All of them experienced thymectomy operation and four(80%) of them completely improved after thymectomy, none of symptoms of diseases were seen. Almost all of patients completely improved after thymus removal. **Conclusion:** We suggest thymectomy could be a valuable therapy for MS/ MG patients. But more research should be done on this issue.
Keywords: Multiple Sclerosis, Myasthenia Gravis, Thymectomy.

2760 P

Immunomodulatory effects of Novel synthetic 2-Amino-4-hydrochromen derivatives from α-Naphthol on growth of peripheralblood mononuclear cells with Anti-Tumor Activity

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Background: Benzopyrones are a group of compounds including coumarins and chromenes, which have shown anti-tumor and anti-HIV activity as well as estrogen antagonist function. We aimed at evaluation of therapeutic importance of some new synthetic chromene derivatives. This study investigated the effects of some new synthetic derivatives on growth peripheral

blood mononuclear cells (PBMCs) from healthy men and on growth inhibition A549 a lung carcinoma Interestingly, the chromene compounds modulated the pattern of cytokine expression in PHA-stimulated PBMC.It can be concluded that these new synthetic derivatives exert potent anti-tumor effects, beside cytokine modulation function. **Methods:** This study investigated the potential of new synthetic derivatives on blood-isolated PBMC from healthy people and growth inhibition of A549 cells (12, 24, 48h & 50, 250, 500, 1000 nM).Microtiter analysis for cell growth, cytotoxicity assay by lactate dehydrogenase. And ELISA for immune reactions were performed**. Results:** Data indicated that some of the compounds exert potent inhibitory effects on growth of A549 cell line (500 nM, 24h), with no considerable cytotoxic effect on PBMCs (24h).**Conclusion:** These studies indicated that the new synthetic derivatives exert potent anti-tumor effects, beside immunomodulatory function.

Keywords: Synthetic chromene derivatives, Immune modualation , PBMC , A549

2931 P

Plasma level of brain-derived neurotrophic factorin Relapsing Remitting multiple sclerosis patients

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Background: Brain-derived neurotrophic factor (BDNF) is thought to play an important role in neuronal repair and plasticity. Recent experimental evidence suggests neuroprotective effects of this protein in multiple sclerosis (MS). We investigated the BDNF plasma concentrations in RRMS patients and healthy subjects. **Methods:** In a case-control study, plasma was collected from healthy subjects as control group (n=43) and patients with relapsing remitting multiple sclerosis (RRMS) (n=45) including patients treated with Interferon beta (INFβ) (n=32) and whom without treatment (n=13). The plasma levels of BDNF were assessed by ELISA method. **Results:** Plasma level of BDNF in MS patients was significantly decreased compared to control subjects (*p*=0.046). We found the significant relationship between BDNF plasma levels and MS patients in comparison with healthy subjects (374.79±14.63 pg/ml versus 437.03±27.43 pg/ml respectively). Also BDNF plasma levels of RRMS patients treated with INFβ was equal to the control subjects and was not influenced by Interferon beta therapy. **Conclusion:** Our findings revealed the reduced BDNF level in patients group in comparison with healthy subjects. These results demonstrated the importance of BDNF during autoimmune disease in human which may suggested the suppressive or therapeutic role of BDNF on neuroinflammatory disease.

Keywords: Relapsing Remitting Multiple sclerosis (RRMS)**,**Brain-derived neurotrophic factor (BDNF)**,**neuroprotective.

3035 P

Association of plasma Osteopontin (OPN) levels and gene variant in patients with relapsing-remitting multiple sclerosis (RRMS)

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Background: Multiple sclerosis (MS) is a chronic neuroinflammatory disease identified by demyelination of central nervous system (CNS). Osteopontin is a proinflammatory molecule, modulating TH1 and TH17 responses. Several reports suggest its involvement in multiple sclerosis (MS) pathogenesis. **Methods:** In a case-control study, plasma and DNA were collected from healthy subjects as control group (n=45 for ELISA and n=104 for HRM-Real Time PCR) and patients with relapsing remitting multiple sclerosis (RRMS) (n=45& n=117 for ELISA and HRM-Real Time PCR respectively), synchronized age, sex and race. Statistical analyses were performed in order to assess the correlation between OPN plasma level and gene variant (rs186640706) in patients and healthy subjects. **Results:** A significant difference between OPN plasma levels and genetic variant (rs186640706) in RRMS patients in comparison with healthy subjects was observed (*P*-value<0.05).**Conclusion:** This study provided a distinguishing clarification between plasma levels and genetic variation of OPN between investigated groups and reveals the possibility role of disease progression in MS patients. Thus, further studies are required for investigation of MS and its state of curing.

Keywords: Multiple Sclerosis, Osteopontin, Plasma, Single Nucleotide Polymorphism (SNP).

3090 P

The association ofgenetic polymorphisms in Methionine tetra hydrofolate Reductase (MTHFR), cystathionine beta synthase (CBS), and Methionine synthase (MTR) genesand risk of prostate cancer

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Background: It is well known that hereditary factors contribute to prostate cancer susceptibility. Prostate cancer is a form of cancer that develops in the prostate, a gland in the male reproductive system. Studies have shown that genetic variation changes in genes MTHFR, MTR, CBS may cause prostate cancer. **Methods:** A case– control study was designed to examine the genetic polymorphisms relation of MTHFR (MTHFR C677T), CBS (CBS 844ins68), MTR (MTR A2756G) genes and genetic susceptibility to the risk of prostate cancer. HinfI, BSRI, and HeaIII enzymes were used for detecting the genetic variation of MTHFR, CBS, and MTR respectively. Fifty-six cases with histologically proven prostate cancer and 75 age matched healthy controls were recruited for this study. The genotypes were determined by PCR-RLFP. **Results:** Our results shown the association of MTHFRC677T and CBS844ins68 genetic polymorphisms in patients groups compared with healthy subjects (*P*-value>0.000), but in MTRA2756G we

did not observe any association of gene variants within both groups (*P*-value<0.05). We also assessed the correlation of demographic characteristic of the investigated subjects (e.g. age, having family history of prostate cancer, and PSA level) with genetic variation on three genes, and we did not observe any significant relationship. **Conclusions:** Our results indicated that MTHFR C677T and CBS 844ins68polymorphism in MTHFR and CBS geneshave associated with the development and clinical features of Prostate cancer. These important candidate genes warrant further large scale studies in different ethnicities to better elucidate its role in prostate carcinogenesis.

Keywords: Prostate cancer, Single nucleotide polymorphism (SNP), Methionine tetra hydrofolate Reductase (MTHFR), Cystathionine beta synthase (CBS), Methionine synthase (MTR)

3264 P

Asthma control among patients in Saghez, Kurdistan, Iran

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Background: The prevalence of asthma is increasing in the developing world. Asthma, management guidelines have been instituted to provide recommendations for the optimal control of asthma. This study evaluated the current level of asthma control as reported by the patients, which may be a reflection of adherence to guidelines. **Methods:** Asthma patients referred to a respiratory diseases clinic were recruited for the study. The asthma control test (ACT) was administered on the patients and data were also obtained for medication use and disease monitoring. A total ACT score of <20 signified poor control. **Results:** Out of 87 patients, 80 completed the survey (91.9%). The average age of patients was 46 ± 18 years (mean ± standard deviation). The average ACT score was 14.4 ± 4.8 79.9% of patients had poor control. 63.1% of patients who perceived their asthma to be well or totally controlled were objectively assessed to be poorly controlled. More than half of the patients used short acting a2 agonist alone and only 20% used inhaled corticosteroids for maintenance therapy. Thirty five patients made unscheduled emergency room visits in the past 12 months and 69.8% could not use their inhaler devises well. Emergency room visits (odds ratio [OR] 9.5) and poor inhaler technique (OR 18.9) was independent predictors of poor asthma control. **Conclusion:** The current level of asthma control among patients in saghez is below guideline recommendations. Management of patients did not appear to follow guideline recommendations and patients tend to over−estimate their disease control.

Keywords: Asthma, Control, guidelines, Recommendations, Saghez

Late Abstracts

Poster Presentations:

3383P

A Homozygous mutation (R35Q) of CARD9: a 25 year-old man with intractable colitis

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Background: The dectin-1/CARD9 recognition pathway seems to be of high importance for mucosal antifungal host defense, whereas systemic immunity to Candida largely relies on alternative immune recognition pathways. **Methods:** We report a 25 year-old man with five-year intractable colitis despite of multiple courses of different medications. He has a history of nocardial brain abscess followed by invasive sinusitis with Nocardia during childhood. **Results:** The histopathologic study and culture of colon specimen were compatible with ulcerative colitis and invasive candida infection. A complete immunologic evaluation was unremarkable. Genetic analysis revealed a homozygous mutation (R35Q) of CARD 9 (caspase recruitment domain family member 9). After a modest recovery, during two years of itraconazole, blood transfusion was required frequently due to profound anemia. Another colonoscopy was performed before colectomy. The tissue culture reported to be Candida glabrata, thus caspofungin was initiated. After two weeks, complete recovery was observed. **Conclusion:** The interesting findings in this case are unusual presentations of candida infection in a CARD-9 deficient patient consisting of brain abscess, destructive sinusitis and finally chronic ulcerative destructive pancolitis without any history of any oro-esophageal involvement, which seems to be a unique case in the literature. In addition, intractable colitis non-responding to several courses of treatment with amphotericin B and different azoles was another perplexing finding, which resulted in isolation of naturally resistant candida species to azoles. We conclude to consider investigation for dectin-1 and CARD-9 deficiency in chronic invasive candida infection and identify the candida subspecies in these patients to optimize the correct treatment.

Keyword: dectin-1/CARD9, intractable colitis, mutation

3384P

Primary Immune Deficiencies in Adulthood: Fifteen Years of Experience from Iran

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Background: Primary immunodeficiencies (PIDs) are not solely diseases of childhood. We describe the clinical presentation and outcome for 91 adult patients with previously unrecognized PIDs. **Methods:** All patients more than 15 years old diagnosed as PID between

October 1998 and September 2012 in NRITLD were enrolled. Secondary causes of immune deficiency were excluded. The diagnosis was confirmed by standard immunologic analysis. **Results:** Ninety-one Iranian patientswith a mean age of 29±12 (16-69) were identified. The frequency of humoral, cell-mediated, phagocytic and complement defects were 53.8, 11, 34 and 1.1%, respectively. Overall, the most common disorders were common variable immune deficiency (CVID, 43 cases) and chronic granulomatous disease (CGD, 25 cases). Fourteen patients (including five and four patients with CVID and CGD, respectively) died during follow-up. Respiratory infections were the most common complication followed by involvements of gastrointestinal tract, skin, lymph node, liver, bone and central nervous system. Some other special findings were spindle cell pseudo-tumor in an interleukin 12-receptor (IL-12-R) deficient patient, IL-12** P40 deficiency with recurrent Salmonella infection and disseminated BCG, IL12R deficiency with MDR-TB, IL12R deficiency with chylous peritonitis and obliterating bronchiolitis and psoriasis in a CGD patient. Only 4% of patients had definite diagnosis in childhood, 31% had adult onset symptoms, and 65% were undiagnosed as PID despite the presence of recurrent complications since childhood. **Conclusion:** This series provides unique data regarding PIDs presenting in adulthood, and serves as a timely reminder that physicians must consider the diagnosis of PIDs in their adult patients with recurrent, unusual, chronic and opportunistic infections; unusual autoimmune disorders, some kinds of malignancies and granulomatous diseases. In Iranian registry of PID, 930 cases have registered during 25 years (1981-2006); hence, 91cases of adult PID during 15 years is considerable. It seems, this is the first case series of PID in adults but certainly underestimated, much more work and attention needed.

Keyword: Primary immunodeficiencies, adulthood.

3448P

Cost effective in-vitro propagation of ornamental plants and evaluation of antimicrobial activities against *staphylococci*

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Tissue culturing is extensively used as a successful technique for the propagation of numerous plants. This venture is focused on the propagation of ornamental plants by using simple and low estimated media not including any additional adjuvant. *Ixora coccinea*, *Draceana reflexa* (Song of India), *Alternanthera* (Purple Knight), Kiwi (*Actinidia deliciosa*) and *Cestrum Nocturnum* (Raat Ki Rani) were subjected for the initiation. Among five plants, three plants successfully showed the results while one of them did not show positive results. *Draceana*, *Alternanthera*, *Ixora* successfully nurture in the lab condition while Kiwi and *Cestrum Nocturnum* were unsuccessful to grow in the medium.The successfully propagated ornamental plants (*Ixoracoccinea, Draceana Reflexa* (Song of India), *Alternanthera* (Purple Knight) were then tested for the antimicrobial activity. The leaves aqueous and chloroform extracts showed potential antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The most effective extract which showed 100% efficacy was the aqueous extract of *Ixora coccineae* against *Staphylococcus aureus* whereas the less effective extract among all was the aqueous extract of *Draceana*. Five different antibiotics discs were also tested in comparison to the ornamental plants. Such a cocktail may reduce the threat of rapid resistance development

against several antibiotics.
Keywords: Kiwi *(Actinidiadeliciosa), Alternanthera (*Purple Knight*), Dracaena reflexa (*song of India), *Ixora coccinea*, *Cestrum nocturnum*, MS Medium

3372P
The immunological and neuroimmunological mechanisms of traumatic brain injury
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Background: Cortical spreading depression (CSD) are associated with dramatic failure of brain ion homeostasis, efflux of excitatory amino acids from nerve cells, increased energy metabolism and changes in cerebral blood flow (CBF). There is strong clinical and experimental evidence to suggest that CSD is involved in the mechanism of migraine, stroke, subarachnoid hemorrhage and traumatic brain injury. Therefore, in the present study, we used the spreading depression model to investigate the effects of repeated spreading depression on peripheral and central adaptive immune responses. Moreover, we studied the effect of repetitive SD on dark neurondensityand expression of $GABA_A$α and β, receptors. The results of the present study demonstrate that repeated spreading depression in rats induced elevated lymphocyte proliferation, IFN-γ, pro and anti-inflammatory cytokines in peripheral and central levels. Brain assays also demonstrated reduced alterations in GABAA α and β, GAD and HSP70 expression and enhanced the number of dark neurons. The findings could help to explain the interrelatedness of adaptive immunity, peripheral inflammation, and traumatic brain injury.
Keywords: Neuroimmunological response, Cytokine, Spreading depression, Wistar rats.

3413P
Evaluation of antiapoptotic effect of Neuron Regeneration Peptides 2945 on Epileptic rats
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Background: Delayed neuronal death after seizure attackmay be mediatedby the induction of apoptosis-pathway. Caspase-3, a mammalian cysteine protease, promotes apoptosis after some neurological disorders.Neuron Regeneration Peptides (NRPs) are small synthetic peptides that stimulate neural proliferation, migration, and differentiation with no apparent toxicity and high target specificity in CNS.The aim of this study was to investigate the effect of NRP2945 on the apoptosisafter seizure activity induced by pentylenetetrazol (PTZ) in rats. **Methods:** The effects of different concentrations of NRP2945 (5 and 20µg/kg) were tested onexpression of caspase-3 protein in the temporalcortex and hippocampal area after seizure induction. In addition, the number ofterminal deoxynucleotidyltransferase-mediated dUTP nick end-labelling-positive neurons in the hippocampus and temporal cortexwas investigatedafter NRP2945 application in epileptic rats. **Results:** Application ofNRP2945 at 5 and 20µg/kg decreased the expression of caspase-3 protein in the CA1 and CA3 hippocampal areas and the

temporal cortex. In addition, application ofNRP2945 at 5 and 20µg/kg reduced the number of apoptotic neurons in the both temporal cortex and hippocampal area. **Conclusion:** This study indicates that NRP2945 is able to prevent the neuronal apoptosis induced by PTZ by suppressing of caspase-3 protease. Further studies are needed to elucidate the potential role of NRP2945 as an antiapoptotic drug.

Keywords: Brain, Epilepsy, NRP. Hippocampus

3418P

Effective individual and social factors to delay vaccination children less than 18 month

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Background: Immunization is most important cost-benefit factor to decrease vaccine preventable Disease. Vaccination versus common infectious disease cause to decrease children mortality in recent decades and cause public health increased and this subject is more important in infants whose are sensitive and their immunity system are not complete. This study was designed to determine the causes of delayed immunization of children fewer than 18 months of Khorramabad in 2011. **Methods:** this study was cross-sectional, in khorramabad 2011 year. Information Gathered from mothers who have children under 18 years old in way structured interview with self-organized questionnaire which validity and Reliability confirmed. **Results:** Mean of maternal age was 28.9 ±5.3 years and the mean of children age was 11.24 ± 6.11months and the mean delay was 18.2 ± 40.1days..Most delay implies were no time with 23.8% (48 people) and disease for children with 21/8% (44 people). Based on Pearson correlation and regression tests, there are significant relations between maternal age and number of children in family; it is meaning that with increase maternal age and number of children, delay length increased. **Conclusions:** harmful effects derived from delay vaccination in children should be attended by managers by considering Results in this study and others. More attempts to education and decrease delay time vaccination suggested.

Keywords: Immunization, children, maternal, vaccine, khorramabad

3405P

Immunogenic study of infectious pancreatic necrosis virus (IPNV) VP2–VP3 fusion protein from aquatic birnavirus

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Background: Infectious pancreatic necrosis virus (IPNV) is a member of the family

Birnaviridae that has been linked to high mortality and causes serious economic losses to salmonid aquaculture. **Methods**: The Iranian IPNV isolate was propagated in CHSE cells, and the virus was collected after the second passage in CHSE-214 cells. The coding regions for the major epitopes of structural protein VP2 and structural protein VP3 were amplified from the segment A of the genome of infectious pancreatic necrosis virus (IPNV) and were expressed in Escherichia coli in an effort to develop a vaccine for IPNV in fish. In this study an expression vector was constructed for expression viral protein VP2-VP3 fusion g. The designed vector was constructed based upon PET26b (+) with T7 promoter. A fragment containing the full length of the VP2 and VP3 gene of Iranian Sp strain (NCBI: KC489465) and VP3 gene (NCBI: KC489466) was amplified by polymeras chain reaction (PCR) using genomic RNA of IPNV as template. IPNV VP2–VP3 fusion gene was constructed by splicing overlap extension (SOE) PCR and The ligated DNA was then transformed into *E.coli* BL21.Subsequently, juvenile rainbow trouts were inoculated with the recombinant strains via injection route. **Results and Conclusion:** Our results demonstrated thatEscherichia coli-expressed rVP2-VP3, increased resistance against IPN infection was demonstrated by challenge.

Keywords: Infectious Pancreatic Necrosis Virus, *Escherichia coli*, Vaccine

3388P

PCR assays for the identification of *Hysterothylacium Sp.* with zoonotic potential and hidden allergens in fish

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Background: Hypersensitivity to the allergens of Hysterothylacium sp. is common in patients who haveurticaria/angioedema, anaphylaxis, or both after the ingestion of fish and who have negative skin test responses to fish proteins. **Methods:** A total of 30lizardfish*(Sauridatumbil*) ranging in length from 27- 40 cm and 30 Pink perch (*Nemipterus japonicas*) ranging in length from 21- 30 cm were examined from different ports of Persian Gulf by standard method. Individual anisakid larvae were repeatedly washed in physiological saline (pH 7.3) and identified to species or generic level based on the host andtissue from which they were derived, and the morphology of the parasites.In order to evaluate the quality of the anisakid samples, PCR was used to amplify the entire ITS fragment (including ITS-1, 5.8S, ITS-2) with primers NC5 (forward: 5′GTAGGTGAACCTGCGGAAGGATCATT3′) and NC2 (re verse:5′TTAGTTTCTTTTCCTCCGCT-3′) and specific primer for *Hysterothylacium sp.* **Results and conclusion:** The anisakids found were identified as the third larval stage (L3) of *Hysterothylaciumaduncum*, with a total prevalence of 98% and 100% in *Nemipterusjaponicas* and*Sauridatumbil*respectively.*Hysterothylaciumaduncum*, is the main fish parasites in the two species of fish and it is causing allergic symptoms after fish consumption.

Keywords:

3434P

Comparing Atorvastatin, Simvastatin and lovastatin effect on theserum-Interleukin 6 level In patients with chronic renal failure

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Background: Statins are the most important group of cholesterol lowering drug and are effective in treatment of hyperlipidemia specially on CRF Patients. Statins effect on lowering the inflammatory mediators like Hs –CRP and IL_6 is controversy.Elevating of serum inflammatory mediators which is seen in half of CRF patients is effective in atherosclesis exacerbation and increasing the risk of vascular discases like MI. Statins effect on above variants(IL_6 -and Hs CRP) were assessed in this study. **Materials and Methods:** 138 from CKD (in stage V) patients of Kashan treatment centers; 101 Patients were intered to study and 95 patients resided to and of study. Patients werer allocated in 3 equal groups: a group of 32 patients received simvastatin 20 mg daily second group of 32 patients received lovastatin 40 mg daily and third group of 31 patients received Atorvastatin 10mg daily during two months. Morning blood samples were collected before and after treatment to evaluate the above cases it was used for serum IL -6 level measuring from American Kit Human BMS 313/2 . Data were analyzed with SPSS computer program and statisticed tests for example Pired T test and ANOVA. **Results**: serum IL -6 elevations was seen in 43.2% (41 patients) and HS-CRP elevation is 63.2% (60patients) of total ones before treatment. This result after treatment were respectively 46.3 (44 patients) and 51.6 % (49 patients). serum IL -6 level befor treatment with Atorvastatin , sim vastatin and lovastatin was respectively 2.14 , 1.76 and 1.73 pg/ml and after trietment was 2.06 , 2/22 and 2/18 pg/ml. Atorvastatin was decreased IL -6 level 0.08 pg/ml (pv<0.18 , 1.94) simvastatin and Atovvastatin had not effect on serum IL _ 6 level It was shown that every 3 drug were able to lowering HS- CRP level. Atorvastatim effect was prominent (before 24.4 after 14.2, pv<0.001) and lovuastatin was in the second level (before 14.6 after 8.1, pv<0.02) CRP lowering was not significant in simvastatin group (before 13.6 after 10.2mg/l,pv<0.14). SEM for CRP befor and after was respectively 2.8, 3.7 and 2.02. Atorvastatin and specially simvastatin effect on total cholesterol and LDL, HDL (in decreasing direction) was reported meaning full. LDL decreasing with simvastatin and Atorvastatin was respectively 20.8 (p<0/001) and 12.9 mg/dl (p<0/01) lovstatin had not meaning effect on LDL (decreased 5.2 mg/dl) . Moreover the total cholesterol was decreasted from7.4%-19.6%. In this case, simvastatin(pv<0.0001)was more effective than atorvastatin(pv<0. 01) lovstatin had the least effect.< pv (0/035)TG decreasing was not significant. (with simvastatin meaningfull)< p0/02,dereased 13.2dl/mg).The above treatment didn›t have meaning full Effect on Creatinin GFR and BMl. It was seen briefly increase in CPK in atorvastatin group and ALT in simvastatin group; <p (0/03)but climical myoitis or hepatitis was not seen. **Conclusion**: Protective effect statins on CRF is from pthway lowering of serum lipid including LDL, total cholesterol, and e in flammation including IL_6 and CRP Anti in flammatory atorvastatin and lovastatin effect is more than simvastation, although this effect was by CRP lowering, without meaning fulleffect on IL-6.

Keywords: Simvastatin, lovastatin, serum-Interleukin 6, Atorvastatin

3373P

A case presentation: A 22 years old boy suspected to Neutrophil specific granule deficiency

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Neutrophil specific granule deficiency (SGD) is a rare congenital disorder. The neutrophils of these patients display atypical bilobed nuclei; lack expression of at least one primary and all secondary and tertiary granule proteins; and possess defects in chemotaxis, disaggregation, receptor upregulation, and bactericidal activity. SGD patients suffer frequent and severe bacterial infections. Although the first of five patients worldwide was reported in the early 1970s, the molecular basis for the defect was discovered only recently. The case is a 22 years old boy and history of recurrent generalized abscess with positive cultures for Staphylococcus Aurous. Many investigations performed for him and all of arms of immune system seem normal except that he had a lower normal limit of chemotaxis. In more investigation we could find about more than 90% of neutrophils as bilobed and also we couldn't find any granule in cytoplasm of neutrophils by electron microscope. So the most compatible diagnosis for his disease is Specific granule Deficiency.

Keywords: *specific granule deficiency, bilobed neutrophils, recurrent abscess*

3392P

The study of seroepidemiology of IgG against Helicobacter Pylori in patients referred to the central laboratory in the city of Zabol during the first 11 months of 1392

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Background: Helicobacter Pylori is the main cause of ulcers and inflammation in stomach. One of the antibodies that is produced against it is from IgG class which its titer is important in the disease process and diagnosis of previous patients.Awareness of prevalence of this disease can help to prevent and treat it. **Method**: In this study we investigated the test results of 618 patients who referred to the laboratory and after blood collection and separation of serum, Helicobacter Pylori IgG antibody test with Elisa method was performed on them. **Results**: From the total number of patients 74/12 % (458 persons) were women and 25/88 % (160 persons) were men. The test of 81/22 % (502 persons)were positive which the percentage of positive cases in women were 81/65 % and in men were 80 %. **Conclusion**: The result of this study showed that in this region infection of women to the Helicobacter Pylori is far more than men.

Keywords: Helicobacter Pylori, IgG, Zabol

3409P

Association of Siglec-8 Single Nucleotide Gene Polymorphisms (SNPs) with Allergic Asthma

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Background: Asthma is a chronic inflammatory disease of the airways in which eosinophils, mast cells and basophiles play major roles. Siglec-8 is a newly discovered inhibitory receptor on these cells, up on binding to yet unknown ligand delivers signals induce apoptosis. We aimed in this study to investigate single nucleotide polymorphisms (SNPs) of siglec-8 in allergic asthma patients. **Methods:** 194 patients with allergic asthma and 190 normal subjects were enrolled in this study. DNAs were extracted using salting out method and the frequencies of genotypes and alleles of siglec-8 gene rs36498, rs36491and rs10409962 were determined using by PCR-Single Strand Conformation polymorphism (SSCP) method. Different electrophoretic patterns were seen after polyacrylamide gel electrophoresis (PAGE) and silver nitrate staining, and exact genotype of each pattern was determined by sequencing. Statistical analyses were made by STATA 11. **Results:** rs36498 showed significant association with allergic asthma (p=0.022) and T allele was found as protective allele (OR=0.61, p=0.008). There was no association between rs10409962 and allergic asthma (p=0.127, p=0.18), and rs36491 was not polymorphic in both patients and control groups. **Conclusion:** Our results showed significant statistical association of rs36498 located in promoter region of siglec-8. This polymorphism is thought to influence the expression level of siglec-8 and protective T allele could be considered to elevate surface expression of molecule which in turn leads to increased apoptosis of effector cells and lower probability of allergic asthma. Siglec-8 could be potential new therapeutic target for clinical conditions in which eosinophils play major role.

Keywords: Asthma, Siglec-8, SNP, PCR-SSCP

3390P

The effect of a selected exercise on Interferon-Gamma and body composition,of kidney transplant patients

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Background: Kidney transplant is the selected treatment of chronic kidneydisease and it improves life quantity and quality. Kidney transplant patients are exposed to a lot of different infectious on diseases because of long usage of suppressing immune system drugs and Because exercise has an important effects on regulating and decreasing the Inflammatory cytokine the purpose of this study was investigating the effects of 10 weeks selective exercise IFN-, Percent Body Fat and Body Mass Index in kidney transplant patients. **Methods:** 44 Kidney transplant patients voluntarily and objectively were selected and were divided to two groups of control (n=15) and (exercise=29) randomly. Exercise groups participated in training program for 10 weeks, three days a week each day 60 – 90 minutes. During this time the controlled group did not participate in any regular exercise. The blood samples were taken before and after 10 weeks. Data were analyzed using t dependent and independent test. **Results and Conclusion:** According to the result of this study 10 weeks of selected exercise with the present study features cause fat and obesity control of kidney transplant patients and it doesn't have any effects on IFN-γ production.

Keywords: Interferon-Gamma, Physical Training, Body Composition, kidney transplant patients

3377P

Immunohistochemical Assessment of Vaniloid Receptor Type 1 in Epileptic Rat in CA3 pyramidal cells

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Introduction: Temporal lobe epilepsy (periodic and unpredictable occurrence of seizures) is a particularly devastating form of human epilepsy. Elucidating the mechanisms of epileptogenesis could provide novel therapeutic approaches aimed at the prevention or management of the disease. Transient receptor potential vanilloid 1 (TRPV1) belongs to a family of ligand gated ion channels and permeable to Na^+, K^+, and highly Ca^{2+} ions. In this study we investigated changes to immunoreactivity and electrophysilogical of TRPV1 channels in temporal lobe epilepsy model in CA3. **Methods**: Male rats (100±10 g) were received methylscopolamine (1 mg/kg/i.p) 30 min prior to injection of pilocarpine to reduce the peripheral cholinergic effects of the pilocarpine. Rats were then recieved a single dose of pilocarpine hydrochloride (380 mg/kg, i.p.). Rats experienced status epilepticus within 2 h following pilocarpine injection were included as epileptic animals. Control rats were age-matched with treated animals. For immunoreactivity assessment, after 3 months the animals were deeply anesthetized and perfused transcardially with normal saline followed by 1% paraformaldehide. Brains were removed from the skull, fixed and coronal paraffin embedded sections were cut at 8µm-thick and collected on slides. Immunofluorescent has been performed to assess the percentage of CA3 pyramidal neurons expressing TRPV1. **Results**: Our results showed TRPV1 immunoreactivity was increased in CA3 after 3 month (61.14±3.1, $p<0.001$) in pilocarpine treated rats compared to control aged matched (25.32±1.2). **Conclusion**: This study indicates that TRPV1 channels potentially have role in epileptogenesis and functional significance of these receptors in this disorder should be studied.

Keywords: hippocampus, epilepsy, TRPV1

3415P

MiR-21 transfection can differentiate human naïve T cells to regulatory T cells

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Background: Regulatory T cells are important players of the immune system that down regulate the immune responses. Tregs are considered the main mediators of peripheral tolerance. Their mechanisms of action and clinical applications are subjects of considerable debate currently. These cells are involved in preventing autoimmunity and defective development and function of these cells can result in autoimmune disease. Increasing evidence supports the role of miR-21 in the regulation of Foxp3 expression in Treg cells. In this research we tried to clarify whether miR-21 transfection to naïve CD4+ T cells is useful in the generation of iTregs in vitro. **Methods:** We separated naïve T cells using negative isolation magnetic kit and cultured them in the presence of anti-CD3, anti-CD28 and Treg polarizing cytokines or miR-21 transfection. **Results:** We investigated in vitro differentiation of miR-21- transfected naïve

CD4+ T cells to iTregs and then compared these iTregs to cytokine-differentiated iTregs and negative controls. We showed that expression of Foxp3, TGF-b, and IL-10 are increased in miR-21 transfected naïve T cells in comparison to cytokine-differentiated iTregs and negative controls. **Conclusion:** Our data demonstrate that miR-21 is effective in the in vitro differentiation of naïve T cells to iTregs.

Keywords: miR-21, naïve CD4+ T cell, iTreg

3453P

Assessment of vitamin D effects on Treg cells and Foxp3 molecule in lupus- like syndrome induced mice model

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease which is characterized by auto-reactive T cell and anti-ds DNA antibodies. Treg cells are crucial for tolerance and reduced numbers of Treg cells exist in patients with lupus. Vitamin D has immunomedulatory effect on the immune system and recently has received worldwide attention. In the present study we studied vitamin D effects on Treg cells and Foxp3 molecule in lupus-like induce mice model. **Methods:** Female Balb/c mice were divided in four groups: Group one: Lupu-like disease was induced with activated chromatin; Group two: Two weeks of treatment with vitamin D and then lupus-like disease disease was induced with activated chromatin; Group three: injected with non activated chromatin as a control; Group four: injected with PBS and Ferunds adjuvant as another. Four mice from group one were treated with vitamin D for two weeks after establishing the disease. These mice were considered as group five. On the 10^{th} week of disease spleens were extracted for assessment of Treg cells by the flowcytometery and cytokine genes expression. **Results:** The vitamin D effects were associated with increased expression level of Foxp3 gene. We found that vitamin D can increase the number of Treg cells in lupus-like induced mice model. **Conclusion:** Vitamin D two weeks before lupus induction increased Foxp3 molecule and the number of Treg cells. Vitamin D after lupus induction caused any significant effect. These findings suggested that vitamin D as a prophylactic agent would benefit on SLE disease.

Keywords: Lupus, Balb/c, Mice model, Treg cells, Vitamin D

3430P

Evalution Activity of silver nanoparticles synthesized using (lavandulifilia) extract Mountain north -east of Iran (esfarayen)

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Background: It is a perennial plant that mountain lavandulifilia and a height of 20 to 50 cm thick bushes. nanoparticles synthesized by Chemically methods usually result in some retrntion

of toxic and nano particles in environmental application. This study is applied with use of plant extracts resulted from metal nanoparticales with green chemistry techniques. **Methods:** Making phytochemical properties of silver nanoparticles using (lavandulifilia) extract and using it is as reducing agent to form silver nanoparticles in. During nanoparticle Aqueous medium containing silver nanoparticles can show its power. at a wavelength of 458 nm which is ralated the silver plasmon absorption is rapidly synthesis nanoparticles. morphology and size the silver nanoparticles by transmission electron microscope tem and sem was use by scanning. **Results and Conclusion:** The purpose of this research is to determine the amount of silver nanoparticles tend (lavandulifilia)and synthesis in which silver nanoparticles were detected by using transmission electron microscopy(TEM) and scanning electron microscopy (SEM) after synthesis. as results of good regenerative power produced by (lavandulifilia) extract,silver nanoparticles were synthesized with dimension 100-1 nm.

Keywords: Green chemistry, silver nanoparticles, lavandulifilia

3443P

Effect FIET (Futsal Intermittent Endurance Test) on humoral immune system in futsal players

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Background: Humoral immune dependent intensity, duration and type of exercise, therefore the aim of this study was to evaluate the Effect of a new futsal intermittent endurance test(FIET) on the humoral immune system is futsal players. **Methods:** 18 Football players selected and randomly divided into two experimental groups (n = 7) and control group (n = 7).Futsal Players who are constantly involved in training futsal team. Mean age, weight and height for the experimental group respectively (24.19 years), (64.8 kg), (179.3 cm) and For the control group respectively (22.4 years), (71.4 kg), (181.6 cm). **Results:** The results showed Futsal intermittent endurance test was significant increase in the number of leukocytes, neutrophils and immunoglobulins ($P<0.05$). **Conclusion:** It seems, Futsal intermittent endurance test the stress is not a factor for immune suppression for football player and repeat this exercise,Strengthen the immune system and to improve the performance of the players during practice.

Keywords: FIET, Immune system, Exercise, Futsal

3451P

Demography and seroprevalence of HTLV-1 infection in blood donors in mashhad from 2006 to 2010

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Background: Human T-Lymphotropic Virus (HTLV), a virus from Retroviruses family, is the main factor of Adult T Lymphocytic Leukemia (ATL) and Tropical Spastic Paraprasia (TSP). As blood transfusion is one of the major HTLV transmission ways and the city of Mashad is a

HTLV endemic region in our country, it seems necessary to investigate the HTLV1/2 infection prevalence in Mashad blood donation volunteers. **Methods:** In this descriptive study from 2006 to 2010. all the blood donation volunteers, who were eligible for blood donation but had HTLV serum positive results in both screening and confirmatory tests with Elisa and Western Blot method, respectively, were considered as HTLV infected individuals . The infected group was compared with a group of healthy blood donors as a witness group. The data were imported to SPSS17 software, the results were analyzed, and statistical comparisons were made using the Chi-square test. **Results:** Out of all 1443 infected donor, 1188 (% 82.3) were male and 255 (% 17.7) were female with the mean age 38.75±10.71 years old. The overall seroprevalence of HTLV-1 infection in female and male blood donors was 0.82% and 0.30%, respectively. It was a significant relation between age, sex, marital status, education levels and history of blood donation with HTLV seropositivity ($p< 0.001$). **Conclusions:** Due to the lower frequency of infection in blood donors and in those with higher education levels, the selection of blood donors from these populations is further considered by the blood transfusion centers. Since the prevalence of infection in the general population of Mashhad has not been declined and this infectious disease is still prevalent, therefore, increased public awareness of the modes of transmission and prevention, screening of pregnant women and advises to the infected mothers against prolonged breast-feeding the infants for the infection control, are recommended.

Keywords: HTLV-1, blood donors, prevalence, Mashad, Iran

3424P

Development avidity ELISA test for discriminating acute from chronic Toxoplasma infection using recombinant proteins

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Background: Primary infection with Toxoplasma in pregnant women can be transmitted to the fetus and cause chorioretinitis, neurologic defects, and death. In addition to primary infection with Toxoplasma IgM existence, Avidity Elisa should be done The total parasite antigen tests are used mainly disadvantages including: 1) non-uniform quality of the antigen, resulting in total failure of the standard kit,and2) the incidence of false results and high levels of borderline. In this study, two recombinant antigen for differentiation of acute and chronic Toxoplasma GRA6 and GRA7 were investigated. **Methods**: In this study, IgG Avidity ELISA method using two proteinsGRA7 and GRA6 to differentiate betweenacute and chronic Toxoplasma infection on 78 serum samples from pregnant women were studied. Recombinant proteins were purified by affinity chromatography. Using standard kits Euroimmun was divided into acute and chronic sera. Serum IgG and IgM were positive belonging to the infection index was down Avidity. Sera from chronic infection ,IgG positive, IgM were negative and the index had high Avidity. **Results**: 20 of 23 (87%) acute sera in the Avidity ELISA by GRA7 index Avidity were lower than of cut-off age and accordingly belonged to acute Toxoplasma infection. On the other hand, 26 of 26(100%) chronic sera in the Avidity ELISA by GRA7 index Avidity were higher than of cut-off age and accordingly belonged to chronic Toxoplasma infection. 20 acute sera in the Avidity Elisa by GRA6 index Avidity were lower than of cut-0ff age and 24 chronic sera in the Avidity ELISA by GRA6 index Avidity were higher than the cut-off. **conclusion**: The results

showed good efficiency of GRA6 and GRA7 recombinant proteins in distinguishing of acute toxoplasma infection than chronic by IgG avidity ELISA, specially in this test, performance of GRA7 better than of GRA6.Usingacombinationof recombinantantigensin Avidity ELISAmay be able toreplace thelysed whole-cellantigensparasites.
Keywords: Toxoplasmosis, Avidity, GRA6, GRA7

3414P
Short-term administration of 5-Florouracil enhances the efficacy of DC-based Cancer immunotherapy in melanoma model

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Background: Role of dendritic cells (DCs) in immunotherapy of cancer is of great importance due to its crucial effect in adaptive immune responses. It is shown that suppressor cells such as myeloid derived suppressor cells (MDSCs) interfere with DC-based vaccine immune responses. Recent studies demonstrated that 5-florouracil (5-Fu) selectively removes tumor-associated MDSCs. The aim of this study was to evaluate administration of 5-Fu on DC-based cancer immunotherapy. **Methods:** B16F10 cell line was used for tumor inoculation. C57/BL6 Mice were received two injections of 5-Fu (50mg/kg) after tumor inoculation. Bone marrow derived DCs were generated in the presence of recombinant mouse GM-CSF and IL-4. Tumor volume and overall survival were assayed after DCs administration. Splenocytes were evaluated for CD107 expression as means of cytotoxic activity of CTLs. **Results:** Our results showed that 5-Fu cooperates with DC vaccine to increase overall survival in tumor bearing mice and to decrease tumor volumes. In this study we demonstrated that combination of 5-Fu and DC vaccine results in increased CD8+/CD107+ T lymphocytes in the spleen of tumor-bearing mice compared with single injection of 5-Fu or DC vaccine. **Conclusion:** Based on our experiments short-term 5-Fu therapy in combination with DC vaccine increases anti-tumor immune response through strengthening cytotoxic T cell response.
Keywords: DC, MDSCs, 5-Fu, Tumor

3393P
Production of polyclonal antibody against recombinant growth hormone and designing an ELISA kit for measuring and comparing some of its diagnostics index that with commercial kit

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Background: Human growth hormone (hGH) is a secretory protein of the blood which is used extensively in the biotechnology and medical sciences. Recombinant human growth hormone production is still one of the priorities of the Iranian Ministry of Health. One of the sensitive and accurate techniques for the measurement of this hormone is the ELISA method. The scientists need to produce antibody against hGH to measure the serum level of hGH in the controls patients. The commercial foreign ELISA kits are commonly imported from other countries. Therefore, the current study aimed to produce a polyclonal antibody against hGH to make a domestic ELISA kit to measure this hormone. **Methods:** Rabbit polyclonal antibody against hGH was produced following 10 week of immunization by the antigen injection, The antibody characteristics were evaluated by the immunoblotting. The purified antibody was used to design the ELISA kit. Designed sandwich ELISA method was standardized concerning the amount of antibody coating and antibody conjugate. Ultimately, the sensitivity and specificity of the designed kit was compared with the foreign kits. **Results:** The polyclonal antibody against hGH was produced in rabbits and purified successfully. This anti body can be used in the western blot and ELISA methods. The diagnostic value of the designed kit was 98 percent, compared with the foreign kits. **Conclusion:** This kit has a diagnostic value in the assessment of hGH, therefore; it can be a good alternative to the commercial kits, so that with massive Production of this antibody and design of ELISA kit we can go ahead towards the self-sufficiency.

Keywords: growth hormone, polyclonal antibody, ELISA

3423p

Study of Plant Food Allergen Families in Allergen Sequence Databases

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Background: Plant food hypersensitivity is constantly increasing in the world. Also the incidences of plant food allergenic-induced anaphylactic reactions have increased significantly during later years. A plant food allergy is a reaction of Immunoglobulin E (IgE) to an allergen which is a protein or glycoprotein in plant food. Many plant food allergens are glycoproteins and their molecular weights range from 10 kD to 70 kD. The most common food allergies are: milk, eggs, peanuts, tree nuts, soy, wheat, fish and shellfish. Among plant food allergens; peanuts, soybeans, and tree nuts after ingestion have most severe allergic reactions. **Methods:** In this work, we studied Plant Food Allergen Families in Pfam and many of the allergen databases including; IUIS, AllAllergy, Allergome, InformAll, AllergoPharma, SDAP, EVALLER, ALLERDB, Biopep, Alegpred, FARRP, ADFS, and etc. **Results:** Based on our results, as of January 2014, there were 14831 recognized protein families in the Pfam database, yet only ~2% of those families are represented among allergens. Plant food allergens families include Prolamin superfamily, Cupin superfamily, Profilin, EF-hand domain, PR-10, Heveinlike domain, Class I chitinases, Oleosins, Beta-1,3-glucanase, Papainlike cysteine protease, Thaumatinlike protein, Expansin, Enolase. **Conclusion:** About 65% of the plant food allergens belong to three superfamilies including the prolamin superfamily, the cupin superfamily, and the pathogenesis-related proteins (PR-10) family.

Keywords: Plant, Food Allergen, Families, Sequence Databases

3425P

The Necessity for Improvement of the Algorithms Used for In Silico Allergenicity Assessment of Novel Proteins

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Background: Prevalence of allergic diseases is increasing worldwide, particularly in low and middle income countries. Also upsurge in the prevalence of allergies is observed as countries become more urbanized. Allergy is a global public health problem and greater efforts should be made towards its prevention and optimal treatment. Prediction and In Silico allergenicity assessment is very important for safety evaluation of foods, Protein-based therapeutics, and other uses of recombinant proteins. Several bioinformatics approaches have been developed for evaluation the potential allergenicity of recombinant proteins. **Methods:** In this investigation, we study approaches and algorithms used for In Silico allergenicity evaluation of novel proteins. Based on our results there are two types of In Silico-based allergenicity prediction. **Results:** Some allergen sequence databases such as SDAP, Allergenonline, Allermatch, ADFS, PSD, and AllerTool follow FAO/WHO guidelines and searches for primary structure of protein and sequence similarity. In this approach we achieve to high sensitivity (true positives/ (true positives + false negatives)) and many false positives and have low precision or positive predictive value (true positives/ (true positives + false positives)). Another approach includes identifying conserved allergenicity related motifs. Some allergen sequence databases such as AlgPred is a motif based server which combines four methods for motif search: Support Vector Machines (SVM), MEME/MAST, IgE epitopes and Allergen Representative Peptides (ARP). **Conclusion:** Deficiencies of the algorithms used for In Silico allergenicity evaluation of novel proteins are; 1) In both approaches allergenicity is assumed linearly and conformational epitopes are not considered. 2) Discovery of new allergen will be restricted by their lack of identity to known allergens. 3) 3D structure of proteins, the positions of IgE epitopes on surface of protein, and stability of IgE-Allergen complex that are important for molecular allergenicty mechanism are not considered. Thus current algorithms used for In Silico allergenicity assessment of novel proteins and FAO/WHO guidelines should be improved.

Keywords: Improvement, Algorithms, In Silico, Allergenicity

3426P

Allergenicity evaluation of 8 genes most introduced into crop plants; a bioinformatics approach

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Background: Eight genes widely introduced into crop plants are *CP4-EPSPS* derived from *Azetobacterium tumefaciens* sp. strain CP4, phosphinothricin acetyltransferase gene derived from *Streptomyces hygroscopicus* (*bar*) and *Streptomyces viridochromogenes* (*pat*), and *Cry1Ab, Cry1Ac, Cry1A(b/c), mCry3A, and Cry3Bb1* derived from *Bacillus thuringiensis*. CP4-EPSPS, bar, pat, Cry1Ab, Cry1Ac, Cry1A (b/c), mCry3A, and Cry3Bb1 proteins contain

455, 183, 183, 1155, 1178, 1176, 652, 589 amino acids respectively. **Methods:** This sequence was aligned using the FASTA35.04 program in the most specific allergen protein databases; FARRP, SDAP, Algpred, ADFS, Allermath, Allergome. Sequence alignment was implemented with the allergen proteins in three matches including: the full sequence matching sequence, matching the 80 amino acids and eight amino acids. **Results:** The results showed no similarity between CP4-EPSPS, bar, pat, Cry1Ab, Cry1Ac, Cry1A (b/c), mCry3A, and Cry3Bb1 proteins and allergen proteins in the full sequence matching, matching the 80 amino acid (Domain) and matching an 8 amino acid to determine the epitope potential. **Conclusion:** We conclude that CP4-EPSPS, bar, pat, Cry1Ab, Cry1Ac, Cry1A (b/c), mCry3A, and Cry3Bb1 proteins has non-allergenic potential.

Keywords: Allergenicity evaluation, Most introduced genes, Crop plants, Bioinformatics

3344p
Inflammatory cytokine detection in adenotonsill and peripheral blood mononuclear cells culture in adenotonsillectomy patients: a comparative study

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Background: Tonsils and adenoid hypertrophy is a major respiratory symptom in children which is partly due to recruitment of inflammatory cells in upper airway lymph nodes as a result of the effects of synthesis and release of different inflammatory cytokines. It seems that infections play role in concert with these cytokines leading to tonsilar hypertrophy and other pathologic consequences. It is proposed that cellular infiltrate of tonsils and adenoids may secrete different quantities of these cytokines compared with peripheral blood mononuclear cells (PBMC) cultures .**Methods:** Among patients who were admitted for adenotonsillectomy to the ENT ward. 37 patients, under 1-12years old patients with fulfill criteria selected to include the study. Excised adenoid and tonsils cultured and inflammatory cytokines Interferon-γ (INF-γ), Interlukine-1 (IL-1), IL-6, IL-8 and tumor necrosis factor-α (TNF-α) measured in cellular culture supernatant. The same cytokines measured in PBMC cultures . **Results:** The data shows that there is a significant difference between IFN-γ and IL-8 amounts in adenoid tissue culture supernatant and PBMC culture of our patients. Furthermore, the amounts of IFN-γ, IL-1 and IL-8showed considerable difference between tonsilar tissue culture supernatant and PBMC culture of these patients. Although there is a significant correlation between IL-6 amounts in tissue culture supernatant and PBMC culture (P=0.02), the respective data for TNF is only almost significant .**Conclusion:** Inflammatory cytokines may have significant role in the early provoke of inflammation occurred in hypertrophied tonsils and adenoid. The majority of these cytokines increase the expression of adhesion molecules on epithelial cells and influence the recruitment of leucocytes and inflamed tonsils. On the other hand lack of sufficient cytokine release may lead to persistent infections and may cause chronic inflammation and hypertrophied tissue.

Keywords: inflammatory cytokines, adenotonsillectomy patients, adenotonsill, PBMCs

3439P

In vitro differentiated encephalitogenic TH17 cells induced experimental autoimmune encephalomyelitis

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Background: Experimental autoimmune encephalomyelitis (EAE) is a rodent model of multiple sclerosis (MS), induced by autoreactive Th cells which mediate inflammation and demyelination of CNS. Interleukin (IL)-17-producing T helper (Th)-17 cells have recently been explained as a distinct population of CD4+ T cells which play an important role in autoimmunity. But whether Th17 cells are encephalitogenic has been controversial. **Methods:** We generated in vitro Myelin oligodendrocyte glycoprotein (MOG)-specific Th17 cells in the presence of TGF-β, IL-6, IL-23 and peptide MOG35-55. Th17 development was confirmed by assessment of relevant transcription factors and secreted cytokines by flowcytometry , ELISA and real time-PCR. In addition, Th17 to Th1 plasticity was monitored. Upon adoptive transfer encephalitogenic activity of this cell subset was characterized. In order to generate encephalitogenic lineage to develop a direct method for induction of MS animal model, The encephalitogenic Th17 cell fusion with Sp2/0 cell line were performed. **Results:** Although in our present study, we developed a protocol to induce MOG-specific Th17 cell from naïve T cells. Our results showed that myelin oligodendrocyte glycoprotein-specific Th17 cells induce EAE upon adoptive transfer. **Discussion:** These data confirm that effector Th17 cell subset with specificity for myelin Antigens can induce CNS autoimmunity. Although we obtained a hybrid cell line with specificity for myelin Antigen, ability to secret IL-17 and expression of RORγt, as the main transcription factor of Th17 subset, but we cannot induce EAE in recipient mice. It is may due to its property to induce tumor.

Keywords: EAE, TH17, MOG35-55

3447P

Identification of antigens of *Plasmodium falciparum & P. vivax* for vaccine production using bioinformatics methods

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Background: Malaria remains one of the most prevalent infectious diseases worldwide and is, therefore, a global health problem despite substantial efforts to control the disease over the past few decades. Approximately 3.3 billion people are at risk, and 250 million cases each year were reported in the period 2006–2008, primarily in Africa. **Methods:** The amino acid sequence of some proteins of *Plasmodium falciparum & P. vivax*, retrieved from UniprotKB and NCBIp databases. At third step, by using of immunoinformatics servers allergenic peptide (epitopes) were predicted and analyzed. **Results:** Our results revealed many antigenic epitopes: there were 8 antigenic epitopes for O96272 between 6-28 amino acid lengths, for O974747 between 7-31, Q8IEU210 between 7-30, Q8IJN012 between 6-22, Q8I4P9 12 between 6-26, Q8I6697 between 7-55, O9627312 between 6-27, Q7RIT8 27 between 6-27, Q7RCZ9 27 between 6-25, Q7R838 19 between 6-39, Q7RPV3 4 between 9-16, Q7RS62 18 between 6-16, Q7RP59 19 between 7-43, Q7RC97 31 between 7-41, Q7RC988 between 7-15, Q7RS60 8

between 7-24, Q7RC96 5 between 8-20. **Conclusion:** Bioinformatics analysis results showed that these sequences have several antigens, and we can use these epitopes for other purposes. For example production of drugs and vaccines.
Keywords: *Plasmodium falciparum*, *Plasmodium vivax*, epitopes, Antigenicity

3386p

Investigating the effect of rs3783605 SNP on the activity of VCAM-1 promoter in human umblical vein endothelial cells

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Background: Vascular Cell Adhesion Molecule-1 (VCAM-1) plays a role in cell adhesion to the vascular endothelium. It is suggested that the expression level of VCAM1 is associated with a number of human diseases. Such expression level is regulated by many factors including the promoter activity that is possibly affected by the single nucleotide polymorphisms (SNPs) present in the promoter. There are previous reports suggesting an important role for rs3783605 at -420 position, in the pathogenesis of VCAM1-associated diseases. Therefore, present study was designed to investigate the effect of rs3783605 on the activity of VCAM-1 gene promoter in Human Umbilical Vein Endothelial Cell line (HUVEC). **Methods:** To achieve this, two appropriate expression vectors containing VCAM1 promoter with different alleles of rs3783605 were constructed to express the Green Fluorescent Protein (GFP). Expression vectors were transfected into HUVECs and their EGFP expression level was assessed by the fluorescent microscopy and real-time PCR. **Results:** The EGFP expression level in the cells transfected by promoter-less vector is about 0.00001 folds of cells transfected with vector containing CMV promoter (P-value < 0.001). The expression level in the cells transfected by vector containing A allele of rs3783605 is 0.14888 folds and G allele is about 0.37851 folds of cells transfected by vector having CMV promoter (P-value < 0.001). Moreover, HUVECs transfected by G allele of rs3783605 showed about 2-fold higher transcriptional activity compared with the A allele, P-value < 0.04. **Conclusion:** Our findings showed that rs3783605 polymorphism may play a role in VCAM-1 gene expression.
Keywords: VCAM-1 gene, CMV promoter, rs3783605, SNP, HUVEC

3404P

Antigenic epitopes analysis and antigenicity evaluation of different proteins of *Leishmania donovani*

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Background: Leishmaniasis is endemic in about 88 countries and is responsible for the annual loss of 2.4 million disability adjusted life-years and over 59,000 deaths. The visceral form of

the disease is mainly caused by *Leishmania donovani*, *L. chagasi*, or *L. infantum*, and 500,000 new cases of visceral leishmaniasis (VL) occur each year.This protozoan parasite displays immense adaptability tosurvive under extremely harsh conditions. **Methods:** The amino acid sequences of *Leishmania donovani* and most identity proteins from *Leishmania donovani* retrieved from UniprotKB and NCBIpdatabases.Then sequences aligned and the most highly conserved and variable region and kind of amino acids in these regions were evaluated. At third step, by using of immunoinformatics servers antigenic peptide (epitopes) were predicted and analyzed. **Results:** Our results revealed many antigenic epitopes: there were 31 antigenic epitopes for E9BB03 between 7-37 amino acid lengths, for E9BJI0 22 between 6-33, E9BB00 35 between 6-25 , E9BRN2 27 between 7-53, E9BDV8 12 between 7-30, E9BAY6 33 between 6-56, E9BB54 59 between 6-53, E9BRN1 19 between 6-19, E9BNA3 27 between 7-50, E9BAY8 25 between 7-45, E9BRN4 11 between 6-49, E9BAX5 11 between 7-31, E9BAZ1 28 between 7-77, E9BAX9 19 between 6-28, E9BRM8 10 between 8-48, E9BJH9 11 between 8-30, E9BRM5 10 between 6-34, E9BB07 113 between 6-73, E9BSJ6 10 between 7-33, E9BAX4 12 between 6-76. **Conclusion:** Our result shows that these proteins have many antigenic proteins that can cause leishmaniasis.Thus antibody against these proteins may explain clinically relevant symptoms due to this disease.

Keywords: *Leishmania donovani*,epitopes, Antigenicity

3374P

Evaluation of cartilage oligomeric matrix protein (COMP)in chronic development of collagen-induced arthritis

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Background: Cartilage oligomeric matrix protein (COMP) is found at elevated concentrations in sera of patients with joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA).We recently showed that COMP activates complement via the alternative pathway and that COMP-C3b complexes are present in sera of RA patients, but not in healthy controls. We now set out to elaborate on the information provided by this marker in a variety of diseases and larger patient cohorts.The development of arthritis, like collagen-induced arthritis (CIA), involves cartilage as a target tissue. We have investigated the development of CIA in COMP-deficient mice. **Methods:** COMP-C3b levels in sera were measured by using an enzyme-linked immunosorbent assay (ELISA) capturing COMP and detecting C3b. Serum COMP was measured by using ELISA. **Results:** COMP-deficient mice showed a significant early onset and increase in the severity of CIA in the chronic phase, whereas collagen II-antibody titers were similar in COMP-deficient and wild-type controls. COMP antibodies were not found in wildtypemice. Finally, COMP-deficient and wild-type mice responded similarly to collagen antibody induced arthritis, indicating no difference in how collagen II antibodies interact with COMP-deficient cartilage during the initial stages of arthritis. **Conclusion:** COMP deficiency enhances the early onset and development of chronic arthritis but does not affect collagen II autoimmunity. These findings accentuate the importance of COMP in cartilage stability.

Keywords: COMP, rheumatoid arthritis, Cartilage, collagen

3410P

Assessment serum level of vitamin D in asthmatic children and comparing of them by non-asthmatic groups and also analyzing the influence of serum level of vitamin D in intensity of asthma

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Background: Vitamin D deficiency has been rediscovered as a public health problem worldwide. It has been postulated that vitamin D deficiency may explain a portion of the asthma epidemic. The purpose of this study is to assess the serum vitamin D levels in children with asthma compare to non-asthmatic population and to investigate the association of serum vitamin D level with severity of asthma. **Methods:** We measured serum 25-hydroxyvitamin D level in 50 children with mild intermittent to moderate persistent asthma at the time of enrollment and 50 age- and sex matched non-asthmatic in a case-control study. Independent sample t-test, χ^2 and spearman correlation coefficient were used to analyze the data. **Results:** Mean vitamin D level was significantly lower in subjects with asthma than in non-asthmatic subjects. In these children, lower vitamin D levels were associated with increased asthma severity. **Conclusion:** Taking into consideration the lower 25-OH vitamin D in asthmatic children, it seems that by improving vitamin D status holds promise in primary prevention of asthma and in decreasing the severity of disease. Because vitamin D deficiency is prevalent even in sun-replete areas, clinical trials are needed to definitively answer questions about the role of vitamin D in asthma.

Keywords: Asthma, Vitamin D, Prevention

3395P

Terbutaline, a β2 adrenergic receptor agonist, inhibits progression of MLDS-induced diabetes mellitus in C57 mice

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Background: Type 1 diabetes mellitus is an autoimmune disease characterized by the selective destruction of pancreatic β-cells. The proinflammatory immune responses have a very prominent role in the pathogenesis of this disease. Recent trends in the treatment of this disease has focused on the shifting of the immune response towards anti-inflammatory profile. It has been shown that stimulation of β adrenergic receptors (especially β2 adrenergic receptors) of immune cells can diminish proinflammatory responses. The Goal of this study was to evaluate the preventive effect of terbutaline, a β2-adrenergic receptor agonist, on the progression of multiple low dose streptozotocin (MLDS) induced diabetes mellitus in inbred male C57 mice. **Methods:** Terbutaline was administered with a dose of 60 µg every 12 hours intraperitoneally, for a total duration of consecutive 12 days (1 day before, 5 days concomitant with, and 6 days after the induction of diabetes). **Results:** Our results showed that treatment with terbutaline significantly inhibited increase of the level of fasting blood sugar, decreases the level of TNF-α, increases the level of IL-10 and ameliorates the severity of the pancreatic inflammation. **Conclusion:** Terbutaline prevents the development of MLDS-induced diabetes mellitus in C57 male mice through decreasing and increasing proinflammatory and antiinflammatory immune responses, respectively.

Keywords: Type1 diabetes mellitus, Beta blocker, Inflammation, neuroimmunology

3351P

Co-transplantation of VEGF-expressing human embryonic stem cell derived mesenchymal stem cellsto enhance islet revascularization in diabetic Nude mice

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Background: Pancreatic islet transplantation has emerged as a promising treatment for type I diabetes. However, its efficacy is severely hampered due to poor islet engraftment and revascularization, which have been resulted to partially loss of transplanted islets. It has been shown that local delivery of vascular endothelial growth factor (VEGF) could accelerate transplanted islet revascularization, although permanent high level of VEGF may lead to undesirable side effects. In this study we investigated the effects of conditional cell-based delivery of VEGF through collagen-fibrin hydrogel on transplanted islet function and revascularization. **Methods:** RH6 human embryonic stem cell derived mesenchymal stem cells (ES-MSCs) have been transduced by two lenti viruses containing rtTA and VEGF-A. Tet-on expression of VEGF from these cells was shown by tube formation assay and was confirmed through VEGF ELISA. After co-transplantation of these cells and mouse isolated islets through collagen-fibrin hydrogel in the omental pouch of diabetic nude mice, the blood glucose, body weight, glucose tolerance and serum C-peptide was measured after 28 days. As control groups, islets were transplanted alone and with non-transgenic ES-MSCs. **Results:** The results showed improved islet functionality and micro-vessel density, compared with control groups. **Conclusion:** We conclude that conditional expression of VEGF from ES-MSCs during islet transplantation could enhance islet functionality and revascularization. This result can be used to improve the outcome of clinical islet transplantation.

Keywords: Pancreatic islet transplantation, VEGF, ES-MSCs

3452p

Effects of vitamin D on Th17 cells and some related molecules in systemic lupus erythematosus induced by active chromatin in BALB/c mice

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Background: Systemic lupus erythematosus is an autoimmune disease, which is characterized by production of autoantibodies against self- antigens specially DNA. Disease is multifactorial and the main cause of it is still unknown. Thus animal models of SLE can be usefull for studying the cellular and molecular mechanisms which are involved in the disease pathogenesis. This study aim to stablish a murine model for SLE-like syndrome by immunization with active chromatin for assessment of vitamin D effects on Th17 cells and related cytokines in SLE. **Methods:** 30 female Balb/c mice, 6-8 weeks, divided into four groups: Group one: Lupus-like disease was induced with activated chromatin; Group two: Two weeks of treatment with vitamin D and then lupus-like diseas was induced with activated chromatin; Group three: injected with non activated chromatin as a control; Group four: injected with PBS and Ferunds

adjuant as another control. Four mice from group one were treated with vitamin D for two weeks after establishing the disease. These mice were considered as group five. At the end of study mice splenocytes extracted and the mRNA expression of IL-17, IL-23 and IFN-γ were analyzed using Real-Time PCR and ELISA techniques. **Results:** In this study mRNA expression levels of IL-17, IL-23 and IFN-γ in the groups which received vitamin D declined. **Conclusion:** Vitamin D reduced anti-ds DNA antibody, IL-17, IL-23 and IFN-γ levels in mice which received this vitamin for two weeks before lupus induction. Therefore vitamin D may have beneficial effects on preventing lupus disease.

Keywords: Systemic lupus erythematosus, cytokines, Th17 cells, vitamin D

3354P

Evalution of pH and temprature on absorption of Tetanus toxoid on Aluminum phosphate

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Background: Tetanus is a medical condition characterized dy a prolonged contraction of sketelal muscle fibers which is caused by tetanospasmin, neurotoxin produced by the Gram positive, rod – shaped obligate anaerobic bacterium *Clostridium tetani*. The DTP (Diphtheria, Tetanus, Pertussis) vaccination started form 1940 caused eradication of Tetanus diseases throughout the world. The Aluminum phosphate or alum has been commonly used as a adjuvant for Tetanus vaccine. It is formulated by Aluminum phosphate and Aluminum hydroxide. In the present study the different factors like pH and temprature wich have major role on absorption of Tetanus toxoid were stuied. **Methods:** In this study to evaluate the optimum condition for absorption of Tetanus toxoid the different factor like pH & temprature were evalvated. The different pH (5,5.2,5,4,5.6,5.8,6) temprature (4^0c, 25^0c, 37^0c) to find pH (6.4,6.6,6.8) were studied find the optimum condition for absorption of Tetanus toxoid. **Results and Conclusion:** our results indicated that the optimum condition for absorption of Tetanus toxoid on Aluminum phosphate gel is pH 5.8, temprature 4^0c and final pH 6.6 our observation showed 92% absorption of Tetanus toxoid on Aluminum phosphate.

Keywords: vaccine, Tetanus, temperature, pH, Aluminum phosphate

3355P

Evalution of pH and temprature on absorption of diphtheria toxoid on Aluminum phosphate

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Background: Diphtheria is a respiratory traet illness wich cased by *Corynebacterium* diphtgeria. The Diphtheria is a Gram positive bacteria without capsul and spore. The DTP (Diphtheria, Tetanus, Pertussis) vaccination started form 1940 caused eradication of Diphtheria diseases throughout the world. The Aluminum phosphate or alum has been commonly used

as a adjuvant for Diphtheria vaccine. It is formulated by Aluminum phosphate and Aluminum hydroxide. In the present study the different factors like pH and temprature wich have major role on absorption of diphtheria toxoid were stuied. **Methods:** In this study to evaluate the optimum condition for absorption of Diphtheria toxoid the different factor like pH & temprature were evalvated. The different pH (5,5.2,5,4,5.6,5.8,6) temprature (4^0c, 25^0c, 37^0c) to find pH (6.4,6.6,6.8) were studied find the optimum condition for absorption of Diphtheria toxoid. **Results and Conclusion:** our results indicated that the optimum condition for absorption of Diphtheria toxoid on Aluminum phosphate gel is pH 5.8, temprature 4^0c and final pH 6.6 our observation showed 92% absorption of Diphtheria toxoid on Aluminum phosphate.

Keywords: vaccine, diphtheria, temperature, pH, Aluminum phosphate

3403P

Evaluation of VEGF peptide based vaccine on inhibition of proliferation in tumor cell lines

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Background: Already, In silico analysis especially bioinformatics help us to decrease in projects time and costs and increase in reliability of research studies results. In this study, we used this approach to design of VEGF peptide based vaccine, which have an inhibitory effect on tumor growth and metastasis. **Methods:** VEGF-A isoforms sequences were obtained UniProt database and aligned by mega4 software. The similar and conserved sequences were selected. To study B cell and T cell epitopes that are suitable for stimulate immune responses we used IEDB analysis resource and finally to avoid of sequence overlapping with other human antigens we have used BLASTp alignment search tool in NCBI database. **Results:** So with using the bioinformatics analysis in immunoinformatics branch that is focused specifically on immunology and vaccinology we have chosen peptide sequence of VEGF A that has minimum overlapping with other proteins in body. Also, this sequence has enough antigenicity, with suitable and accessible epitopes for broken tolerance and ability to stimulate anti-tumor appropriate response. **Conclusion:** In summery our analysis reinforce the potential of this peptide based vaccine to stimulate body to generate VEGF blocking antibodies and elucidate an appropriate immune response against the tumor cells to inhibit tumor metastasis and prevent further uncontrolled growth of cancer and following the success in In silico phase in future phases peptide vaccine will be evaluate In vitro and In vivo studies.

Keywords: Immunoinformatics, Peptide vaccine, VEGF, Angiogenesis

3412P

The effect of aerobic exercise on Hs-CRP and body composition indexes in non-active obese men with emphasis on Ramadan fasting

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Background: Arterial inflammation has emerged as central to the progression of atherothrombosis. One of the markers of inflammation, the high-sensitivity C- reactive protein "hs-CRP" is the most studied, with evidence that it may also play a direct pathogenic role in atherosclerotic lesion formation. Purpose of the study was to evaluate the effect of aerobic exercise and Ramadan fasting on the high-sensitivity C-reactive protein as a marker of inflammation in non-active obese men. **Methods:** In this study, 18 obese men aged 50-40with a BMI over 30 kg per square meter as a public call among the 70 subjects were selected randomly and after it were divided in to fasting (N=9) and fasting and exercise (N=9) groups. Then while the first group would do only fasting, fasting and exercise group in addition to the intervention fasting group, exercise to be carried out in 27 sessions. In addition, for check the desired changes in the month of Ramadan, blood samples were taken from four different times. Finally, using repeated measures analysis of variance in the level of p <0/05 theories were put to the test. **Results:** Ramadan fasting significantly decreased the high-sensitivity C-reactive protein ($p < 0.05$) as well as aerobic exercise significantly were reduced high-sensitivity C-reactive protein ($p < 0.05$) in both groups. **Conclusion:** aerobic exercise and Ramadan fasting has a beneficial effect on the reduction of low-grade inflammation.

Keywords: high-sensitivity C-reactive protein, aerobic exercise and Ramadan fasting

3356p

Designing and evaluation of Dot-ELISA for diagnosis of *Fasciola* infection in Cattle

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Background: *Fasciola* causes considerable economic loss to the meat industry. Clinical signs and symptoms appear three weeks post-infection. Furthermore, early diagnosis is not possible because eggs are not found in faeces until flukes reach maturity, usually between 10 and 14 weeks after infection, hence a fasciolosis coprological diagnostic method has presented a challenge.The purpose of this study was to describe an in-house dot- ELISA using two extracted *Fasciola* (crude and excretory–secretory) antigens for diagnosis of fasciolosis in cattle. **Methods:** The sera specimens of slaughtered cattle were taken and examined for *Fasciola* infection. Sera from two groups of cattle, one infected with fasciolosis (n = 60) and the other non-infected with fasciolosis (n = 60), were used in the dot- ELISA test. *Fasciola* crude worm antigen was prepared as described by Oldham and Williams (1985) with some modification. *Fasciola* excretory/secretory antigen was prepared according to Simsek et al. (2006). Dot-ELISA was conducted as described earlier, with some modification (Pappas MG, 1988). **Results:** All sera were tested and evaluated. Except specificity, other parameters such as, sensitivity, accuracy, positive and negative predictive values of Dot- ELISA with E/S

Ag were better than these values of Dot- ELISA with Cr Ag. **Conclusion:** In conclusion, excretory/secretory Ag was the best coating antigen in Dot-ELISA for the serodiagnosis of fasciolosis in cattle due to its high sensitivity, specificity, and precision rates.
Keywords: *Fasciola*, Cattle, Dot-ELISA

3362P
Silencing Pten by siRNA, substantially increases HL60 differentiation to neutrophile by DMSO and ATRA

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Background: A characteristic feature of leukemia cells is a blockade of differentiation at a distinct stage in cellular maturation.Aberrant cell differentiation, especially suppression of terminal cell differentiation, exists in all tumors. Terminal differentiation causes proliferative cell loss within any cancer cell population. PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene that It's deletion or mutation is associated with a large number of human cancers and several autosomal-dominant disorders. **Methods:** PTEN Si RNA successfully transfectedto HL-60 cells as shown by western blotting .HL60 differentiation to neutrophile assessed by flow cytomeretry and NBT assay.Cell death was evaluated by trypan blue staining. **Results:** Hl-60 cells transfected by PTEN Si RNA showed increased level of differentiation in the presence of DMSO and ATRA. **Discussion:** It has been shown that ATRA and DMSO up regulate PTEN in HL-60 cells and consequently potentiate the inhibition of growth and cell cycle progression of these cells. Our data strongly supports the idea that although active maintenance of cell-cycle arrest is an important aspect of the differentiated state, Cell survival and inhibition of apoptosis is necessary for cells to differentiate.
Keywords: Silencing Pten, Si RNA, differentiation DMSO, ATRA

3363P
Evaluation of interferon-gamma receptor mutations in patients with disseminated BCG infection

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Background: Mendelian susceptibility to mycobacterial disease (MSMD) is a clinical syndrome that predisposes otherwise apparently healthy individuals to infections caused by weakly virulent mycobacteria, such as BacilleCalmette-Guérin (BCG) and environmental mycobacteria .MSMD-causing mutations have been identified in 6 genes. Five of these genes are autosomal and encode the IFNGR1 and IFNGR2, STAT1, the p40 subunit of interleukin

IL12B, and the β1 IL12RB1, whereas the sixth gene is X-linked and encodes NEMO. These defects impair IFN-γ-mediated immunity. **Methods:** We assessed IFN-γ production by Il_12 stimulation of peripheral mononuclear cells gathered from patients using ELISA and Il-12 production by IFN-γ stimulation .Any defects in these path ways were further studied by flow cytometric analysis of the related receptor and then defected receptor gene was sequenced. **Results:** From 45 patients involved in our study 25 patients showed IFN_γR deficiency and didn't produce IL-12 and 20 patients with Il_12BR deficiency who are incapable to produce IFN γ. **Discussion:** Complete abrogations of IFN-γR1 or IFN-γR2 are strongly associated with an early onset of severe and often fatal infection with low virulence mycobacterial species, such as non-tuberculosis mycobacteria or BCG. In these patients the infection continues despite the instauration of an appropriate anti-tuberculosis treatment. Our results showed both INF γ and IL-12 pathway are equally involved in disseminated BCG infection.

Keywords: disseminated BCG infection,mutations,interferon-gamma receptor

3435P

Immunological Testing Reveals Exposure to and Active Plasmodium infection in the non-endemic region of Iran

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Background: Prevalence of malaria infection was investigated by using malaria antibody- and antigen-detection ELISA tests in the non-endemic province (Bushehr) located in the south western part of Iran. **Methods:** No active malaria case was reported by traditional microscopic examination of 1955 blood smears. Serum samples were tested for malaria antibodies for assessing possible exposure to infection. **Results:** 13.8% (270 out of 1955 tested) samples showed detectable level of malaria antibodies. Further testing for *P. falciparum* specific malaria antigen yielded no positive results indicating no exposure to falciparum infection in Bushehr region. However, six samples (three from traveler and three from resident group) representing a 0.3% of total population indicated active malaria infection based on positivity of plasmodium lactate dehydrogenase antigen, a marker for active Plasmodium infection. A low level exposure to possibly non-falciparum Plasmodium parasitic infection was indicated as assessed on the level of pLDH. Malaria species confirmation is needed on the six cases that were positive for pan pLDH antigens. This suggests that they may be infected, but additional studies are needed to assess whether malaria infection is potentially being transmitted or not in the Bushehr region. **Conclusion:** This study has showed practical usage of immunological testing and considered to be more sensitive tools for investigating Plasmodium infection in the hypo-endemic region. This information may be valuable for future investigations.

Keywords: ELISA antibody detection, antigen detection, pLDH antigen, malaria exposure, active malaria infection, hypo-endemic, Iran

3456P

Immuno-biochemical study of bacterial lipopolysaccharide (LPS) as inducer of the immune system to prevent the development of murine Leishmaniasis caused by *Leishmania major* in Balb/c mice *via* nitric oxide pathway

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Background: Leishmaniasis is a zoonotic disease that is caused by *Leishmania* species and its cutaneous leishmaniasis (CL) is still a major health problem with symptoms sporadic form in Iran. This study may be useful ways to prevent, improve or reduce pathophysiological symptoms of this disease. In this study, the Immuno-biochemical role of bacterial lipopolysaccharide (LPS) as inducer of the immune system to prevent the development of murine Leishmaniasis caused by *Leishmania major* in Balb/c mice via nitric oxide pathway was investigated. **Methods:** The specific amount of the bacterium *Salmonella abortus* LPS was injected to Balb/c mice in the test group and the same volume of saline was injected to mice in the control group, after then 17 weeks the immunological changes in the nitric oxide (NO) levels in suspensions of the liver and spleen and also in serum were evaluated, Biochemical changes in the serum Zn and Cu level, lesion size, body weight, survival rates, number of amastigotes inside macrophages(MQ) and the values of hepato-splenomegaly all were assessed. **Results:** In spite of positive effects of LPS among outbred mice, LPS stimulation didn't increase NO activity in Balb/c mice and showed no sufficient effects on the pathophysiology of Balb/c mice, compared to NMRI outbred mice. **Conclusion:** Therefore could not be expected as a standard stimulator to induce inflammation, or as a factor to increase the immune responses of Balb /c mice against virulent *Leishmania major* parasites in this strain of laboratory mice.

Keywords: Leishmaniasis, No, LPS, Balb/c

3400P

Evaluation of probiotic immunostimulatory effects of Lactobacillus bacteria isolated from *Barbus grypus* intestine

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Background: Lactic acid bacteria (LAB) are the most common type of microbes used as probiotics. Isolation, identification and effectiveness of lactic acid bacteria (LAB) from *Barbus grypus* intestine, based on their probiotic effects, were determined in this study. **Method:** Fifty *B. grypus* were captured from natural water resources of Khuzestan province and bacterial flora of fish intestine were assessed. Isolated LABs were evaluated and ranked based on the probiotical indication tests. Finally one isolates including *L. delbruecki sp. bulgaricus* were selected as the highest potent probiotic LABs of *B. grypus* intestine. In the next step the effects of dietary supplementation of isolated Lactobacillus from gut and *Lactobacillus casei*

PTCC 1608 on the immunity and disease resistance of juveniles *B. grypus* against *Aeromonas hydrophila* infection, were evaluated and Immune parameters was examined at 30 and 60 Days post-feeding, following which the test diets were withdrawn and control diet was fed up to 75 days. **Results and Conclusion**: Dietary administration of *L. delbruecki* significantly increased the serum lysozyme and complement activity, and respiratory burst activity in *B. grypus* at days 30th of the experimental period. The highest bactericidal activity ($P < 0.05$) was observed in the fish which fed diet containing *L. delbruecki*. These results collectively suggest that dietary supplementation of gut Lactobacillus of *B. grypus* are not only compete with commercial probiotics but also in various respects are better that. *L. delbruecki sp. Bulgaricus* have higher effects in *B. grypus* welfare`s that other probiotics.

Keywords: Lactic acid bacteria, probiotics,

3366P

The cortical and thalamic metabotropic glutamate type-1α receptor reduction in the absence epileptic rats

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Background: Modulatory function of metabotropic glutamate type 1 receptors affects the spike and waves discharges (SWDs) in the WAG/Rij rats, a valid genetic animal model of absence epilepsy. In this study, we describe the alteration of mGlu1α subunit of metabotropic glutamate receptors expression in WAG/Rij rats in somatosensory neocortex as well as in latrodorsal (LD) thalamic nucleus. **Methods:** Experimental groups were divided into four groups of six rats of both WAG/Rij and Wistar strains with two and six months of age. The expression level of mGlu1α receptors was assessed by Real-time PCR, western blotting and immunohistochemical staining. **Results and Conclusion:** The mRNA level of mGlu1α protein was significantly decreased in tow- and six-month-old WAG/Rij rats in the somatosensory cortex and LD thalamic nucleus. The protein level of cortical mGlu1α receptors increased in two-month-old WAG/Rij and decreased in six-month-old WAG/Rij compared to aged- matched Wistar rats. The protein level of LD mGlu1α receptors decreased in six-month-old WAG/Rij rats. The distribution of mGlu1α receptors in fourth cortical layer was higher in two-month-old and lower in WAG/Rij rats compared to age-matched Wistar rats. The distribution of mGlu1α receptors in fifth cortical layer and LD thalamic nucleus was lower in six-month- old WAG/Rij compared to age-matched Wistar rats. The microinjection of mGlu1α receptors agonist within the LD nucleus reduced the absence seizures in epileptic WAG/Rij rats. In contrary, the microinjection of mGlu1α receptors antagonist within the LD nucleus increased the absence seizures in epileptic WAG/Rij rats. The disturbance of mGlu1α receptors expression in the thalmocortical circuit emphasizes the crucial role of metabotropic glutamate receptors in the SWDs generation.

Keywords: Genetic models of epilepsy, Spike-wave discharges, DHPG, LY367385, brain

3436P

Evaluation of the correlation between serum and urinary TGF-B , IL-6 , IL-17 , IL-23 factors with poly cystic kidney

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Introduction: This study, demonstrating IL-17-related cytokines in ADPKD, tries to suggest the possible application of these cytokines in diognosis, evaluating the prognosis and treatment of the disease. **Methods:** selecting 54 patients with ADPKD and 54 normal individuals without any underlying inflammatory diseases, serum levels of IL-17, IL-23, IL-6 and TGF-β were measured through ELISA. Statistical analysis was done by the means of *t* and Mann-whitney u tests and covariance analysis. **Results:** The mean serum levels of IL-17 in ADPKD and control group were 74.2±19.7 and 13.7±8.3 and its urinary levels were 94.3±49.3 and 9.5±9.1 (P<0.001).These levels for IL-23 were in turn 15±5.4, 5.7±5.2 and 18.8±11.8, 4.6±6.2 (P<0.001). For IL-6, such measures were 1.7±0.9, 1.6±1.5 (P=0.53) and 2.7±1.3, 1.32±1.35 (P<0.001) and for TGF-β the levels were 46.3±15, 15.4±0.5 (P=0.5) and 30.2±9.7, 29±13.3 (P=0.56). After adjustment for age, sex, hemoglbin level and blood pressure, there were still significant differences between serum and urinary levels of IL-17 and IL-23 and urinary level of IL-6. **Conclusion:** it is likely that the IL17-IL23 pathway is involved in the pathogenesis of ADPKD and it might be beneficial to be considered in early diagnosis. Moreover, pharmacological block of such pathway might be an alternative treatment method. Although affecting the inflammatory base of the disease, IL-6 & TGF-β are not as appropriate diognostic options as IL-17 and IL-23.

Keywords: ADPKD, IL-17, IL-23, IL-6, TGF-β

3455P

Evaluation of correlation between serum level of interleukin 17 and Disease activity of Rheumatoid arthritis in patients referred to rheumatology clinic during 2012- 2013

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Introduction: Rheumatoid arthritisis a chronic systemic autoimmune inflammatory disease of synovial joints. Various studies showed that higher levels of IL-17 have been observed in serum and synovium of RA patients. The aim of this study is to find out the correlation between the serum level of IL-17 and disease activity. **Methods**: 60 patients fulfilled the ACR criteria for RA were accepted for this cross sectional study. To evaluate the correlation between serum level of IL-17 and disease activity, serum samples were taken and analyzed in laboratory. The DAS28 was calculated for all patients and scores lower than 2.6 were considered as controlled RA whilst higher scores were considered as active. **Results**: Based on das28 score from 60 patients, 33 patients were identified to have controlled RA with mean IL-17serum level of 144.81±47.83ng/l .Furthermore, 27 patients were diagnosed with active disease. The mean serum level for the active patients was 237±93.08ng/l. The analysis showed that there is a significant correlation between serum IL-17 level and DAS 28 in both groups. Furthermore, a strong correlation between IL-17 and disease activity in patients with active RA was

noticed. The study revealed that the level of serum IL-17 was significantly higher in patients with moderate RA activity(DAS28<5)as opposed to the ones with severe activity(DAS28>5). **Conclusion**: This study showed that there is a substantial correlation between serum level of IL-17and disease activity score.The observation sugesst that IL-17 is a good indicator of the disease severity of RA and can be used in patients follow up.
Keywords: disease activity score (DAS28), Rheumatoid arthraitis, serum IL-17level

3403P
Evaluation of VEGF peptide based vaccine on inhibition of proliferation in tumor cell lines

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Background: Already, In silico analysis especially bioinformatics help us to decrease in projects time and costs and increase in reliability of research studies results. In this study, we used this approach to design of VEGF peptide based vaccine, which have an inhibitory effect on tumor growth and metastasis. **Methods:** VEGF-A isoforms sequences were obtained UniProt database and aligned by mega4 software. The similar and conserved sequences were selected. To study B cell and T cell epitopes that are suitable for stimulate immune responses we used IEDB analysis resource and finally to avoid of sequence overlapping with other human antigens we have used BLASTp alignment search tool in NCBI database. **Result:** So with using the bioinformatics analysis in immunoinformatics branch that is focused specifically on immunology and vaccinology we have chosen peptide sequence of VEGF A that has minimum overlapping with other proteins in body. Also, this sequence has enough antigenicity, with suitable and accessible epitopes for broken tolerance and ability to stimulate anti-tumor appropriate response. **Conclusion:** In summery our analysis reinforce the potential of this peptide based vaccine to stimulate body to generate VEGF blocking antibodies and elucidate an appropriate immune response against the tumor cells to inhibit tumor metastasis and prevent further uncontrolled growth of cancer and following the success in In silico phase in future phases peptide vaccine will be evaluate In vitro and In vivo studies.
Keywords: Immunoinformatics, Peptide vaccine, VEGF, Angiogenesis

3380P
Antibody Screening in Patients With Thalassemia Major

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Background: The development of hemolytic alloantibodies and erythrocyte autoantibodies complicates transfusion therapy in thalassemia patients. **Methods:** The frequency causes and prevention of this phenomenon in 90 transfused thalassemia patients were evaluated at Fatemeh Zahra Hospital in Bushehr in a cross-sectional study. **Results:** In our study, the age

of onset of symptoms ranged from 40 days to 12 years (1.72 ± 1.88 years). Hemoglobin (Hb) levels per transfusion in these patients were 8.40 ±0.82%. Red cell alloantibodies were detected in 9 patients (10%). The red cell antibodies developed in this report were mainly Kell and C system. Our data showed that alloimmunization to minor erythrocyte antigens and erythrocyte autoimmunization of significant clinical variables are frequent findings in transfused thalassemia patients. **Conclusion:** There is no relation between the number of blood units transfused and antibody formation in thalassemia, but it is an important factor for increased alloimmunization in these patients.

Keywords: antibody screening, thalassemia major, alloantibodies, autoantibodies

3347P

Evaluation of allergic rhinitis frequency in patients with migraine headaches without aura

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Background: In the recent years, the correlation between allergic diseases and migraine headaches has increased. This may due to inflammatory mediators with vasoactive function that play an important role in these diseases. This study aimed to evaluation of allergic rhinitis frequency in patients with migraine without aura. **Methods:** This analytical-descriptive study was carried out on 212 patients with migraine without aura who refers to neurology clinic of a university hospital in Mashhad, Iran. All of these patients evaluated for allergic rhinitis. The tool of gathering the data was a demographic questionnaire and a questionnaire asking about clinical symptoms of allergic rhinitis; also total serum IgE levels were estimated in patients with clinical symptoms of allergic rhinitis by using enzyme linked immunosorbent assay (ELISA) kit. **Results:** A total of 212 patients (59% female and 48% male, mean age 30.9 years) participated in the study. The frequency of allergic rhinitis in migraine patients was 28.3%. There was a significant difference between frequency of allergic rhinitis and frequency of migraine attacks ($P < 0.0001$), but there wasn't significant difference between frequency of allergic rhinitis with age, sex, marital status, family history and clinical symptoms of allergic rhinitis. **Conclusion:** We propose that inflammatory mediators play a key role in triggering migraine by means of vasodilation and inflammation in the pathogenesis of migraine headaches. Thus, the avoidance of conditions that cause allergic reactions in migraine patients may be a simple and effective way for prophylaxis or their treatment.

Keywords: Migraine without aura, IgE, allergic rhinitis

3360P

The extract of *Staphylococcus aureus* decrease the activity of neutrophils and monocytes of cattle

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Background: *Staphylococcus aureus* is one of the most virulent pathogens with low cure rates involved in bovine mastitis. This study was done to investigate the effects of the extracts of *S. aureus* on phagocytic activity of the neutrophils and monocytes of healthy and staphylococcal infected dairy cattle. **Methods:** Milk samples from five infected cattle (mastitis) and 5 healthy cattle were collected. Simultaneously, heparinized blood samples taken from healthy and infected cattles and neutrophil and monocyte from sampels isolated using meglumine compound and histopaque respectively. Moreover, the extract of *Staphylococcus aureus* was produced and the effects of extraction factors on neutrophils and monocytes phagocytic activity from healthy and infected cattle were determined. **Results:** In healthy cattle, extract of *staphylococcus aureus* hasn't any effect on ingestion process and respiratory burst of monocytes. In infected cattle, ingestion process is severely decreased in monocytes, but the rate of respiratory burst hasn't any significant change. Results showed that the respiratory burst of neutrophils in the healthy cattle is increased after challenge with bacteria and their extracts; however the phagocytic activity of neutrophils is decreased after exposure to extracts. In infected cattle, the phagocytic activity and the respiratory burst of neutrophils are diminished following exposure to extracts. **Conclusion:** Altogether it seems that following the progression of the staphylococcal mastitis, the function of phagocytic cells dramatically decreased. Thus, improvements in management practices in order to prevention and rapid detection and treatment of staphylococcal mastitis is very essential.

Keywords: *Staphylococcus aureus*, Phagocytosis, Neutrophil, Monocyte

3419P

Construction of a genetic vehicle for expressing of recombinant human interleukin-2 in *E.coli*

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Background: Interleukin-2 (IL-2) is a central regulator of immune responses and produced mainly by activated $CD4^+$ T cells. Human IL-2 is a 15 kDa protein containing 153 amino acid residues and a disulfide bond between the residues cys58-cys105. The recombinant form of hIL-2 is used as a drug in variety of cancers especially renal cell cancer and melanoma. To produce active recombinant hIL-2, we employed a bacterial expression vector (pET) and *Escherchia coli* Rosseta-gami as the expression host. Recombinant IL-2 was synthesized in the structure of a fusion protein with thioredoxin and poly-histidine tags to facilitate correct folding and protein purification respectively. Additionally, a self-cleaving coding sequence with inducible proteolytic activity was predicted to excise the recombinant hIL2 from mentioned tags without any additional or deleted amino acids. **Methods:** The coding sequence of hIL-

2 was synthesized and cloned into pCYB2 vector. To introduce *Eco*RV & *Xho*I sites at tail and head of designed DNA fragment respectively, this fragment was amplified using PCR with specific primers and pCYB2 as template. Then Intein-hIL-2 fragment was sub-cloned in pET32b. The recombinant vector transformed into *E.coli* for expression of hIL-2 in soluble, active form. **Results:** Recombinant expression vector was constructed correctly as checked by sequencing. The hIL-2 was produced and purified in a single step using Ni^{++} resin. We confirmed the accuracy of produced hIL-2 by SDS-page, western blotting. Biological activity of the protein was determined by MTS-assay. **Conclusion:** The active hIL-2 was successfully produced and purified using self-cleaving tag in *E.coli* Roseta-gami (DE3) as an efficient host.
Keywords: Interleukin-2, self-cleaving tag, *Escherichia coli* Rosseta-gami

3359P
Study of the relationship between allergies and depression in patients with allergies in Sabzevar

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Background: Allergy is an immunologic reaction to various allergens that affects many people worldwide. Suffering from discomforts of allergic reactions can lead to mood disorders, anxiety and nervous states. Large studies show that the risk of depression in people with severe allergies is about twice of those without allergies. Women with major depression are more likely to have allergies and allergies seem to be more common in men with nervous and anxious characters. There is a peak of suicide in spring, late summer and early fall. A greater seasonality of suicide is reported in those with a history of allergy than in those without such a history. Sensitization and exposure to aeroallergens, which peak in spring, may be conductive to seasonal exacerbation of suicide risk factors.Histamine is a chemical that is released during allergic reactions and may affects mood, when attaches to its receptors on the brain. During allergic reactions, the brain churns out substances called pro-inflammatory cytokines such as IL-1β, TNF-α and IL-6. Also, expression of Th-2 cytokines is elevated in the human orbito-frontal cortex in suicide victims. **Methods:** In a study conducted in Sabzevar city on 60 patients with allergy, they were asked to explain their psychic and moral mood in the onset of allergy symptoms. **Results:** Of the 60 patients, 45 patients declared that in the onset of allergic symptoms, they are violent, aggressive and have nervous mood. Of these 45 patients, 20 patients stated that their symptoms are severe and other declared that their symptoms are moderate. **Conclusion:** The relationship between depression, nervous disorders and allergies has been investigated in many studies. Researches show that pro-inflammatory cytokines can have depression promoting effects, such as dips in the brain's production of the "feel-good" chemical serotonin. Also, researchers concluded that the increase of Th-2 cells and related cytokines such as IL-6 and IL-10 lead to increase of neurological and behavioral disorders and this effect is caused by HPA axis activation. In the conducted study, we are witnessing that %75 of patients admit that in the onset of allergy symptoms are nervous and aggressive and %45 of patients with nervous symptoms declared that the severity of their nervous symptoms is too high. These results confirm other results that researchers have obtained in other studies.
Keywords: allergy, depression, suicide, nervous disorders, pro-inflammatory cytokine

3450P

Preparation of liposome for drug delivery by lung

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Background: N-Acetylcysteine is a drug used in the clinic for the treatment of acetaminophen-induced hepatotoxicity and as amucolytic agent. It possesses free radical-scavenging properties which are attributed to the nucleophilicity and redox interactions of its thiol group. Additionally, NAC is a source of cysteine, often the limiting precursor of *de novo* GSH synthesis. It has been shown that intravenous administration of NAC alone at a dose of 25mg/kg was not effective in conferring any significant protection against LPS-induced hepatotoxicity while an equivalent dose of NAC delivered as a liposomal formulation conferred protection. Results from pharmacokinetic studies have shown that following intravenous administration, NAC undergoes rapid and extensive metabolism in the liver resulting in bioavailability of about 10% . This finding further supports the role of liposomes in their use as a drug delivery system because of their capability of delivering hepatoprotective agents to the liver, an ideal approach to increase local concentration of the agent, to reduce adverse effects, and to achieve maximal therapeutic efficiency. Similarly, results presented by other investigators have shown that intravenous administration of drugs as liposomal formulations prolong their circulation time in blood and increase their distribution to major organs, including the lung. **Methods**: Liposomes can be prepared by several techniques. We employed both film hydration and freeze drying method, in order to determine if there are any differences in the incorporation of NAC in liposomes. The most appropriate method was then used further. Lipid films were prepared by mixing the lipid solutions in the desired composition with solution of the active drug NAC in a round bottom flask. After mixing the desired components, the solvents were removed on a rotary evaporator with vacuum pump at 45 °C. After , the round bottom flask was removed from the water bath for about 3 hours to remove traces of solvent and obtain a dry film. The lipid compositions of the different formulations which were prepared. **Results**: There is considerable interest towards the development of drug-delivery systems that would result in the selective delivery of antioxidants to tissues in sufficient concentrations to ameliorate oxidant-induced tissue injuries. Liposomes are biocompatible, biodegradable, and nontoxic artificial phospholipid vesicles that offer the possibility of carrying hydrophilic, hydrophobic, and amphiphilic molecules. We wanted to employ the film methods for Liposomal Antioxidants for Protection against Oxidant-Induced while administered as a liposomal formulation directly to the the lung injury. The purpose of the comparison of film and freeze-drying method was to determine if there are any significant differences in the incorporation capacity of NAC in the liposomes when using these two methods. **Conclusion:** N-acetylcysteine (NAC), a thiol-containing compound, when administered in its conventional form did not protect against the prolonged shock-induced acute lung injury but when administered as a liposomal formulation directly to the lungs of animals protected against the lung injury. The protective effect conferred by the liposomal NAC occurred at low NAC doses, since liposomes are known to prolong the retention of the antioxidant in the lungs. L-NAC was also shown to have a prophylactic effect against both LPS-induced lung injuries.

Keywords: NAC: N Acetyl L Cysteine , Oxidative stress , fluimucil

3357p

The role of Mir-199a in Multiple Sclerosis according to Bioinformatical prediction

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Background: Multiple sclerosis (MS) is a chronic inflammatory response against constituents of the central nervous system. It is known that regulatory T cells (Tregs) play a key role in the autoimmune balance and their improper function may facilitate the expansion of autoaggressive T cell clones. Recently, microRNAs (miRNAs) have been involved in autoimmune disorders and their loss-of-function in immune cells was shown to facilitate systemic autoimmune disorders. Here we specifically aimed to find essential miRNAs in the pathway of differentiation to Th17 by means of using bioinformatics databases. Our candidate miRNAs could be used for further therapeutic purposes. **Methods:** In silico studies for identifying miRNAs which has roles in differentiation of naïve CD4+ cells to mature Th17 cells revealed several miRNAs. These miRNAs are probably applicable to inhibit the differentiation of Naïve T cells into Th17 cells to reduce the progress of MS. For this cause, we gathered information about different miRNAs in variant autoimmune diseases that Th17 was involved with. On the other hand we determined 64 genes that were invovled in different pathways of Th17 differentiation. Using 10 different databases we analyzed the interaction of these genes with miRNAs. **Results:** Among the datas , it is seen that 8 miRNA, have strong interaction on their corresponding genes. Mir-199a is one of this miRNAs which its role in other autoimmune diseases like Rheumatoid Arthritis and Psoriasis has been detected. The Mir is probably a inhibitor of negative regulator on these genes. **Conclusion:** According to our results Mir-199a can be a key miRNA in progression of symptoms of Multiple Sclerosis by inducing the differentiation to Th17 cells,However *in-vitro* and *in-vivo* experiments is needed to confirm our computational analysis which is an ongoing research of our team.

Keywords: Mir-199a, Th17 subset, differentiation, autoimmune disease

3364P

Possible association of IL-4 VNTR polymorphism with susceptibility to pre-eclampsia

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Background: Preeclampsia is a pregnancy-specific disorder that may be cause of motherly mortality and morbidity. Growing evidence indicated that cytokines are involved in the pathogenesis of Preeclampsia. Interleukin-4 VNTR polymorphism has been implicated in altering the risk of Preeclampsia. The aim of this study was to evaluate the possible association

between VNTR polymorphism IL-4 and susceptibility to Preeclampsia in Iranian women population for the first time. **Methods:** Genetic polymorphism was evaluated in 192 PE and 186 healthy control women. **Results:** We found that the VNTR polymorphism of IL-4 gene has significantly increased the risk of Preeclampsia (RP1/RP2 vs. RP1/RP2, OR, 2.81[95% CI, 1.66 to 8.76]; P=0.0001 RP2/RP2 vs. RP1/RP1, OR, 1.06 [95% CI, 1.01to1.10]; P=0.002; RP2 vs. RP1,OR, 3.07 [95% CI, 1.91 to 4.93]; P=0.0001). **Conclusion:** The results showed that IL-4 VNTR variant has positive association with Preeclampsia susceptibility.
Keywords: IL-4, Preeclampsia, VNTR polymorphism

3365P
Lack of Association betweenIL-1 receptor antagonist gene 86bp VNTR Polymorphism and Leiomyoma

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Background: Uterine Leiomyomas (ULs) is the most common gynecological tumor and a significant health concern for many women.The interleukin-1 receptor antagonist (IL-1Ra) isa naturally occurring cytokine that inhibits interleukin- 1 (IL-1) activity by binding to the IL-1 receptors without signal transduction. The aim of this study was to investigate the association between interleukin-1 receptor antagonist gene VNTR polymorphism and ULs in the South- East of Iran. **Methods**: We studied 99 patients with leiomyoma and 102 controls. Genotyping of IL-1Ra (VNTR) polymorphism was determined by gel electrophoresis after PCR amplification. Frequency of alleles and genotypes in patients and control group was statistically analyzed using χ 2 test or fisher exact test. **Results:** The alleles 1, 2 and 3 frequency of IL-1Ra VNTR polymorphism were %71, %27 and %22 in control group and %74, %20 and %6 in the ULs patients , respectively and there were no significant differences between two groups. No statistically significant differences were observed between the frequency of IL-1Ra genotypes in the study and control groups. **Conclusions**: This study showed that 86bp VNTR polymorphism of IL-1Ra gene is notassociated with leiomyoma.
Keywords: Uterine Leiomyomas, interleukin-1 receptor antagonist, polymorphism

3438P
Evalution Activity of silver nanoparticles synthesized using (ziziphora tenure) extract Mountain north -east of Iran (esfarayen)

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Background: nanoparticles synthesized by Chemically methods usually result in some retrntion of toxic and nano particles in environmental application . This study is applied with use of

plant extracts resulted from metal nanoparticales with green chemistry techniques. **Methods:** Making phytochemical properties of silver nanoparticles using (**ziziphora tenure l**) extract and using it is as reducing agent to form silver nanoparticles in. During nanoparticle Aqueous medium containing silver nanoparticles can show its power. at a wavelength of 458 nm which is ralated the silver plasmon absorption is rapidly synthesis nanoparticles . morphology and size the silver nanoparticles by transmission electron microscope tem and sem was use by scanning. **Results and Conclusion:** The purpose of this research is to determine the amount of silver nanoparticles tend (**ziziphora tenure l**)and synthesis in which silver nanoparticles were detected by using transmission electron microscopy(TEM) and scanning electron microscopy (SEM) after synthesis. as results of good regenerative power produced by (**ziziphora tenure l**)extract,silver nanoparticles were synthesized with dimension 100-1 nm.
Keywords: Green chemistry , silver nanoparticles, ziziphora tenure l

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