13th International Congress of Immunology and Allergy of Iran

Tabriz-Iran 26-29 April 2016

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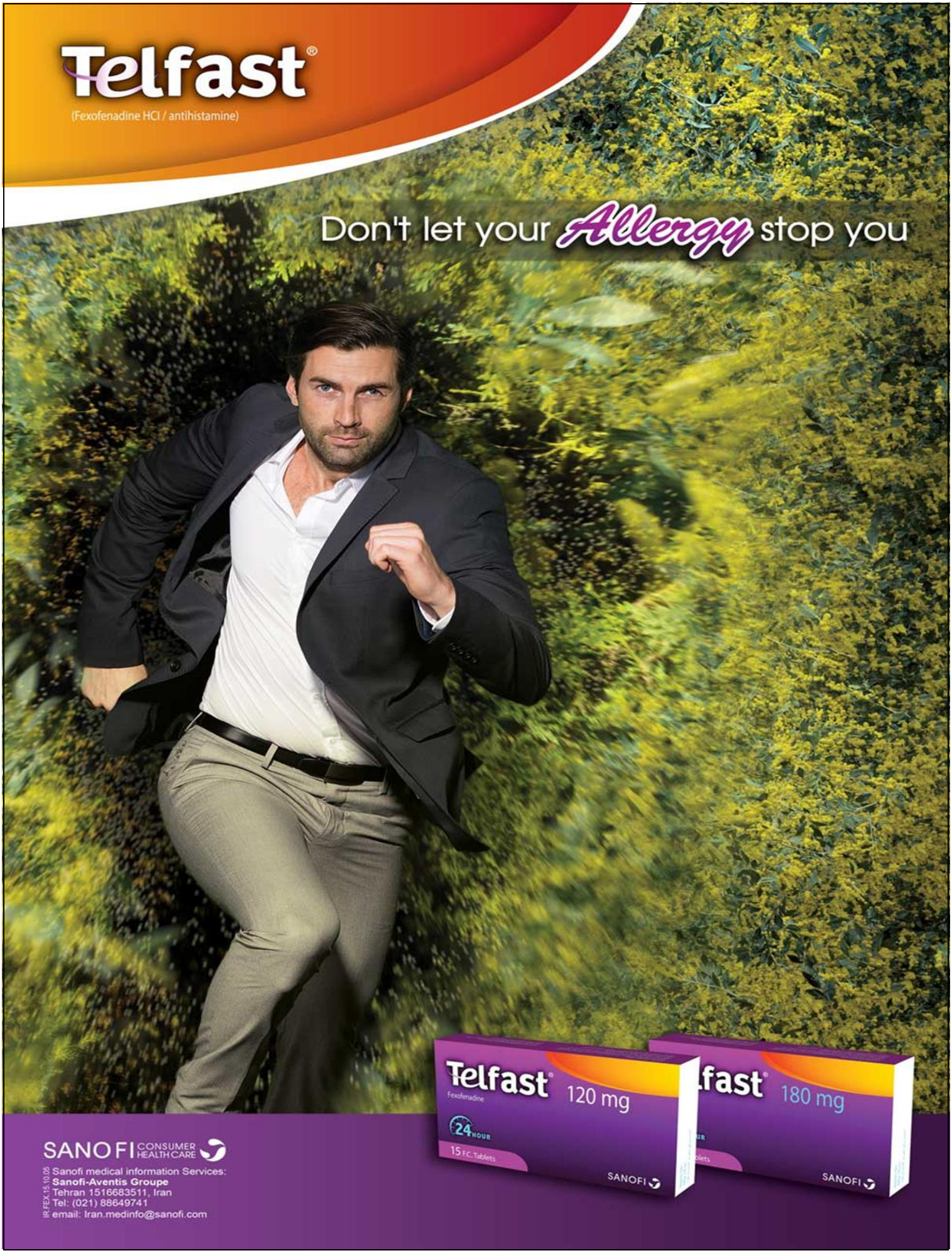
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Tabriz Petrochemical Recreation Complex-Tabriz-Iran

"Immunology: Prevention, Diagnosis and Treatment"

26th- 29th April, 2016

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Welcome Message

In the Name of God

We are all thankful of God who gave the human being superiority to the other creatures by granting him/her the excellence of learning and thinking. We are delighted and happy to host thirteenth Iranian congress of immunology and allergy in cooperation with Iranian Society of Immunology and Allergy in Tabriz, Iran. There is no doubt that this congress and congresses like this could provide an invaluable opportunity for both scientist and researchers of outside or inside of our country, Iran, to meet their colleagues and share their findings with each other and improve their knowledge while students and young investigators of Immunology will have the opportunity of meeting the pioneers of the field and got motivated by them. We are hopeful to be an appropriate host for this congress and provide all participants a memorable and sweet stay at Tabriz, the city of the firsts. One of the important goals of this congress is cooperation of basic and clinical sciences in order to gather scientists in the field of immunology and specialists including rheumatologists,oncologists,allergists,cardiologists,endocrinologists, nephrologists, and etc. together and help both scientists and clinicians to share their experiences and become up to date. Planned keynote speeches by Iranian and International scientist, poster presentations, workshops and advanced courses on Immunology are some sections of this impressive scientific event.

At the end, as scientific secretory of congress and on the behalf of Iranian Society of Immunology and Allergy, I would like to appreciate of your participation. I would like to express my warm and kind thanks to our sponsors, the companies, universities of medical sciences which help us, and Tabriz City Council and municipality and other municipal bodies of Tabriz which their precious supports help us hosting this scientific event in Tabriz.

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Plenary & Key Abstracts

Regulation of NF-kB activation by p21 in macrophages and its implications in endotoxin tolerance and sepsis

Dimitrios Balomenos, National Center for Biotechnology/CSIC, Madrid/Spain

Gram-negative bacterial infection can lead to systemic inflammation and sepsis — an intricate pathology that is leading cause of death in hospitals worldwide. This complex syndrome is characterized by two pathological phases. First following systemic infection, circulating proinflammatory cytokines are secreted due to decontrolled macrophage responses. Subsequently, macrophages enter a state hyporesponsive state and become tolerant to further challenges. This immune paralysis correlates with sepsis progression and death, as it may lead to increased risk of secondary infection. Macrophage unresponsiveness is known as endotoxin tolerance and is induced in mice after secondary lipopolysaccharide (LPS) challenges, and is intimately associated to reprogramming of M1-to-M2 cells. We identified a role for p21 in controlling macrophage polarization, which was independent of its cell cycle inhibitory function. LPS-rechallenged $p21^{-/-}$ macrophages showed impaired ability to polarize from an M1 state to an M2 status, as they did not reduce IFN-β. Biochemical analysis showed that p21 shifted the balance between active p65/p50 and inhibitory p50/p50 NF-kB and controlled macrophage reprogramming by regulating the DNA-binding affinity of p50/p50 homodimers. Thus, p21 favors repressing p50/p50 homodimer binding, which limits IFN-β production and promotes M1-to-M2 macrophage reprogramming. p21 knockdown in human monocytes validated its role in IFN-β regulation. The impact of our findings was emphasized, since immunosuppressed monocytes from sepsis patients showed increased p21 levels, which correlated with IFN-β downregulation. Overall, the data provide new mechanistic insight into monocyte/macrophage immunosuppression, and designate p21 as a key molecule in regulating the balance between inflammatory and hyporesponsive states.

Opportunities and challenges for mucosal immunity and systems analysis of immune responses to vaccines

Ali M. Harandi, Department of Microbiology & Immunology, Institute of Biomedicine, University of Gothenburg, Sweden, ali.harandi@microbio.gu.se

Most human pathogens invade the body through or establish infections in the mucosal tissues. Hence, the development of vaccines to counter mucosal infections represents a top global health priority. Mucosal immunization has attracted much interest as a means of inducing protective immunity against mucosally transmitted pathogens. However, only a handful of mucosal vaccines based on live attenuated or whole killed pathogens are currently approved for human use. Other immunization approaches are hence required to elicit immunity at the mucosal tissues. Recent advances in cutting edge Omics technologies such as transcriptomics, mirnomics and metabolomics combined with systems biology approaches have enabled systems analysis of human responses to vaccines and adjuvants. This talk will give an overview of the state-of-the-art mucosal vaccines and adjuvants and that how systems vaccinology approaches can provide new insights into mechanisms of action and biomarkers of human vaccines and adjuvants.

Novel immunomodulating therapeutic approach for the infectious diseases

Mostafa Haji Mollahoseini, Department of Immunology, School of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran

Immunomodulators are substances that help correct immune system that is out of balance. There are two types of immunomodulators: immunosuppressants and immunostimulants. Immunomodulation has now come to the front stage as a major tactic in anti-infective strategy. More thanthousand infectious agents are recognized as responsible for human diseases and the list will grow in the forthcoming years. Therapeutic strategies based on modulating the immune response at least have two potential advantages over the use of traditional antimicrobials. First, immunomodulators do not act on microorganisms directly, so they may circumvent the problem of rapid emergence of resistance. Second, they offer the potential of a broad spectrum of activity against different microbialdiseases. Chitin is a biopolymer that a key component of insects, fungi, and house-dust mites. Humans are armed with chitinases as well as chitinase-like proteins (CLPs) to defend themselves against chitin-bearing pathogens and modulate their immune responses. Chitinase and CLPs mediates many inflammatory processes through the direct stimulation of different inflammatory mediators, thus increasing the migratory capacity of many immunological cells. Recent studies are interested in the chitin immunomodulatory effects through the processes of chitin recognition and induction of immune response to kill different types of pathogens. It seems that this method be effective in the treatment of Leishmaniasis.The development of targeted therapies either to suppress chitinsaes activity in inflammatory disorders or to specifically enhance its targeted activity to potentiate immunity against infections will not be far in the future.

In vitro allergy diagnostics

Mehrnaz Mesdaghi MD, PhD, Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Most tests used for allergy diagnosis are tests that evaluate allergic sensitization, or the presence of allergen-specific IgE. Most allergic patients have demonstrable allergen-specific IgE that specifically recognizes that allergen, making these tests suitable tools in the diagnosis of allergic diseases. The clinical response of a sensitized individual to an allergen is a physiologic event with multiple variables, among which the presence of allergen-specific IgE is one. So, allergy tests must be interpreted in the context of the patient's clinical history, and the diagnosis cannot be made based on a laboratory result. In vitro testing has some advantages comparing skin testing for example it poses no risk, it is not affected by medications, and it is not affected by skin disease. The most commonly-used in vitro tests are immunoassays. Different immunoassays are used for this purpose like ELISA, RAST, and chemiluminescent immunoassays. The sensitivity and specificity of immunoassays vary with the system being used and the quality of the allergen. The general sensitivity ranges from 60 to 95 percent and specificity from 30 to 95 percent. Other tests that have been largely used in research and recently are used in routine clinical studies include immunoblotting, several tests of basophil activation and function, tests for levels of eosinophil-derived mediators, and microarray testing. Total serum IgE levels are of limited value in the diagnosis of allergic disorders. A high total IgE level may indicate an atopic condition, but it provides no information about the allergens the patient is sensitive to.

Prevention of allergic diseases

Farahzad Jabbari Azad , Mashhad University of Medical Sciences, Mashhad, Iran

The prevalence of asthma and allergic diseaseshas increased in recent years, particularly in the industrializedworld - as evidenced by the ISAACstudy.The disorder usuallymanifests initially in the form of food allergy andatopic dermatitis, followed in later stages by respiratoryallergy with rhinitis and/or asthma. This has ledto the adoption of preventive measures in those childrenwith a high risk of atopy, based on the following considerations:

1) A family history of allergic diseases (asthma, eczema, and/or allergic rhinitis)
2) Apersonal history of atopy such as atopic dermatitis, particularly when associated to food allergy

3)The existence of allergic sensitization, particularly topneumoallergens, of early or late onset, but persistentduring childhood.

Prevention is established at three different levels:

1- primary prevention, avoiding sensitization
2- Secondaryprevention, avoiding appearance of the disease. secondary prevention, destined to avoid symptomsin those patients that are already atopic.
3- Tertiary prevention, avoiding the symptoms. The objective of tertiary prevention is to controlthe allergic disease, preventing (in the case of asthma) the exacerbations and reducing maintenancemedication as far as possible.

Autohemotherapy in chronic urticaria

Sheikhi A[1,2], Azarbeig A[3], KarimiH[4]

1. Department of Immunology, Faculty of Medicine, Dezful University of Medical Sciences, Dezful, Iran.
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4. Department of Internal Medicine, Faculty of Medicine, Dezful University of Medical Sciences, Dezful, Iran.

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.

Anti-inflammatory, Immunomodulatory, Anti-tumor, Analgesic and Neuroprotective effects of *Scrophularia striata* as an Iranian Medicinal Plant

Abbas Azadmehr (Ph. D), Immunology Department, Babol University of Medical Sciences, Babol, Iran

Scrophularia striata Boiss (Scrophulariaceae) is a medicinal plant that has been used as a traditional herb for various purposes such as sedative, scrophulas, scabies, eczema, psoriasis and tumors in folk medicine. Our previous studies demonstrated the inhibitory effect of *S. striata* extract on the nitric oxide (NO) and pro-inflammatory cytokines (TNF-α, IL-1β and PGE2) production by macrophages. In addition, our findings indicated that *S. striata* extract could inhibit human leukemia cell growth and breast cancer through inducing G2/M phase cell cycle arrest and apoptosis. Moreover, our results showed the ability of *S. striata* to decrease reactive oxygen species (ROS) generation and neuron cell apoptosis. Also, this medicinal plant indicated analgesic activity. Phytochemical assay demonstrated that the main component including phenolic compounds, phenyl propanoids and two flavonoids, quercetin and isorhamnetin *3-O*-rutinoside, were identified from this medicinal plant. Future studies suggested

Exercise and changes of inflammatory factors

Mehri Ghafourian, Immunology Department, Medical Schoole, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Inflammation has a significant role in the pathogenesis of several chronic diseases including cardiovascular dis¬ease CVD, type 2 diabetes mellitus, Alzheimer's disease, osteoporosis, and certain cancers. Development of cardiovascular diseases has inflammatory basis, and general (systematic) inflammation plays a central role in atherosclerosis development and progression. It is believed that a strong association exists between physical inactivity and low-grade inflammation and its adverse health outcomes. Exercise probably changes the level of anti-inflammatory and pro-inflammatory factors such as C-reactive protein (CRP), Interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), Interleukin-10 (IL-10), Interleukin-1β (IL-1β), soluble inter¬cellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule(VCAM-1). There is an amount-response relationship between the activity rate and the immune response. There is, also, a significant overlapping between the intensity of the activity and to a specific extent the level of preparation in determining the response of these markers.While the recommendation to exercise for people with age-related chronic disorders is one of the treatment pro¬tocols, these exercise prescriptions might differ in terms of intensityand du¬ration of the exercise period.A single exercise session induces a transient increase in inflammatory sta¬tus, and this increase ranges from mild to severe. Exercise with lower intensity is more effective than with higher intensity in reducing inflammation. This observation may help patients with age-related chronic disorders and may also be useful for clinicians to make better choices about the type and in¬tensity of exercise that they prescribe.

The impact of KIR/HLA-I combination on the prognosis of allogenic BMTin patients with leukemia

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Haematopoietic stem cell transplantation (HSCT) is usually a final treatment for haematologic malignancies. To decrease the side effects of myeloablative procedures, nonmyeloablative regimen combined with donor lymphocyte infusions (DLI) from HLA-mismatched donors is used for effective killing of residual leukemic cells. However graft versus-leukemia (GVL) is an important approach for prevention of relapse, there is a risk of graft versus host disease (GVHD). Like T cells, NK cells also facilitate engraftment, combat infection and control malignant cells in HSCT but do not cause GVHD. Since donor KIRs encounter non-self-recipient HLA class I alleles, Incompatibility between KIRs in the donor and KIR ligands in the recipient improves the results of HSCT by reducing the risk of acute GVHD and relapse. **Keywords**: Haematopoietic stem cell transplantation, KIR, HLA class I

Obstacles and key challenges against knowledge-based companies' success

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Since 2010 the year knowledge-based companies law was legislated, several and occasionally contradictory functions have been introduced. Some ministries have gone further and according to the "IRAN OUTLOOK 1404"determined and enforced the number of knowledge-based companies as well as academic members to be engaged in this law. The office in charge of enforcing this law has even published the list of products to be referred to by applicants nationally. Accordingly, this law is to play a pivotal role to transform or channel the knowledge to wealth in Iran. In order to support those related, many rules and regulations have also been officially documented. The key question is whether these legislations will incorporate and associate academic researches with economy and wealth creation in mid or long-term nationally? If not, what are the obstacles and key challenges against knowledge-based companies' success? Having tracked the obstacles, which authorities are responsible to ease the situation and solve the problems?

This article, aims to elaborate and discuss the barriers and main challenges against knowledge-based companies' success in the country.

Application of Antiviral and Immunomodulatory Agents in Treatment of Adult T Cell Leukaemia and HAM/TSP Patients

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Human T-lymphotropic virus type 1 (HTLV-1) is a pathogenic retrovirus that infects human $CD4^+$ T lymphocytes. Approximately 15-20 million people worldwide are infected with HTLV-1 (4) with the majority of them residing in Japan, Africa, the Caribbean Islands, and Central and South America. We have previously reported that the cities of Mashhad and Sabzevar, in North east Iran, are also endemic for this virus, with prevalence being 2.1 and 1.6%, respectively. The virus is associated with two main types of diseases; a chronic neurologic disease known as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and the T-cell malignancy named adult T-cell leukemia (ATL).The mechanism underlying why less than 5% of HTLV-I-infected individuals develop HTLV-I associated diseases remain understood, however it has been shown that viral factors and Immune response have a pivotal role in immunopathogesnesis of HTLV-I-associated diseases. After many years of research on HTLV-I and associated diseases, treatment of ATL and HAM/TSP patients remains a challenge. We have applied different treatments in ATL and HAM/TSP patients including anti viral agents and immunomodulatory agents. The clinical, viral factors and immunological finding after treatment will be discussed.

Current knowledge of the immunology of leishmaniases

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Leishmaniases are a set of intracellular parasitic infections caused by *Leishmania* species. The considerable morbidity and the economic burden caused by leishmaniases have been a major impediment to development and economic progress, especially in the developing countries. In recent decades, these infections are widening geographically due to the international conflicts in the endemic regions. Due to their coevolution with their hosts, the leishmania species are able to escape the human immune system or divert it by many means to ensure their survival. In this regard, the outcome of such infections can be widely different due the interdependence of multiple parameters, namely the parasite, the host, the vector (sandflies) and the environment. In this session, after a brief introduction regarding the regional distribution of various forms of leishmaniases, the current knowledge on the host-parasite immunology with special emphasis on the potential risk factors, the genetic polymorphism, the modes of avoiding the immune detection and the role of tissue resident memory cells will be reviewed. Furthermore, the outstanding questions and the challenges ahead for the researchers in this field, regarding the complex immunological interactions of leishmania species with their human host, will be presented.

The Role of Allergens in the Pathogenesis of Atopic Dermatitis (Eczema)

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The main role of allergens in the pathogenesis and the course of atopic dermatitis (AD) and has been difficult to establish, recently. Approximately 33% of infants andyoung children with AD will show clinically relevant reactivityto an allergen. Therefore, specific IgE and T cellshave been cloned fromperipheral blood and skin lesional of patients with AD. Our previous study on 1100 allergic patients in Bushehr revealed that 12% (133) were AD. Findings of the study indicated that the prevalence of food allergens were peanut (48.1%), egg (44.4%), and shrimp (39.1%), respectively. Also, the prevalence of inhaled allergens were house dust mite (63%), Russian thistle spore (62.3%) and fungi (54.7%), respectively. Furthermore, eliminationof allergens results in amelioration of skin and clinical symptoms disease. **Key words:** Food and Aeroallergen, Atopic dermatitis (Eczema)

Chemo-immunotherapy: from bench to bedside

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Over the past 10 years, there has been main stream acceptance that some chemotherapies can be combined with immunotherapy in animal models, combining synergistically to lead to an improved outcome. What has proven more difficult is translating that knowledge to positive, practice-changing clinical trials. Two Drugs have been studied (in our lab) in animal model and transfer to clinical trials on the patients with invasive ductal carcinoma Stage IIIb and chemo resistant patients (End stage patients). These molecules were tehranloid and shark cartilage. In animal model we evaluate four parameters include tumor size, IFN γ /IL-4, animal survival, T regulatory cell and CD4/CD8 ratio in both Tehranloid and shark cartilage treated animals. In human trial research were evaluated CBC levels, Tumor marker, quality of life and the clinical features of the patients. The results indicated that Tehranloid could significantly decrease in tumor marker, *incre*ase in the quality of life and improve the clinical feature of the patients after sixty days of treatment. Shark cartilage could increase the level of IFNγ and decrease the level of IL-4, increase the quality of life and clinical features of the patients. Our data concluded that the correlation of shark cartilage and Tehranloid in treated animal and treated patients was significantly correlated. Both drugs improve the clinical and Para clinical parameters of the patients.

Immunomodualory and anti-inflammatory effects of Zingiberofficinale (Ginger). Focus on experimental autoimmune encephalomyelitis

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Introduction: Ginger, an herbal product, havebroad immunomodulatory and anti- inflammatory effects which is used as an alternative medicine in a number of disease. The aim of this study was to evaluate the effects of the ginger extract on the expression of some pro- or anti-inflammatory cytokines in a model of experimental autoimmune encephalomyelitis (EAE). **Materials and Methods**: EAE was induced in C57Bl/6 mice by immunization with myelin oligodendroglial glycoprotein emulsified in complete Freund's adjuvant. The mice were intra peritoneal administered with PBS in control groups or ginger extract (200 or 300 mg/kg, every other day) in treatment group, from day +3 to +30. At day 31 mice were scarified and the spinal cord and brain were harvested. The expression of the IL-17, IL-23, IL-27 and IL-33 mRNA in the CNS determined by using real time-PCR. Moreover, the seum levels of cytokines were measured by ELISA method. **Results:** In PBS-treated EAE mice the expression of IL-27 was significantly lower than that in normal group ($P<0.001$). The mRNA expression of IL-17, IL-23 P19, IL-23 P40 and IL-33 in CNS and serum levels of IL-17 and IL-23 were significantly higher in PBS-treated EAE mice than healthy control group ($P<0.003$, $P<0.001$, $P<0.001$, $P<0.01$, $P<0.05$ and $P<0.01$, respectively). In 200 mg/kg and 300 mg/kg ginger-treated EAE groups the expression of IL-27 was significantly higher as compared to PBS-treated EAE mice ($P<0.01$ and $P<0.05$, respectively). Moreover, ginger-treated EAE groups had significantly lower expression of IL-17, IL-23 P19 and IL-23P40 and IL-33 in CNS and lower serum IL-17 and IL-23 levels than PBS-treated EAE group. The mean clinical scores of EAE was significantly lower in ginger-treated EAE groups in comparison with PBS-treated EAE mice. The mean of the pathological scores for ginger treated groups extract was also significantly lower than that observed in PBS-treated EAE group. **Conclusion:** These results showed that the ginger extract modulates the expression of the pro- or anti-inflammatory cytokines in the EAE mice and also ameliorates the clinical symptoms of disease. **Keywords:** Experimental autoimmune encephalomyelitis, Ginger, IL-17, IL-23, IL-27, IL-33, Clinical symptoms.

Enhanced Co-expression of Interleukin-6 and Parathyroid Hormone-related Peptide: A Potential Prognostic Marker for Hormone-insensitive and Apoptotic-resistant Prostate Cancer.

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During the early stages of prostate cancer, tumor cells are dependent on androgen as a growth factor, thusa withdrawal of androgen results in apoptotic cell death and clinical remissions. However, tumor recurrences after therapeutic interventions are commonly associated with increasing androgen independence of the tumor cells and increasing resistance to pro-apoptotic and chemotherapeutic agents. Interleukin-6 (IL-6) and Parathyroid Hormone-related Peptide (PTHrP) are multifunctional cytokines and immune responses regulators. Overexpression of both IL-6 and PTHrP have been implicated in prostate cancer progression and bone metastases. Serum IL-6 and PTHrP levels are elevated in patients with untreated metastatic prostate cancer, and are correlated with poor prognosis and chemo resistance. We have shown that the expression of PTHrP is greater in poorly differentiated carcinoma as compared with the well-differentiated tumors. We have also reported thatIL-6, PTHrP mRNA, and protein are highly expressed in androgen-insensitive prostate cancer cells, andthe co-culture of androgen-insensitive prostate cancer cells with bone marrow mesenchymal stem cells (BM-MSCs) leads to an increase of IL-6 and PTHrP expression. In addition, the expression of PTHrP may contribute to the apoptosis-resistant phenotype in androgen-insensitive prostate cancer cells, and repression of PTHrP expression increases the sensitivity of androgen-insensitive prostate cancer cells to apoptosis. Furthermore, PTHrP induces the expression of IL-6 and its soluble receptor (IL-6sR) during the progression of prostate cancer. Recent findings indicate that IL-6 balance differs in pathological and normal prostates suggesting that changes in the expressions of IL-6 in prostate carcinoma samples could be used as a prognostic marker of disease progression. To date, the best prognostic indication is a combination of the clinical stage, Gleason grade, and serum Prostate Specific Antigen (PSA) at diagnosis. However, the inherent biological variability of PSA levels improves usefulness of PSA only marginally, limiting its value for prostate cancer screening and prognostication. Therefore, the combination of PSA and other immune markers will substantially improve prostate cancer detection.

Obesity and autoimmunity

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Obesity a newly recognized chronic disease has a rapidly increasing prevalence throughout the world. According to reports of WHO, there are more than one billion overweight and 300 million obese individuals in the world. Obesity is the second leading cause of preventable death exceeding by cigarette smoking and is associated with numerous health problems including hypertension, cardiovascular events, type 2 diabetes mellitus and other health problems. Recently, the studies have shown that the chronic low grade inflammatory nature of the obesity play a major role in the etiology of obesity-related disorders. Researchers found that obesity leads to the breakdown of the body's protective self-tolerance, creating the optimal environment for autoimmune diseases, and generates a pro-inflammatory environment likely to worsen the disease's progression and hinder its treatment. Investigating the classic pro-inflammatory cytokines like IL-6 or TNF-α give very limited information about immune function in obesity. The most reliable assays for studying the immune function in obesity are specifically the ones that measure the immune function in a “functional manner” particularly the $CD4^+$-T cell function. Obesity has a direct impact on $CD4^+$-T cells compared with $CD8^+$-T cells; adipose tissue- secreted inflammatory cytokines like TNF-α stimulates $CD4^+$-T cells’ differentiation to Th1 cells; whereas, these pro-inflammatory cytokines has no effect on $CD8^+$-T cells. On the other hand, the imbalance between two important subsets of $CD4^+$-T cells including Th1 and Th2 cells in obese individuals are associated with numerous health consequences including atherosclerosis, acute coronary syndrome and autoimmune disorders. Interferon γ (IFN) γ, the Th1 secreted inflammatory cytokine, is involved in adipose tissue expansion and triggers inflammation in adipose tissue. Moreover, increased Th1 and Th17 cells in

obese individuals are associated with numerous pathologic conditions. Therefore, therapeutic strategies are needed to shift Th1/Th2 and Th17/T-regulatory balance toward Th2 and T-reg cells respectively. Among nutritional strategies, the potential benefits of weight reduction and several micronutrients like vitamin A, E, D and microelements should be considered. **Key words:** Obesity, Autoimmunity, Nutrition

Prenatal and postnatal genetic diagnosis of primary immunodeficiency disorders: From routine methods for diagnosis to new molecular assessments for newborn screening

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The primary immunodeficiency disorders (PIDs) are a heterogeneous group of diseases and the genes responsible for many of them have been identified. Prenatal diagnosis of PIDs that are caused by mutations and genetic defects appears to be important to prevent the birth of infants with chronic and disabling diseases. Fortunately, such a possibility for prenatal diagnosis of all common primary immunodeficiency diseases such as Chronic granulomatous disease (CGD), Severe combined immunodeficiency (SCID), X-linked agammaglobulinemia (XLA), Wiskott–Aldrich syndrome (WAS), using PCR method and gene sequencing analysis has been established in Immunology, Asthma and Allergy Research Institute (IAARI). In addition, this method can be applied for confirming the diagnosis of patients suspected to PIDs following clinical evaluations and immunological screening for innate and adaptive immune systems. Recent studies show that the incidence rate of PIDs is higher than all of the previously reported cases in scientific literatures especially in countries (such as Iran) with a high rate of consanguineous marriages. Considering the importance of early diagnosis of PIDs, particularly B and T cell deficiencies and related mortality due to the risks of recurrent infections and even death following BCG vaccination, several approaches have been made in order to screening the newborns early after birth. One of the recent, precise method with a high percentage of specificity and sensitivity would be measuring the circular DNA fragments known as T-cell receptor excision circles (TREC) and κ-deleting excision circles (KRECs) using Multiplex Real Time PCR. This technique is currently used in more than 20 states in America as the neonatal screening program and gradually also in European countries. Newborn screening for T/B cell deficiencies especially SCID would be a valuable way to screen the patients, immediate immunological evaluations and finding the best donor for bone marrow transplantation (BMT). BMT is considered as the definitive treatment approach with the highest success rate in SCID patients if done before 3 to 3.5 months after birth.

High throughput HLA typing

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Beside their role in normal and pathologic immune responses, Human Leukocyte Antigen (HLA) molecules also play pivotal role in transplantation process. HLA genes are the most polymorphic genes in the whole genome and determining the alleles of HLA, so-called HLA typing, for both donor and recipient is more important in hematopoietic stem cell transplantation. Because of high cost and technical challenges, typing of such highly polymorphic alleles is a main struggle for registries to find potential volunteer donors. Newly-introduced molecular PCR-base techniques help us to perfume high-resolution HLA typing. They are cost-effective and more accurate than conventional low- and medium-resolution HLA typing methods including restriction fragment polymorphisms (RFLP), sequence specific primers (SSPs), sequence based typing (SBT), sequence-specific oligonucleotides (SSOs)

and single strand conformation polymorphism (SSCP). Of several approaches introduced by different study groups, next-generation sequencing (NGS) and SSOP-based Luminex method are more interested and used for HLA typing.

Interaction Between Skeletal Muscle and Immune System Following Exercise Training

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The immune system is a system that normally protects us from the many bacteria and viruses that exist in the world. On the other hand, this system could help for skeletal muscle adaptation following exercise training. Skeletal muscle was identified as an organ that produces and releases cytokines. Therefore, it has been suggested that cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert paracrine, autocrine, or endocrine effects should be classified as "myokines". It is now known that skeletal muscle has the capacity to produce and express cytokines that belong to distinctly families, the list includes IL-6, IL-8, IL-15, LIF, and brain-derived neurotropic factor. Given that skeletal muscle is the largest organ in the human body, the discovery that contracting skeletal muscle secretes proteins established a novel paradigm: skeletal muscle is an endocrine organ that produces and releases myokines in response to contraction, which can influence metabolism in other tissues and organs such as the liver and adipose tissue. The discovery that exercise provokes an increase in a number of cytokines suggests a possible link between skeletal muscle contractile activity and immune system changes. Myokines have contribution to Th1/Th2 immune responses. On the other hand, one of the main factors determining the function of satellite cells within injured skeletal muscle is infiltrating immune cells. It seems that immune cells could active satellite cells to induce skeletal muscle adaptation following exercise training.

Molecular engineering and pharmaceutical applications of monoclonal antibodies

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Molecular engineering is an ideal method to modify antibodies in numerous ways, to meet various clinical requirements. We can modify the molecular size, immunogenicity, stability, binding affinity, specificity and functionality of antibodies by antibody engineering methods. Moreover, it is possible to conjugate antibodies with various biological molecules such as toxins and imaging mediators to develop novel therapeutic agents or diagnostic procedures. Thus, production of safe and efficacious antibodies through molecular biology methods has become one of the main focuses in the last three decades in biopharmaceutical industries. Nowadays, more than twenty well characterized monoclonal antibodies are in routine clinical use. Moreover, some types of antibodies such as bispecific antibodies, diabodies and SCFV are not found in their natural forms and should be constructed by recombinant technologies. In this presentation we will discuss the recent trends in antibody engineering for improvement of their clinical applicability.

Immunologist and production of laboratory kits

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Mostly, immunologists spent much of their time behind research or clinical laboratory bench. However, increased demand for development and production of innovative immunoassays has forced them toward industrial ideas. Nowadays, most of improvements in the medical diagnostic methods, mainly occur in the field of immunodiagnosis. Therefore, identification, purification and production of immunogenic molecules and raising specific monoclonal and polyclonal antibodies could help them in development of novel immunoassays. In this presentation we will focus on this demand to introduce this opportunities for immunologists. In this way we hope that they will transfer their research-based products from bench to industry as an effective knowledge transfer technique.

Practical Research and Development in Pharmaceutical Industry

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In this paper we are going to have a distinguishable meaning of practical R&D to emphasize on differences between Research and Research & Development. It could be guide for R&D specialists as to how they can quickly evolve skills built around three factors -Man, Mind and Time. It covers the management of scientific personnel, management within a variety of R&D organizational structures, creating a climate of innovation, the management of projects including the time management and communication aspects of the job. As such, it reaches to the vital holistic approach to the R&D specialty in pharmaceutical industry, which are not taught at university, providing a deep and detailed insight into the intricacies of R&D concept. We suggest these concerns and knowledge to all of the R&D staffs and leaders: Harnessing the human resource, building the scientific skills base of the group, developing the people who form the skills base, the R&D team management, innovative environment, the structural components of an R&D organization, The provision of the appropriate support ,a financially sound, healthy, safe and quality environment, creativity and innovation, the protection of intellectual property, the project management of innovation, the selection and evaluation of R & D targets, the innovation chain, the project management skills and finally professional ethics.

Evaluation of serum level of IL-33 in periodontitis patients with and without type II Diabetes

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Introduction: Periodontal disease refers to the pathological inflammation of periodontal tissues surrounding the teeth that leads to the gingivitis or periodontitis. Type two diabetes is a form of hyperglycemia and altered lipid metabolism which also causes many pathological changes in the oral cavity. The relationship between these two diseases under the effect of immunological and non-immunological factors has been examined. This study has been conducted and aimed to determine the serum levels of interleukin-33 (IL-33) in periodontal patients, suffered from type two diabetes and in non- diabetic periodontal patients. **Materials and Methods:** This case-control study was done on periodontal patients suffering from type two diabetic and non-diabetic referred to periodontology section of dentistry faculty, and diabetes clinic of Ali Asghar Hospital of Zahedan during 2014-2015 .The method of sampling was easy non-probability or available that after obtaining informed consent from individuals 5 ml`s peripheral blood was taken and serum levels of IL-33 in patients was measured by ELISA method and to describe the data and from the central index and dispersion, T-independent test, Mann-Whitney U test and Chi square test were used for normally distributed variables, variables that were not distributed normally and Qualitative variables respectfully. **Results:** The results of IL-33 serum analysis showed that serum levels of IL-33 in patients with diabetic plus periodontitis was lower than non-diabetic, periodontitis patients that was statistically significant (P = 0.002). **Conclusions:** The serum levels of IL-33 in periodontal patients with type II diabetes, decreases significantly compared to non-diabetic patients. **Keywords:** Serum levels of IL-33, type II diabetes and periodontal disease

Exercise in polluted air and immune function

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Air pollution in large, industrial cities has become a serious health concern. On the one hand, living in polluted cities is inevitable, and on the other hand, its consequences for the life of their citizens make them worried. Cars, factories, etc. account for the most part of the air pollution in large cities, with Carbon monoxide (CO), Nitrogen oxides (NOX), Ozone (O_3), Particulate Matters (PMs), Sulfur dioxide (SO_2), and Volatile organic components (VOCs) being the primary pollutants. Among these, PMs has attracted special attention due to its ability to penetrate deep into the lungs and then affect internal tissues and organs. According to unofficial statistics, the mortality rate due to air pollution in 2013 in Tehran included 2658 deaths from PMs, 15 deaths from Carbon monoxide, 72 deaths from Ozone, and 896 deaths from Sulfur dioxide; meanwhile, the total statistical value of deaths caused by Tehran air pollution was estimated to be more than 328 million USD in the same year. Many government bodies have been concerned with this issue and each has applied some temporary measures. However, none has been able to develop a working solution to date. Among the proposed solutions to tackle this problem have been closing schools and offices, and imposing a traffic plan according to vehicles plate number being odd or even.

Air pollution has direct relationship with development of acute inflammation and respiratory symptoms, and exacerbation of chronic inflammatory conditions such as asthma and cardiovascular diseases. Many inflammatory mediators play an important role in both cellular and physiological responses to air pollution particles, such as cytokines and chemokines. Cytokines are proteins that are secreted by cells of the innate and adaptive immune system and mediate the function of these cells. These are produced in response to microbes and other antigens and each has different effects on cells involved in immune and inflammation. Chemokines are a large family of cytokines with a similar structure that stimulate leukocyte movement and regulate the migration of leukocytes from

the blood to tissues. One of the problems facing the population in polluted cities is doing physical activity in these conditions. The general opinion, advocated by physicians, is to avoid physical activity. Exposure to air pollution is associated with increased risk for developing diseases, including cancer, asthma, Alzheimer's, cardio-respiratory disease among others. Exercise is characterized by increased metabolism and respiratory rate, which means a greater volume of contaminants, will enter the body through respiratory tract. Nonetheless, can we consider that doing exercise in polluted air is harmful? Recent studies have shown that physically active people in polluted cities refer to the hospital and medical centers less frequently than others and are less likely to develop disease or die from air pollution compared with inactive people. Recent studies have shown that risk of diseases such as lung cancer, asthma and allergies, cardiovascular disease and Alzheimer's disease could be reduced by having an active lifestyle. Sports activities enhance the immune system and improve the body's anti-inflammatory system, reducing the risk of developing diseases. So it is safe to recommend people in polluted cities to adopt an active lifestyle. Of course, it should be noted that on those days when pollution levels are extremely high, physical activities should be limited to indoors equipped with air conditioning systems. To sum up, people living in polluted cities may develop resistance against the adverse effects of air pollutants through pursuing an active lifestyle.

The roles of bacteria and immune system in pathogenesis of periodontal diseases

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Periodontitis is defined as an inflammatory disease of tooth supporting tissues. Periodontal disease results from a complex interplay between the subgingival biofilm and the host immune–inflammatory events that develop in the gingival and periodontal tissues in response to the challenge presented by the bacteria. Periodontal disease is a unique clinical entity. It is not an infection in the classic sense of the word. With most infections, a single infective organism causes the disease. With periodontal disease, a large number of species are identifiable. Of course, the Gram-negative asaccharolytic bacterium *P. gingivalis* has long been associated with human periodontitis and its capacity to induce the disease in rodent or non-human primate models appears to confirm its role as a causative organism. *P. gingivalis* can impair host defenses in ways that alter the growth and development of the entire microbial community, thereby triggering a destructive change in periodontium. Specifically, by subverting innate immune signaling including the crosstalk between complement and Toll-like receptors (TLRs) which leads to exacerbation of destructive inflammatory response by inflammatory cytokines.

Recent studies suggest that *P. gingivalis* could additionally modify the adaptive immune response. Specifically, the interaction of *P. gingivalis* with dendritic cells induces a cytokine pattern that favors T helper 17 polarization at the expense of the TH1 lineage. TH17 has potent role in destruction of bone which is an important feature of periodontitis. Moreover, *P. gingivalis* inhibits gingival epithelial cell production of TH1-recruiting chemokines and synthesis of interferon- gamma (IFN-γ). On the contrary of TH17, TH1 has modulatory role in periodontitis, thereby pathogen cause the breakdown of balance of immune responses.

Allergy & Asthma

Oral Presentations:

9736O

Cloning and Expression of the Recombinant Major Allergen of Salsola kali Pollen (Sal k 1) in Probiotic Lactococcuslactis

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Introduction: The recombinant major allergen of Salsola kali pollen is a 40 KDa protein that can cause allergic rhinitis. The aim of this study was cloning and expression Salsola kali allergen in probiotic Lactococcuslactis as a live vaccine for allergy treatment. **Material and Methods:** To produce recombinant Sal.k1 allergen, the S. k1 gene was amplified by PCR method. Then it was inserted into the PNZ 8148 vector and transported into competent E. coli strain MC 1061.next, recombinant PNZ 8148 was extracted and transferred to competent NZ9000 Lactococcuslactis. In order to consider the recombinant L. lactis expression to be taken into account, Mouse Sal k1 against polyclonal Antibody was produced. **Results:** The Sal. K1 DNA was properly inserted into PNZ vector. The transformed L.lactisjust expressed recombinant Sal k1 protein. Sal k1 against polyclonal Ab produced in mouse. **Conclusion**: This study would be useful to the studies in endemic regions of allrergic rhinitis that are managed by Sal k1. Recombinant L.lactis can be used as a live mucosal vaccines for allergy treatment.

10897O

The comparison of different mouse models of allergic asthma

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Allergic asthma is a chronic obstructive pulmonary disease characterized by inflammatory infiltrating cells in lungs, predominantly by eosinophils, mucus hypersecretion, antigen specific immunoglobulin and air way hyperresponsivenessin response to Metacholine. According to WHO, Asthma affects more that 300 million people worldwide. Treatment of allergic asthma is one of the obstacles of medicine. Animal models are great tools for exploring the pathogenesis of allergic asthma and for testing new drugs for treatments. The availability of a proper model is one of the specific goals of scientists. Therefore, in this study we induced allergic asthma in mice through different methods and compared the result based on inflammatory infiltrates in bronchial alveolar lavage and lungs, airway hyper responsiveness, cytokine production in lymph nodes and Spleen, allergen specific IgG 1 and IgE.This study would help scientist to choose the right model for their experiments.

10825O

Investigating the association of ADMA33 single nucleotide polymorphisms (SNPs) with susceptibility to asthma in Azeri population of Iran

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Introduction: Asthma which is affecting growing number of population is a clinical condition with complex cellular and genetic factors. Single nucleotide polymorphism (SNP) in gene coding for molecules playing major roles in the immunopathogenesis of asthma have been interested recently as genetic predisposing factors. Possible association of two SNPs in ADAM33 (a disintegrin and metalloprotease 33) which participates in airway remodeling with susceptibility to asthma was studied in this study. **Material and methods:** 190 patients with asthma and 180 healthy controls were enrolled in this case-control study. Using conventional PCR method, specific bands were amplified and the frequency of genotypes of T1 (rs2280091) and V4 (rs2787094) ADAM33 SNPs were determined by digestion with NcoI and PstI, respectively. **Results:** Frequency of genotypes of T1 and V4 were not significantly different between patients and controls (p=0.54 and p=0.85, respectively). In addition, no significant differences were seen in allele frequency of both T1 and V4 SNPs (p= 0.47 and p= 0.15, respectively). **Conclusion:** In contrary to some other similar studies in different populations, our results showed no association between frequency of genotype or alleles of both T1 and V4 SNPs in ADAM33 gene and predisposition to asthma in Azeri population of Iran. Genetic differences in different ethnic groups might be involved in such inconsistent results. More studies in populations with larger number of patients and healthy individuals are needed for concluding remarks for involvement of ADAM33 SNPs in asthma.

10902O

Evaluation of the skin prick test sensitivity to Birjand pine's pollen and commercial pine extracts among allergic rhinitis patients in Birjand city, Iran

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Introduction: Allergic rhinitis is the most common allergic disease around the world. Plant' Pollens trigger symptoms in patients especially in dry and windy areas. Pine trees are widespread throughout eastern part of Iran and are very abundant in Birjand city. In spite of their importance as a major source of pollination, there is no data about the allergenicity of pine pollen in this area. The aim of this study was to compare the allergenicity of Birjand's pine extract and commercial pine extract in a group of allergic rhinitis patients referred to Birjand Allergy Clinic during the Persian year of 1993.**Material and methods:** Pure pine pollen collected from pine trees in the city and aqueous extract prepared. Total protein content measured by Bradford method and SDS-PAGE was done for both domestic and commercial extracts. Allergic rhinitis patients participated in this study and skin prick test performed with a battery of common allergic extracts including domestic and commercial pine extracts. **Results:** SDS-PAGE

identified three different bands of 40, 50 and 70 kDa in Birjand's pine extract. The prevalence of skin prick test sensitivity to domestic and commercial extract was 9% and 15% respectively with no cross reaction. Grass and weed pollen were the most frequent allergens among the patients. **Conclusion:** The result of this study showed that in spite of the abundancy, pine's pollen is not a major allergen in this area and there is not much similarity between domestic and commercial pine extracts in case of allrgenicity. **Key words:** Allergy, pine pollen, Birjand, allergic rhinitis.

11177O

Evaluating the correlation between the number of eosinophils, Tregs, and T cells in esophageal tissue of patients with. Eosinophilic Esophagitis.

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Introduction: Eosinophilic Esophagitis (EOE), is a primarily polygenic allergic disorder, involving mechanisms that fall between pure IgE-mediated and delayed Th2 responses. There is an eosinophil-rich inflammation in the absence of known causes for eosinophilia. There is a strong correlation between allergy and EOE. Regulatory T cells may have an important role in allergies and eosinophilia. There are limited data on the association of Treg and EOE. In this study, we evaluated correlation between the number of eosinophils, Treg, and T cells. **Materials and methods:** Any pediatric patient with clinical symptoms of EOE, who had a normal pH probe study and had at least 15 eosinophils/HPF in esophageal tissue was considered to have EOE. We studied FOXP3+, CD3+ cells by immunohistochemistry assay. Those cells that were stained by anti- FOXP3 and anti- CD3 were considered as Tregs and those that were stained just by anti- CD3 were considered as Tcells. **Results:** Eosinophils, Tregs, and T cells were counted in HPF (×400) for 10 patients and the average of 3 HPF was recorded. There was a negative correlation between number of eosinophils and T cells (Pearson Correlation = -0.66, PV=0.05). No significant correlation was seen between the number of eosinophils and Tregs. There was a positive correlation between the number of eosinophils and the ratio of Tregs/ T cells (Pearson Correlation= 0.68 , PV=0.04).**Conclusion:** There is no significant correlation between the number of eosinophils and Tregs in esophageal tissue of EOE patients.

11150O

Comparative Predictive Analysis to Identify Human Allergens

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Introduction: Allergy has been considered as a key cause of disease worldwide, since 20% of people are affected by allergic rhinitis and have atopic dermatitis at some point in time. This occurs mostly by substances entitled allergens causing an allergic reaction. In some people, the immune system recognizes allergens as foreign or dangerous. As a result, the immune system reacts via producing a type of antibody called IgE to defend against the allergen. The identification of these components via experimental methods seems to be costly and time-consuming however most experiments do not show reliable outcome. Therefore, computational approaches due to their flexibility and high-throughput results are widely applicable in this task. **Material and Methods:** In this work we applied machine learning algorithms to detect whether one protein is allergen or not. These algorithms were trained with protein-driven features whereK-fold cross validation was applied to evaluate how reliable the algorithms are. The best algorithm was chosen to develop a predictor in order to identify allergens. To this, all proteins were used as input of predictor. **Result:** The results of this study showed high performance compared to experimental methods. The evaluation of algorithms in terms of accuracy, sensitivity and specificity were outstanding. This suggests that predictions made by our model, were reliable. **Conclusion:** Our developed predictor introduced potential allergen which can be used prior to any experimental task. This technique would ease the difficulty of experimental works in the field of allergen immunotherapy. These results can be widely used as a complementary method in detection of allergens. **Keywords:** Computational Method, Allergy, Allergen, Machine Leaning

Poster Presentations:

3469P

Production of polyclonal antibody against class IV chitinase by acrylamide as an adjuvant

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Introduction: Freund's adjuvant has been used for decades as an immune enhancer in immunization of laboratory animals. But it is not usable for insoluble unpurified recombinant proteins. To overcome this problem, we use polyacrylamide embedded proteins as a convenient and time-saving procedure. In this study we have succeeded in producing a specific antiserum against a cyctein-rich recombinant protein, class IV chitinase, in rabbit. **Materials and Methods:** The proteins were separated through electrophoresis in 12.5% SDS-polyacrylamide gel. After gel staining, the chitinase bands (32 kDa) were dissected, homogenized and injected subcutaneously into the seven sites and intramuscularly into the one site of rabbit, without any further purification steps. After 3 boosters (200 µg/ injection), the heart blood was collected and cross absorbed with BL21 bacteria to remove non specific antibodies. Finally, the antiserum was purified with protein A chromatography and confirmed with SDS-PAGE. The sensivity and specifity of the purified antibodies were determined with indirect ELISA and western blotting. **Results:** The resulting polyclonal antibody demonstrated high specificity and no cross-reaction on western blot. SDS-PAGE analyses of polyclonal antibody showed pure 50 kDa heavy chain and 25 kDa light chain. Furthermore, The dilution limit of this antiserum for indirect ELISA was up to 1:500. **Conclusion:** This study showed that polyacrylmide colud be a good choice of adjuvant for rabbit immunization. This procedure is more convenient and has a lower cost than freund’s adjuvant.

3476P

The effect of allergic asthma on mononuclear cells phagocytosis in comparison of healthy people

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Introduction: In many infections and diseases, induction and increasing power of immune system is necessary to cure and decrease the pathological effect. Phagocytosis is a primary step of immune system activation and pathogen elimination. Allergic asthma is an important immunological disorder with high prevalence in the world. **Materials and Methods**: In this investigation, strengthening the Monocyte phagocytosis is surveyed. For this purpose, 47 asthmatic patient and 60 healthy people were selected. Blood samples were taken and PBMCs were separated from each Heparin Blood specimens in the Faikol-Hypak style. Finally, the two tests, reduction Nitro Blue Tetrazolium (NBT) renewal and latex bed florescence phagocytosis were performed. **Results:** The results have shown that phagocytosis of asthmatic patients has no significant difference from healthy people ($P<0.05$). **Conclusion:** According to our study, allergic asthma has no effect on phagocytosis of PBMC and Monocyte-Macrophage phagocytosis pathway in innate immune systemdoes not change during allergic reactions. **Key Words:** Allergic asthma, PBMC, phagocytosis

3479P

A comparative different aspects quality of life in health adolescents with asthma adolescents referred to Ghaem hospital in Mashhad

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Introduction: The complex nature of asthma and negative effects on patient body, mind and spiritual state can affect their quality of life. The aim of this study was to compare the different dimensions of quality of life between healthy and hemophilia teenagers. **Materials and Methods:** In this descriptive-comparative study, 64 teenagers with similar demographic characteristics like age, sex, and socioeconomic status were randomly divided in two groups of case and control in Seyed Al-Shohada hospital. Data were collected through questionnaires filled out at two times by the researcher. Data were analyzed by using SPSS15, paired t-test, independent t-test and Man Whitney. **Results:** Average scores of physical, psychological and environmental aspects for quality of life and average total score of quality of life in the control group were significantly different from the case group ($p> 0.001$). The average scores of social dimention of quality of life in both groups in the first and second evaluation were not significantly different. **Conclusions:** Regarding the poor quality of life in teenagers with hemophilia compared with healthy teenagers, measures to improve the quality of life in all its aspects seem to be essential. **Key words:** Quality of Life, Asthma, Adolescent

3480P

Comparative study on the quality of life amongst asthmatic boys and girls aged between 7-17 in Mashhad

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Introductions: The quality of life in patients with chronic disorders is highly affected by the diseases, especially when such variables as gender and age interfere with the situation. Youngsters suffering from asthma face numerous physical, social and psychological problems. The aim of this study was to compare the quality of life amongst asthmatic boys and girls aged between 7-17 in Mashhad. **Materials and Methods:** In a descriptive-comparative study, 290 asthmatic children aged between 7-17 "minimum after six month of the disease were selected by consecutive sampling method. Demographic information form and Asthma Quality of Life Questionnaire (AQLQ) were completed by parents. The data were analyzed by SPSS software using T test, Mann- Whitney U and analysis of variance. **Results:** The study findings showed the mean score quality of life was (50.56, SD=19.45) for girls and (36. 02, SD=16.49) for boys. Statistical analysis showed a significant difference between the quality of life amongst asthmatic boys and girls (p=0.012). **Conclusions:** The difference between the quality of life in asthmatic boys and girls should be considered when planning programs to enhance their quality of life. Thus, it is proposed the quality of life to be tested in both genders, in different ages with different chronic diseases. **Key words:** Quality of life, Asthma, Children

3481P

Prevalence and Risk Factors of Asthma and Allergic Diseases in School Children Age Range (6-7 and 13-14 Years) from Assalouyeh City, Bushehr Province by using ISAAC Protocol

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Introduction: Asthma and allergic diseases present a major health burden. The prevalence of these diseases is increasing in various parts of Iran as well as the world. The aim of this study was to assess the prevalence of asthma and allergic diseases and their association with various risk factors among school children age range from Assalouyeh region, Bushehr Province. **Materials and Methods:** The ISAAC Phase I and III questionnaires were completed by parents of 190 children aged 6-7 years and self-completed by 223 students aged 13-14 years old. **Results:** The prevalence of atopic eczema, allergic rhinitis and asthma among6-7 year-old students were 11.6%, 13.7% and 5.8%, respectively. While, the prevalence of these diseases among 13-14 year-old students were found to be 14.3%, 21.5% and 15.2%, respectively. Consumption of fast and sea foods, pet keeping and parents smoking were associated with these diseases in both the groups (P<0.05). **Conclusion:** In our study the prevalence of asthma and allergic diseases in school children in Assalouyeh was high. Also there were strong relation between allergic diseases and fast and sea foods, pet keeping and parents smoking as risk factors. **Key words:** Asthma, atopic eczema, allergic rhinitis, ISAAC

3482P
Relationship between Food Allergens and Allergic Diseases based on Skin Prick Test in Bushehr

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Introduction: The prevalence of allergic diseases is increasing in recent years. Identification of reactive food allergens is important in the diagnosis and treatment of these diseases. The aim of this study was to determine frequency and relationship of common food allergens in patients with allergic diseases in the province of Bushehr. **Materials and Methods:** Skin prick test (SPT) was done by using 21 food allergens in 1100 patients with allergic diseases who referred to Shohadaye- Khalije- Fars university hospital. **Results:** The frequency of allergic rhinitis, asthma, chronic and acute urticaris and atopic eczema were 54.2%, 23%, 12.4%, 4.1% and 12%, respectively. The severe form of SPT reactivity were occurred with shrimp (P= 0.01), cow's Milk (P= 0.02) and peanuts (P= 0.04). The highest prevalence of food allergens also belonged to peanuts (46.6%), egg yolk (43.1%) and shrimp (42%). **Conculsion:** Findings of this study indicated that the severe reaction of SPT and relation between asthma and allergic diseases was seen with shrimp, cow's Milk and peanuts. **Key words:** Allergic diseases, Asthma, Food allergens, Skin prick test

3483P
Relationship between Aeroallergens and Allergic Diseases based on Skin Prick Test in Bushehr

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Introduction: Allergic diseases are increasing in the world as well as Iran. Aeroallergens have a main role in the pathogenesis of allergic diseases. The aim of this study was to assess the prevalence of aeroallergens and their relationship with the diseases based on skin prick test (SPT). **Materials and Methods**: SPT was done by using 22 aeroallergens in 1100 patients with allergic diseases who referred to Shohadaye- Khalije- Fars university hospital. **Results**: The severe form of SPT reactivity were occurred with Russian thistle (21.1%, P=0.001), Chenopodium album pollen (14%, P=0.001) and DermatophagoidPteronyssinus (9%, P=0.001). While, the highest prevalence of aeroallergens belonged to House Dust Mite (HDM) (69%), feather (60.8%), Russian thistle (59.9%) pollen and Alternaria mold (59.6%). **Discussion**: The study indicated that severe SPT reactivity with Russian thistle and Chenopodium album pollen as an outdoor allergen occurred in our patients . Meanwhile, the prevalence of indoor aeroallergens including HDM, feather and mold was high. **Key words**: Allergic Diseases, Asthma Aeroallergen, Skin Prick Test

3484P
Prevalence and Risk Factors of Asthma and Allergic Diseases in Primary Schoolchildren Living in Jam City from Bushehr Province by Using ISAAC Protocol, 2014

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Introduction: Asthma and allergic diseases have presented a major health problem throughout the world in recent years. The prevalence of these diseases has been increasing specifically in Iran. The aim of this study was to assess the prevalence of asthma and allergic diseases and their association with various risk factors among Primary school children age range (7-6and 14-13) from Jam City, Bushehr Province by using Phase I, III ISAAC Protocol. **Materials and Methods:** The ISAAC Phase I and III questionnaires were completed by parents of 516 pupils aged 7-6years and self-completed by 569 pupils aged 14-13 years. **Results :**The prevalence of atopic eczema, allergic rhinitis and asthma among 7-6years old pupils were 13.8 %, 20% and 3.9%, respectively. While, the prevalence of these diseases among 14-13 years old pupils were assessed 19.5%, 19.2% and 6.7%, respectively. There was an association between asthma, rhinitis and eczema ($P< 0.05$). Exposure to smoke, pet keeping and gas for cooking as risk factors were associated with these diseases in both the groups ($P< 0.05$). **Discussion:** In our study the prevalence of asthma and allergic diseases in school children was high. Also there were strong relations between allergic diseases and exposure to smoke, pet keeping and gas for cooking as risk factors. **Key words:** Prevalence**,** Asthma, atopic eczema, allergic rhinitis, ISAAC,

5496P

Evaluating Immunomodulatory Effects of Estrogen on IL-6, IL-17, IL-23 and TGF-B Expression in Phytohemagglutinin (PHA) Stimulated-peripheral Blood Mononuclear Cells of Patients with Asthma

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Introduction: TH-2 and TH-17 cells are involved in Asthma. Some cytokines such as IL4, IL5 , IL13 and IL-17 play important roles in the pathogenesis of asthma . In this regard , the present study aimed to investigate the impact of female sexual hormones on IL-6 , IL-17, IL-23 and TGF-B expression in PBMCs of patients with asthma .**Materials and Methods:** Blood samples were collected from 30 patients with asthma and 20 healthy individuals. PBMCs were separated and cultured in medium in four different conditions: Low concentration estrogen (10- 8M) , High concentration estrogen(10- 7M) , PHA alone , Without stimulation .The expression of IL-6, IL-17, IL-23 and TGF-β were measured by qRT-PCR.**Results:** No significant difference was revealed in the expression of IL-6 and TGF-β in PBMCs treated with estrogen. The patient group showed a higher IL-17 expression in low concentration estrogen in comparison with the other wells. The expression of IL-23 in patient group was higher in high estrogen concentration ($P=0.01$) . While IL-23expression in the control group both chambers with estrogen had a higher levels. Expression of IL-6 gene was higher among patients group ($P=0.002$) while the expression of IL-23 gene was lower ($P=0.5$) . **Conclusions:** Estrogen with its concentration-dependent manner play a major role in inflammation by secreting IL-17 and IL-23 and decreasing TGF-β. hormone therapy with estradiol in menopausal women should prescribe with precautions to prevent aggravation of asthma.

7564P

Role of Zn in Activation of Mast Cells in Allergic Reactions

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Introduction: The mechanism by which IgE triggers allergic reactions involves tissue mast cells (MCs) and basophiles. The two cell lines are granulated, distinct and functionally similar, contain histamine, heparin and have

high affinity IgE receptors (Fc_ERI) on their surfaces. They release histamine upon binding to IgE. However, their derivations and mechanisms of response differ greatly. Basophiles derive from the bone marrow and leave the marrow already mature, then remain in the peripheral blood where they comprise 1% of the circulating leukocytes. The MCs also derive from precursor cells in the bone marrow and migrate to specific tissue sites to mature. They are present from birth in maintained stable concentrations throughout life and play a central role in allergy reactions and asthma. The granules of MCs contain various chemical mediators and inflammatory cytokines that are released upon Fc_ERI cross- linking and activation. **Materials and Methods:** Two sites are used: http://www.ncbi.nlm.nih.gov/pubmed/16818790, http://www.ncbi.nlm.nih.gov/pubmed/21212618. **Results:** The granules of MCs are known to be rich in zinc (Zn). However, the precise molecular mechanism/s of Zn in MCs activation have not been clarified but it has been reported that there are multiple Zn-dependent mechanisms in MCs activation. **Conclusions:** The results of many different studies indicate that Zn chelators inhibit histamine release, and cytokine production in MCs. Interestingly, the inhibitory effects are rescued by ZnSo4 supplementation. In fact, Zn is essential for granule translocation to the plasma membrane, a process to be important for MCs degranulation and FC_ER1 mediated cytokine gene expression. **Key words:** Mast cells- IgE – Zinc- Histamine- Basophiles

7606P

Potential molecular changes on TLR4 at DNA and mRNA levels in canine leukocytes affected by atopic dermatitis

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Introduction: Canine atopic dermatitis (CAD) is a highly common skin disease in dogs especially terrier breed; its pathogenesis is multifactorial and associated with genetic factors. AD leads to loss of skin function withintermittentinflammatory reactions in humans and dogs. TLRs, especially TLR4, play a very important role in inflammatory responses to allergens. **Materials and Methods:** TLR4 mRNA expression in PBMC was comparatively analyzed in three groups of dogs (with AD, allergic skin diseases andcontrol or no allergy, n=8). Blood samples were collected from 28 dogs. The TLR4 transcription of PBMC was measured using qPCR. Additional experiments were done on leukocytes of 12 AD dogs for DNA extraction withdesigned primers to obtain desired fragment of TLR4 gene (1925bp); the PCR productswere then sequenced and analyzed with software. Also, we analyzed the single nucleotide polymorphism (SNPs) and sequence of desired fragment and then a molecular condition was designed for digestion of fragment with XceI endonuclease as PCR-restriction enzyme (RE) digestion method. **Results:** Compared to controls, expression level of TLR4 transcript in PBMC of AD dogs significantly down-regulated. But, only a slight increase in allergic TLR4 transcript was observed. We also confirmed well-known nonsynonymous SNPs in the desired fragment of TLR4gene in some AD dogs; RE digestion of desired fragment showed that one of the SNPs occurred exactly in the restriction site of XceI endonuclease. **Conclusion:** Considering the broad roles of TLR4 and PBMC in immunobiology, we provide new insights to understand the molecular mechanisms and clinical implications of TLR4in AD. **Key words:** Allergy, Atopic dermatitis, dog, TLR4, PCR, polymorphism

7617P

Determining the Fecal Release and Comparing the rsults with the Release of Cultural PCR of tcdA and tcdB Samples from *Clostridium Difficile* and Lac Lactobacillus in Patients with History of Allergies

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Introduction: Allergy is one of the problems of advanced societies. *Clostridium difficile* and Lactobacillus are from intestinal bacteria. The purpose of this study was to investigate the relationship between fecal release of *Clostridiumdifficile* and lactobacillus genes with the cultural release in allergic subjects. **Materials and methods:** 80 stool samples (40 samples from allergic subjects and 40 samples from non-allergic subjects), were taken. The samples were cultured for Clostridium on specific CCFA medium and for lactobacillus on specific MRS agar medium. DNA was extracted from stool cultures, fecal and cultural PCR was performed to amplify tcdA, tcdB and Lac genes. Data was analyzed by statistical analysis. **Results:** Out of 80 subjects, 65 cases (81.3%) of cultural PCR and 59 cases (73.8%) of fecal PCR were Lac positive. 13 cases (16.3%) of cultural PCR and 4 cases (5%) of fecal PCR were tcdA positive. 20 cases (25%) of cultural PCR and 5 cases (6.25%) of fecal PCR were tcdB positive. Lac cultural PCR,tcdA and tcdBfecal PCR had significant relationship with history of allergies.**Conclusion:** During the study, it was observed that there was inverse relationship between the presence of lactobacillus in feces culture and history of allergies, and also there was direct relationship between the presence of *Clostridium difficile* genes in stool (tcdA and tcdB) and history of allergies. **Keywords:** Allergy, Clostridium difficile, Lactobacillus.

7685P

Identification of fungal organisms in allergic patients with chronic Rhinosinositis(CRS)

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Introduction: Chronic Rhino-sinusitis (CRS) is considered as a chronic disease that influences life of more than 5% people in world. Allergic Fungal Rhino-sinusitis (AFRS) is the most common form of fungal rhino-sinusitis in patients, who suffer from chronic rhino-sinusitis. These infections have been mostly triggered from Aspergillus and Dematiaceous species. The main goal of present study is to identify a fungal organism in the nasal discharge/purulence/post nasal specimens of patients with CRS. **Materials and methods**: allergic patients, who had facial pain/pressure and nasal obstruction/blockage symptoms, were selected for a cross sectional analysis. After collecting nasal discharge/purulence/post-nasal specimens by Dacron swap, samples were cultured in mycology media and microscopic analysis was executed. Finally, positive samples were identified with respect to morphology and slide culture technique. **Results**: 40 samples of allergic patients suspected to fungal rhino-sinusitis were analysed. Among all these, 14 ones reported positive with respect to fungal features, in which 8 cases have been recognized as Aspergillus Fumigates, 2 cases as Aspergillus Niger, 1 case as Aspergillus Flavus, 1 case as Mucor and 1 case as Rhizopus. **Conclusion**: According to the fact that fungi spores are abundant in the environment, problems such as allergic Aspergillosis, fungal sinusitis, fungal rhino-sinusitis, as well as other fungal allergic infections might be considered in patients with allergic diseases. Rapid diagnosis of fungal infections and adding an anti-fungal treatment protocol can contribute to the healing process of allergic patients. **Key words**: Allergic patients, chronic rhinosinositis, fungal rhino sinositis

9802P

The most common food allergens in patients with allergy symptoms referred to Mofid Children's Hospital since 2013-15

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Introduction: Food allergy (FA) is a kind of heterogeneous diseases, affecting multiple organs like skin, respiratory or gastrointestinal tracts, which have different forms from a local allergy to a systematic anaphylaxis. The prevalence of FA is about 4% among children in the world and the prevalence is increasing recently. Th2 and IgE-mediated reactions play an important role in this disease. In this study we determined the most common food allergens in children referred to our hospital by measurement of specific IgE against common food allergens. **Materials and methods:** This cross-sectional study was performed on 416 patients with skin, respiratory and gastrointestinal allergies, which referred to Mofid Children's Hospital since 2013-15. Allergen specific IgE was measured via an immunoblotting method using AlleisaScreen® system kit and their data were collected. **Results:**Of 416 patients, 257 (61.8%) were male and 159 (38.2%) were female, and average age of patients was (6.58^{+} 3.2.) The values over 2+ were considered to be positive. The most common allergens were milk (36.5%), wheat flour (15.1%), egg white (12%) and sesame seeds (6.9%) respectively. **Conclusion:** This study showed that the most common food allergen was milk and the order of common food allergens in this study was different from other reports; this might be due to the different food habits and /or ethnic diversities. **Key words:** prevalence, food allergen, milk, wheat flour, egg, sesame seeds.

9811P

Chitin microparticlesdownregulate expression of CHID1 gene by in *vitro*mixed leukocyte culture.

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Introduction: chitin and its derivatesmicroparticles (MPs) have immunomodulatory activities. We examined the effect of size, purity and acetylation degree of chitin MPs on CHID1- encoding chitinase like protein, SI-CLP, involved in inflammation- gene expression in mixed leukocyte culture. **Material and Methods**: Small and medium-sized chitin MPs were prepared by sonication, and they were used in treatment of leukocyte mixed culture in comparison with chitosan and shrimp shell small-sized MPs. Neutral red uptake assay and microscopic examination of apoptosis were used to assess cytotoxicity of MPs and finally following cell treatment with MPs (100 µg/mL) for 48h, IL-6 production were measured by ELISA and expression levels of CHID1gene were determined by Quantitative Real Time PCR. **Results**: chitinous MPs of different concentrations had no cytotoxic effects. In gene expression analysis, Small-sized chitin MPs (<40 µ) resulted in down regulation of CHID1 gene expression (P=0.004), while other MPs didn't change it significantly. **Conclusions**: Size, purity and acetylation

degree of chitin MPs influence immune cells interactions and it seems small-sized chitin MPs can potentially modulate immune responses through decreasing CHID1 gene expression. Using small-sized chitin MPs may be effective to treat allergies which their treatment strategies rely on modulating the immune responses. **Keywords**: Chitin; chitosan; chitinase; immune modulation.

9842P
Study of relationship between serum vitamin D level and IgE in allergic children

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Introduction: In recent years, the role ofvitamin D deficiency in the incidence and aggravation of types of allergy has been highly considered and several studies have been done in this regard. Becausevitamin D deficiency as well as the prevalence of allergyis the important issuesin the health field, thisstudy was done toinvestigate the relationship between vitamin DlevelandIgE in children inTehran. **Material and Methods**: In a cross sectional study, 112 allergic children aged less than 12 years (64 boys and 48 girls) were evaluated. Vitamin D was classified in three levels as deficient (<20 ng/mL), insufficient (≥20 and <30 ng/mL), and sufficient (≥30 ng/mL). Vitamin D and total IgE levels were measured by ELISA. Prick and/or patch Skin test were used to confirm the presence of specific IgE. Finally, the data was analyzedstatistically. **Results**: The mean of vitamin D level was 27.14± 17.64 ng/ml that fall into insufficient range. The abnormal level of vitamin D was present in 61.9 % (20.5% insufficient and 41.4% deficient). Vitamin D deficiency was significantly higher in girls(Pvalue=0.03). Although vitamin D deficiency was increased with age, the mean of vitamin D deficiency was not different between age groups.however43% oftotal IgElevelswerehigher thannormal range, there was no association between serum vitamin D and IgE level. **Conclusions**: Given the role of vitamin D in the metabolism of children and its impact on the incidence of allergic diseases, design a national project to determinate the vitamin D deficiency levels and its associated risk factors in Iran, and screening for vitamin D deficiency at different ages seems necessary. **Keywords**: Childhood Allergy, Vitamin D deficiency, Serum IgE level

9844P
Prevalence of food allergens in patients with atopic dermatitis referred to the asthma and allergy clinic in Tehran during 2014

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Introduction: Atopic dermatitis is a common chronic inflammatory disease affecting the skin, and food allergy is one of the main factors for onset and severity of the disease. Understanding the pattern of sensitivity to food allergens in the community can play an important role in previous awareness and prevention of recurrence and severity of atopic dermatitis. The aim of this study is to recognize common food allergens in patients with atopic dermatitis in Tehran by skin prick test in 2014. **Materials and Methods:** In a cross sectional study in Tehran, all patients referred to the Asthma and Allergy Clinic during 2014 were examined and if positive for allergies, were enrolled. After the diagnosis of atopic dermatitis, skin pricktestwas performed forcommonfoodallergensin them. Finally, the data was analyzed by SPSSversion20. **Results:** Out of 1012patients with allergy, 282 ones(28%approximately) were diagnosed with atopic dermatitis, and 87patients (52female and 35 male) were sensitive to at least one food allergen (positive prick test). In patients with atopic dermatitis having a food allergy, the prevalence of asthma, allergic rhinitis and urticarial was 8.6, 11.4 and 5.4 %, respectively. There was no association between the sex and the food allergy in atopic dermatitis. The most common food allergens were egg yolk (38.4%), egg white (36.5%), hazelnut (33.3%), and peanut (28.7%), and lowest prevalence were found in rice (4.6%), barley (5.7%), and meat (6.9%), respectively. **Conclusion:** The prevalence of atopic dermatitis in allergic patients in this study was more than expected. Sensitivity to eggs and nuts as the most common allergens should be considered by families and allergy specialists.

9845P
Prevalence of Aeroallergens in patients with allergic rhinitis based on skin prick test in Tehran

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Introduction: Allergic rhinitis is one of the most common allergic diseases with increasing incidence worldwide and has an important role on life quality of patients according to hygienic and economic situation. Identification of aeroallergens in each region as one of the main causes of disease prevention and treatment of allergic rhinitis in these patients is important. The purpose of this study was to determine the prevalence of aeroallergens in patients with allergic rhinitis in Tehran. **Materials and Methods:** From January of 1391 until 1392, 400 asthmatic patients attended to the Khorshid Allergy and immunology Clinic in Tehran were enrolled. After recording patient information and according to the inclusion criteria, patients with allergic rhinitis were identified and their sensitivity to 12 common allergen extracts was determined by skin prick test. The data were analyzed by SPSS software, version 20. **Results:** The prevalence of allergic rhinitis was 40% with the same ratio between men and women. All patients were susceptible to at least one allergen, and 1.3 % were susceptible to all allergens. The most frequent positive skin reaction was observed for outdoor allergens (i.e., pollen) with a frequency of 20/54%. Indoor allergens included mites (66/46%), hair and skin of animals (78/18%), and fungi (37/17 %), respectively. Moreover, indoor inhaled allergens were more prevalent in the age group 1-6 years. **Conclusion:** The prevalence of allergic rhinitis in this study was high and similar to the global statistics. According to the high prevalence of allergic rhinitis in Iran,

identifying the causative factors can lead to appropriate measures to control and prevent severe allergic outcomes in patients. **Keywords:** Allergic rhinitis, Aeroallergens, Skin prick test.

9847P

Investigating the relationship between vitamin D levels and the amount of IgE in adults with allergic disease in Tehran

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Introduction: In recent years, vitamin D deficiency is a common problem of the world that has many complications, especially in adults. Studies suggest a relationship between low levels of vitamin D and allergic diseases. Given the role of vitamin D in the regulation of immune system, it seems this factor can be one of the effective factors in the development and exacerbation of allergic diseases. Hence, the present study examined the relationship between vitamin D levels and the amount of IgE in adults with allergies (type 1) in Tehran. **Materials and Methods:** In a cross-sectional study, 80 people with allergies (28 males and 52 females) older than 16 years old referring to the Khorshid Allergy and immunology Clinic Were evaluated. Vitamin D and total IgE levels were determined by ELISA. Three levels of vitamin D deficiency (less than 20ng / ml), insufficient (30-20ng / ml) and sufficient (more30 than ng / ml) were classified. In order to confirm the presence of specific IgE, skin prick test was used. Finally, the data was analyzed by SPSS software version 20. **Results:** The mean levels of vitamin D in the all subjects were 21.59 ng / ml that fall into insufficient range (less than 30 ng/ ml). Based on the results, the abnormal level of vitamin D was present in 76.25% (65% insufficient and 11.25 % deficient) and only 23.75 % of them had sufficient vitamin D levels. The results also showed abnormal level of vitamin D in women was more than men (67.21 % to 32.78 %). In addition, vitamin D deficiency was significantly associated with female group (p=0.01). **Conclusions:** In this study, Because of the prevalence of vitamin D deficiency, identifying the effective factors on vitamin D deficiency and also its impact on the incidence of allergic diseases, study of vitamin D deficiency and how to prevent it seems necessary. **Keywords:** Serum level of vitamin D, Serum IgE level, Allergies in adults.

9849P

Effect of serum autologous therapy in patients with idiopathic chronic urticarial

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Introduction: Chronic urticaria is one of the problems in the treatment of allergic and skin diseases and common treatments have systemic side effect or show inadequate effectiveness. Achievement to an effective, safe and

affordable therapeutic method in this field is essential. Serum autologous therapy method has demonstrated notable results recently. The aim of this study was to evaluate the effect of serum autologous therapy in chronic urticaria patients admitted to allergy clinics in Besat hospital-Sanandajin 2013-2015. **Materials and Methods:** In this clinical trial after receiving permission from the ethics committee, 64 chronic urticaria patients were selected randomly and were divided in two group, serum autologous treatment group and the control group (treated with standard methods of drug). 2.5 ml of patient serum was injected intra muscular weekly for nine weeks. Dermatology Life Quality Index(DLQI), severity of urticaria activity score (UAS)and some immunological parameters were used to evaluate patient healing atweeks12 and 16. Data was analyzed using SPSS.18software and independent t-test. **Results:** Quality of life means in treatment group34.84±16.21andcontrol group16.78±0.86 had significant difference($p<0.05$). Also UAS score in the treatment group showed more significant improvement than the control group($p<0.05$). Immunologic indices such as WBC, eosinophils and IgE showed no significant differences in the two groups($p>0.05$). **Conclusion:** The findings of this study approved the positive effect of serum autologous therapy in urticaria patients, nevertheless accurate and molecular evaluation of its mechanism are recommended in future study. **Key words:** Serum autologous, Idiopathic chronic urticarial, Skin diseases, Sanandaj

10847P
Evaluating the prevalence of inhaled allergens in patients with allergic rhinitis in Kerman city (2012-14)

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Introduction: Allergens are natural and chemical compounds that cause the hypersensitivity through stimulating immune system that is known as allergy. The only way to cure this disease is to identify and avoid patients from exposuring allergens. The aim of this study is to identify Inhaled allergens in patients with Moderate to severe Allergenic rhinitis in Kerman city using prick test. **Materials and Methods:** This study is descriptive-analytical one. Totally 47 Patients of Moderate to severe allergic rhinitis referred to Afzalipour hospital with regard to conformance of clinical and preclinical tests and condition for prick test involved in the study. All of the study populations had normal Positive histamine and negative saline test .To determine sensitivity of each patient for each of Inhaled antigens, skin prick test done using allergen extracts. Result analyzed with spss 19. **Results:** 84% of patients responded to at least one test. Weed mix was the most common inhaled allergens. 32% of patients had only moderate and 11/1% severe allergy. 56/9% patients, had both moderate and severe allergy. **Conclusion:** With regard to positive skin tests to Inhaled allergens in the patients it seems that avoiding from airborne allergens in different seasons and precautions in dealing with these allergens is necessary. Drug therapy and Desensitization are next steps in treatment of allergic patients.

10890P
The evalution of effect of thymoquinone (constituent of Nigella sativa) on A_{2B} adenosine receptors, gene expressionin lungand blood lymphocytes of asthmatic guinea pigs

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Introduction: Studies demonstrated that the thymoquinone, had preventive effect on asthma and precluded its pathological changes. In asthma, the responses of the immune system and related cells including T lymphocytes have been influenced. One of the regulating factors which are propounded in the inflammation are adenosine and adenosine receptors, therefore,blocking of A_{2B}receptorsinhibitstherelease ofpro-inflammatorymediators and actions ofT cells.This investigation was proposed to demonstrate its prophylactic effect on A_{2B}R gene expression in blood lymphocytes and lung in the presence ofA_{2B}R antagonist; MRS1706, in asthmatic guinea pigs.**Materials and Methods:** Seventy guinea pigs randomly divided into five groups; Control group,Sensitized group(S), S+TQ, S+Anta A_{2B}, S+TQ+Anta A_{2B}.One day after induction period, the animals were killed and 5 ml blood sample and 100 µg of left lung tissue were obtained immediately.RT- PCR was employed to assess A_{2B} genes expression among the target groups. **Results:** Blood lymphocytes**:** Compared to control group,A_{2B}R gene expression in S group,S+TQ group,S+Anta A_{2B} groupincreased significantly ($p<0.001$ to $p<0.05$).In S+TQ+Anta A_{2B} group is non-significant. Lung tissue: Compared to control group,A_{2B}R gene expression in S group,S+TQ group,S+Anta A_{2B} groupincreased significantly ($p<0.001$ to $p<0.05$).In S+TQ+Anta A_{2B} group, A_{2B}R gene expression significantly decreased compared to S+Anta A_{2B} groups ($p<0.01$).**Conclusion:**Gene expression of A_{2B} receptor in blood lymphocytes and lung tissue in groups showed that thymoquinone administration influence more on blood lymphocytes while administration of the selective A_{2B} receptor antagonist was more effective in lung tissue.**Key words:** asthma, adenosine receptor, adenosine receptor antagonist, gene expression

10913P

Evaluation of the skin prick test sensitivity to common allergens in patients with adenotonsillar hypertrophy

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Introduction: Adenoids and tonsils are a part of immune system which are responsible for antigen sampling from mouse cavity and antibody formation. Adenoid hypertrophy is a common problem in children and adenotonsillectomy is the most common operation in small children although is seen in adult as well. In spite of the high prevalence of adenoid hypertrophy, the cause of this problem is unclear and there are some evidences which support the role of allergic reactions in pathogenesis of adenotonsillar hypertrophy. The aim of this study was to evaluate the prevalence of allergic disorders in patients with adenotonsillar hypertrophy referring to Birjand'sEmam Reza hospital. **Materials and Methods**: 180 patients with adenotonsillar hypertrophy (mean age 13.4 years, range 3 to 51 years, M/F ratio: 51/48) were enrolled in our study. For all patients an allergy questionnaire was filled and skin prick test with a battery of 19 common allergen including tree and grass pollen, fruits, mites, molds and cockroach was done for all patients. **Results:** 38.3% of patients had a positive skin reaction to at least one allergenic extract. The most common allergens were weeds including Russian thistle (42.4%), Pigweed (37%), Ragweed (18.6%), chenopodiacea (16.5%) followed by tree's pollen (15.4%) storage mites (15.2%) and whole egg (14.8%) and Melon (12.2%). **Conclusion:** The results of this study show that a fairly large proportion of patients with adenotonsillar hypertrophy are sensitized to allergens particularly aeroallergens and therefore it is possible that allergy hasa role in development of this problem.

10918P

The effect of linalool extract of Ash pollens (*Fraxinus excelsior*L.) on Lymphocytes infiltration BALF in Respiratory allergic in mice model

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Introduction: Respiratory allergy is chronic inflammatory disease of the respiratory tract which is diagnosed by infiltration and edema of airways, lymphocytes, eosinophils and mast cell. Recent studies indicate to the therapeutic role of linalool essence. Due to anti-oxidant properties of linalool, it may prevent increasing free radical generation and be effective in reducing respiratory allergy complications. **Materials and Methods:** In this research, in order to investigate the effect of linalool extract on lymphocyte infiltration, fifty Blab/c mice model were classified into five groups, i.e. negative control (PBS), positive control by ovalbumin (OVA)-sensitized (injection/inhalation) and three groups of sensitized by ash pollens allergens 5%extract that two groups were treat by extract-receiving respiratory allergy with 50 mg/kg doses, respectively for linalool extract and linalool standard. We studied lymphocyte immigration and lymphocyte subpopulations in lung compartments. **Results:** The results showed a significant increase in lymphocytes of the lavaged fluid of sensitive by ash pollens animals in comparison to those of the control group and the number of lymphocyte also significantly reduced in the groups treated with linalool extract in comparison to those of positive control group($P<0.05$). Our data show that lymphocyte immigration is at least in part responsible for the increase in lymphocyte numbers in the BAL and lung parenchyma in this animal asthma model. **Conclusion:** These findings suggest linalool extract has effects on immune cells particularly that T-lymphocyte activation which cause contraction of the respiratory tract due to release of inflammatory mediators.

10938P

Prevalence of allergies among children with adenoid hypertrophy

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Introduction: Adenoids are a part of lymphoid system and have an important role in defense against pathogens especially in young children. Adenoid hypertrophy is common particularly among children and can cause snoring, mouth breathing and sleep apnea and also makes children prone to sinusitis and middle ear infections. Although the exact is not well known, evidences suggest that allergic reactions may have a role in development of adenotonsillar hypertrophy. The goal of this prospective study was to investigate the prevalence of allergies among children with

adenoid hypertrophy. **Materials and Methods:** one hundred forty children (mean age 9 years, range 3-13 years) with diagnosed adenotonsillar hypertrophy by an ENT specialist were interviewed by an allergist and a questionnaire about allergic disorders, family history of allergies and some environmental risk factors were filled. Skin prick test with common regional allergens was performed for all patients and serum total IgE was measured by ELISA method. **Results:** 37% of all patients had history of allergic diseases. 31% of children had a positive history of allergy in their parents or siblings. Body pruritus and Dyspnea were seen in 11% and 5% respectively. 36% had positive skin reactivity to at least one allergen extract. Presence of plants, birds and pets at home were reported by 32%, 10% and 1% respectively. **Conclusion:** Based on the results of the current study, allergies may have a predisposing effect on tonsillar hypertrophy and treatment of allergic disorders may reduce the severity of symptoms in patients with adenotonsillar hypertrophy. **Key words:** Allergy, Adenoid hypertrophy

10966P

Serum levels of IL-17 is a risk factor for asthma: A Meta-Analysis study

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Introduction: Allergic asthma is an airways inflammatory disease, characterized by reversible airway obstruction associated bronchospasm. The role of IL-17 in allergic diseases like allergic asthma has been investigated and several studies have been conducted on this issue. In this meta-analysis, the possible role of IL-17 in the pathogenesis of allergic asthma was studied. **Materials and methods**: Of five studies, 360 patients with allergic asthma and 164 controls for meta-analysis were identified. OR and 95% CI for serum levels of IL-17 and risk of asthma were performed via using fixed- and random-effects models. Heterogenesity of these studies were assessed using I^2. **Results**: The results showed an association between serum levels of IL-17and the risk of asthma. [OR = 1.954 and 95% CI = (0.873-3.036)] and the mean serum levels of IL-17 showed a significant difference between two groups of patients and controls. **Conclusion**: This results of this meta-analysis showed that the serum levels of IL-17 is significantly associated with the risk of asthma and increased levels of IL17 can be considered as a risk factor for asthma. **Key words**: Meta-Analysis, IL-17, Allergic athma.

10972P

Polymorphisms in IL18 as a risk factor for asthma: meta-analysis study

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Introduction: many studies have shown relationship between polymorphisms in IL18 and risk of asthma. Two most common polymorphisms observed in IL18, were IL18-607C/A and IL18-105 C/C. this meta-analysis was performed to identify the precise relationship between this two polymorphisms and risk of asthma**. Materials and methods:** Of 5 studies, 1074 patients and 729 controls were identified for meta-analysis. OR and 95%CI for polymorphisms in IL18 and asthma risk by using fixed- and random-effects models were performed. Heterogeneity of studies was evaluated. **Results**: results show relationship between IL18-607A/A (OR: 2.55; CI 95%: 1.24-455) and risk of asthma but no evidence supports an association between IL18-105 C/A (OR: 0.46; CI 95%: 0.64–1.25) and asthma.

Conclusion: Meta-analysis study showed that polymorphism in the gene IL18-607C / A has significant association with the risk of asthma.

10985P
Expression of a hybrid protein from *Chenopodium album* allergens in pichia pastoris expression system

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Introduction: Chenopodium album is an important source of pollen allergens that elicit rhinitis allergy in desert and semi desert areas of the world with a prevalence of 62.9%, 53% and 70.7% in Iran, Saudi Arabia and Kuwait respectively. Todays, recombinant allergens havea promising future in improvingIn vivo and in vitro diagnostic tests. In this study, we aimed to express a recombinant hybrid molecule (rHM) consisting of Che a 1, Che a 2 and Che a 3, as the most important allergens of C. album, Pichiapastorisexpression system. **Materials and methods:** A1182bp DNA construct has been cloned into the pPICZα A plasmid and expressed in the methylotrophic yeast Pichiapastoris. Maximum yield obtained after 4 days of induction. The secreted recombinant HM was purified by nickel-affinity chromatography. IgE reactivity of the rHM was evaluated by western blotting using 21 sera of C. album allergic patients. **Results:** The results showed that the rHM was expressed mainly as the soluble protein with an apparent molecular mass of 46 kDa in the SDS-PAGE analysis. The patient pooled serum showed high IgE reactivity with rHM in western blotting. **Conclusion:** The findings suggest that posttranslational modifications (e.g., glycosylation), which occur in eukaryotic cells such as yeast, are necessary for the production of a biologically active allergen for diagnostic tests. **Key Words**: Allergen, hybrid protein, pichiapastoris

11059P
Serum Levels of the CC Chemokines CCL2, CCL5, and CCL11 in Food Allergic Children with Different Clinical Manifestations

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Introduction: Food allergies (FA) are frequent in 8% of children under 3 years old and approximately 2% of adults. Chemokine are involved in various allergies such as FA. The present study was aimed to determine CCL2, CCL5, and CCL11 levels in FA. **Material and Methods:** The study population of this cross-sectional study contained 63 patients suffering from FA and 100 healthy controls. Concentrations of CCL2, CCL5, CCL11, and IgE were measured by enzyme-linked immunosorbent assay (ELISA). Eosinophils were counted using Casy I cell counter + analyzer system model SCAREF system GmbH. Differences were considered significant at $P<0.05$. **Result:** Current results showed that FA patients had significantly elevated numbers of circulating periphery eosinophils than the disease-free controls. Serum IgE levels in FA patients were also higher than controls. We also showed that serum levels of CCL2 and CCL11 were significantly enhanced in FA patients compared to controls but CCL5 was not detectable. **Conclusion:** Overall, findings of the present study proposed that serum levels of CCL2 and CCL11

are elevated in FA and these may be considered as useful parameters in diagnosis of disorder. It is also possible to design treatments on the basis of blocking of chemokines expression by application of antibodies against them to overcome allergic complications in patients suffering from FA.

11080P
Upregulation of gelatinase- Bin human monocytesand mouse macrophages by phytohemagglutinin in vitro

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Introduction: Macrophages and monocytes can induce inflammation by different mechanisms including matrix metallo proteinases (MMPs) production. MMP-9 known as gelatinase-B degredates the extracellular matrix and plays an essential role in several inflammatory disorders including allergic asthma. Lectins are carbohydrate-binding proteins exist in many nutrientsespecially plants. Phytohemagglutinin(PHA), a mostly studied lectin,is a T-cell mitogen with allergenic and inflammatory properties. In this study, effect of PHA on gelatinase-B activity in human monocytesand mouse macrophages has been assessed in vitro. **Materials and methods**: Human monocytic U937 cellsand mouse peritoneal macrophages were culturedin complete RPMI medium. Then the cells at logarhytmic growth phase were cultured in serum-free RPMI medium and subsequently were incubated with different concentrations of PHA (1-10 µg/ml)for 24 hours. The cell culture supernatants were collected. Nextthe activity of gelatinase-B wasevaluated by gelatin zymography. **Results:** PHA considerably increased gelatinase- B activity in human U937 cells and mouse peritoneal macrophages dose-dependently compared with non-stimulated control cells. **Conclusion:** Our results showed that PHA could be a potential stimulator of gelatinase-B activity in monocytes and macrophages. Therefore, the inflammatory effects of PHA reported by others may be partly due to its enhancing effects on glatinase-B. Processing the PHA-rich nutrients such as kidney beansto remove, neutralize or decrease the PHA might be useful for prevention or alleviating the inflammatory-based diseases such as asthma in which gelatinase-Bis overexpressed. Besides PHA could be useful in screening of MMPs modulatorsin immunocompetent cells. **Keywords:** Phytohemagglutinin, gelatinase-B, macrophages

11086P
Evaluation the mast cells recruitment post injection of formal saline 5% in rat's plantar dermis

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Introduction: Mast cells determine as induction sensitivity (allergy) that causes symptoms sharply allergy apposed of external antigens, allergens and toxins in dermis and mucus. This study performed to evaluate mast cells recruitment after formalin stimulation in different times. **Materials and Methods:** 36 Wistar rats were randomly and equally divided in 6 groups. Control group was injected normal saline (50µl) in dermis of right plantar skin and in next groups formal saline 5% (50µl) injected in dermis of right plantar skin. After1, 2, 6, 24, 96 hours samples of plantar skin obtainedrespectively. Samples were fixed in formal saline 10%, passaged, and prepared paraffin sections obtained, and hematoxylin-eosin and toluidine blue staining methods were performed. **Results:** Study of mast cells showed that these cells often accumulated around blood vessels and mean distribution of mast cells in superficial regions of dermis were more than the deep regions, and granular mast cells were often more than the degranular cells. This study showed that the mean distribution of mast cells increased significantly ($P<0.05$) in one and two hours after formalin injection, and then the number of mast cells decreased gradually until 96 hours after

formalin injection. Also mean distribution of degranular (active) mast cells in deep region of dermis were more than the superficial region. **Conclusion:** The observations of this study showed that the mean the number of mast cells in the first hours of inflammatory reaction was most deal increased, which causedrecovery trend to berapidly.

11104P

Evaluation of Prevalence food allergens in patients with atopic dermatitis in Kerman city, using skin prick test

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Introduction: Allergens are natural and chemical compounds that through stimulating immune system causes the hypersensitivity that is known as allergy. The only way to cure this disease is to identify and avoid patients from exposuring allergens. The aim of this study is to identify food allergens in patients with atopic dermatitis in kerman city using prick test. **Materials and Methods:** This study is descriptive-analytical one. Totally 62 Patients of atopic dermatitis referred to AfzaliPour hospital in 2011-2014 with regard to conformance of clinical and preclinical tests and condition for prick test involved in the study. All of the study populations had normal Positive histamine and negative saline test. To determine sensitivity of each patient for each of food antigens, skin prick test done with using of allergen extracts. Results analyzed with SPSS (version19). **Results:** Among study populations 29(46.7%) and 33(53.3%) respectively were Male and female. type 3 was common in atopic dermatitis patients that patients with this one were sensitive to food allergens. in food allergens egg white (29.6%), egg yolk (29.2%) and cow milk with 14.8% were prevalent ones. Allergy to egg whites ($P \leq 0.03$), egg yolk ($P \leq 0.002$), tomato ($P \leq 0.02$) was statistically significant among patients and in all of them frequency in males was higher than females. **Conclusion:** With regard to positive skin tests to food allergens in the patients it seems that avoiding from airborne allergens in different seasons and precautions in dealing with these allergens is necessary. Drug therapy and Desensitization are next steps in treatment of allergic patients. **Key words:** Prick test, Food allergens, Atopic dermatitis

11168 P

Detection of Clostridium difficile and Lactobacillus by Stool PCR and Culturing and Their Relationship with the Hematological Parameters in People with Allergies

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Introduction: Lactobacillus is a gram-positive, and anaerobic bacillus and clostridium is a gram-positive and anaerobic bacillus. The purpose of this study was to investigate the relationship among the presence of Clostridium difficile (tcdA and tcdB genes) and lactobacillus (Lac gene) by stool PCR and culturing with the hematological parameters in people with allergies. **Materials and methods**: Blood and stool samples were taken from 80 subjects (40 cases, 40 controls). Stool samples were respectively cultured on CCFA and MRS agar specific mediums for

clostridium. After DNA extraction from cultures and stools, clostridium and lactobacillus genes were amplified by PCR using specific primers. **Results:** In the case group, 28 samples of Lac stool PCR,19 samples of clostridium culture PCR and 1 samples of clostridium stool PCR were positive.Also Lac culture PCR was positive in 28 samples; the average blood PLT became 248.42, which was significantly different from other groups. In the control group, 37 samples of Lac culture PCR,31 samples of Lac stool PCR, 5 samples of clostridium stool PCR and 9 samples of clostridium culture PCR were positive. Of the 9 culture samples, 3 samples were positive for tcdA with an average PLT about 302.66 and 6 samples had tcdB with an average of MCH about 30.66, which showed significant difference to other groups. **Conclusion:** According to the results, it was found that there are significant relationships among Lac and tcdA cultures with blood PLT and also tcdB culture with blood MCH. **Keywords:** Clostridium difficile, Lactobacillus, PCR, Hematological Parameters.

11171P
In Vitro and in Vivo Anti Allergic Activity of Shallot (Allium hirtifolium) extract

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Introduction: Nowadays, the development of phytotherapies has aimed to inhibit allergic reactions. Allium has a long history of being used for the prevention and treatment of human diseases. **Material and methods**: In this study, we evaluated the anti-allergic activity of shallot extract (A. hirtifolium) on Th2 response of BALB/c mouse splenic lymphocytes. First, we prepared a hydro-alcoholic extract of shallot and examined it for toxic dose. The extract was prepared to study the effects of shallot on groups of BALB/C mice. two doses of extract (80 mg/kg ,400 mg/kg) injected intrapritonealy within 10 days in vivo. Also, the mouse splenic lymphocytes were treated with different concentrations of shallot extraction (from 7 to 25o µg/ml) in vitro. And then, Il-4 level assesed using Elisa method. We analyzed the expression of GATA3 level (Th2 transcription factor) by using real-time PCR. Also, The anti-allergic effects of extract were assessed in mice immunized with ovalbumin by IgE levels. **Results:** Our results showed that shallot significantly reduced Th2 response by reducing the expression of GATA3 and IL-4 levels. However, the extract has no considerable effect on ovalbumin-Induced allergic mice model in IgE levels. Considering the in vivo and in vitro results, it is suggested that the anti allergic effect of shallot may occure through IgE-independent pathway. **Conclusion:** Shallots are a safe and rich source of compounds with low toxicity. Therefore, shallots can be used as potential candidates for further investigations and as potential natural sources for the treatment of allergic diseases such as asthma.

11228P
Effects of *Clostridium difficile* Toxin on Fluctuations in IgE in patients referring to central laboratory in Ilam province.

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Introduction: *Clostridium difficile* is the intestinal normal organism thatits prevalence is associated with increased inflammation and allergies. The purpose of this study was to investigate the relationship between IgE as prognostic of allergies and the presence of the genes of toxin A (Tcd A) and toxin B (Tcd B) of *Clostridium difficile* in patients referring to central laboratory in Ilam province. **Materials and methods:** Stool samples from 80 patients referring to laboratory were cultured on specific medium for *C. difficile* in anaerobic conditions, then bacteria colonies were isolated and their DNA were extracted. Then PCR was done for two gene (tcdA, tcdB). Test of IgE was done by Elisa for evaluating Allergy. **Results:** Of 80 samples, 41 cases (51.25%) were positive and 39 cases (48.75%) were negative for Clostridium. 24 patients (30%) had toxin A, which 4 had high IgE, and 6 patients (7.5%) had toxin B, which had no high IgE, as well as 9 patients (11.25%) had both toxins A and B that two cases had high IgE. **Conclusion:** The results of the study showed that the fluctuations of IgE were higher in the group who had toxin A. This notes the possibility that part A by itself has more power in creating allergy, than when both parts A and B are present together.

11300
The Footprint of TGF-β in Airway Remodeling of the Mustard Lung

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Introduction: Mustard lung is a major pulmonary complication in individuals exposed to Sulfur Mustard (SM) gas during Iran-Iraq war. It shares common pathological and clinical features with some chronic inflammatory lung disorders, particularly Chronic Obstructive Pulmonary Disease (COPD). Airway remodeling, which is one of the main causes of lung dysfunction and the dominant phenomenon of chronic pulmonary diseases, is seen in mustard lung. Among all mediators involving in remodeling process, Transforming Growth Factor (TGF)-β plays a pivotal role in lung fibrosis and consequently airway remodeling. **Materials and methods:** In this review, it was searched some keywords: Sulfur Mustard with cellular and molecular mechanisms, oxidative stress, protease and anti-protease, inflammation and signaling pathways in various papers and databases from 1990 to 2015. **Results:** Regarding high levels of this mediator detected in mustard lung patients, it seems that the TGF-β has a key role in airway remodeling (including epithelial layer damage, sub-epithelial fibrosis, and angiogenesis). **Conclusion:** Finally, based on TGF-β targeting, we reviewed new airway remodeling therapeutic approaches.

11304P
Allergenicity study of mature pollen grains in *Rudbeckiahirta* L.

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Introduction: Due to flower stability and drought tolerant, *Rudbeckiahirta* L. is a popular ornamental plant of Asteraceae family, largely used in landscaping and prevention of soil erosion. Since pollen grains have considerable role in allergenic causes and 80-90% of allergens have plant origin, the aim of this research was to investigate the allergenicity of *Rudbeckiahirta* pollen grains in Wistar rat. **Materials and Methods:** In order to evaluate the experimental study, pollen grains were collected from eastern area of Tehran, Iran. Pollen extracts were prepared by incubating pollen grains in phosphate buffered saline, pH 7.4 in three different concentrations of 110 µg/mL, 210 µg/mL and 310 µg/mL, respectively. The allergenicity experiment was done on the Wistar rats with the same weight and age. The injections were continued within 3 weeks, once per week intraperitoneally with concentration of 100

µL. **Results:** The skin tests in Wistar rat treated with pollen extracts indicated wheal with diameter larger than control group. In clinical tests, the percentage of eosinophils and lymphocyte were significantly increased ($p<0.01$) in concentration of 310 µg/mµL compared with control group. Moreover, percentage of Monocyte in group treated with 110 µg/mL and 210 µg/mL showed significant difference in comparison with control and concentration of 310 µg/mL. Also the IgE measurement showed significant changes in sample injected compared with non-injected Wistar rat. **Conclusion:** It can be concluded that the usage of *Rudbeckiahirta* as decorative species should be limited in the crowded places in particular hospitals and kindergartens. **Keywords:** Allergen, *Rudbeckiahirta*, IgE, Pollen, Wistar rat

Cell & Gene Therapy

Oral Presentations:

6514O

Suppression of Snail1 inhibits cell migration and proliferation in esophageal cancer

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Introduction: The transcription factor Snail1 induces epithelial-to-mesenchymal transition (EMT) in tumor epithelial cells, a process associated with the emergence of stemness, invasion and cancer malignancy. In this study, the aim was to clarify the effect of a specific Snail1 small interference RNA (siRNA) on sensitivity of TE-8 human esophageal squamous carcinoma cell line to investigate cell proliferation and migration. **Materials and Methods**: TE-8 cells were transfected with specific Snail1 siRNA. Relative Snail1 and Vimentin mRNA expressions were measured by Quantitative real-time PCR. Western blot analysis was performed to determine the protein levels of Snail1. In vitro wound healing assay of TE-8 with or without Snail1 siRNA was evaluated. MTT assay was used to evaluate the proliferation of TE-8 cells after down-regulation of Snail1 gene. The number of apoptotic cells was determined with the TUNEL assay. **Results:** It was found that siRNA effectively inhibited Snail1 expression at both mRNA and protein levels in a concentration-dependent manner and repress the expression of Vimentin at mRNA level. Moreover, Knockdown of Snail1 by siRNA significantly diminished the activation of cell migration. Down-regulation of Snail1 inhibited the proliferation of TE-8 cells. Compared with the control group, siRNA targeting Snail1 significantly promoted cell apoptosis. **Conclusions:** The results indicated that down-regulation of Snail1 inhibits the proliferation of TE-8 cells by promoting cell apoptosis and significantly inhibits epithelial-mesenchymal transition and suppresses the migration of esophageal squamous carcinoma cell line TE-8.
Keywords: Esophageal cancer, Snail1, apoptosis, epithelial–mesenchymal transition

7520O

Suppression of cell migration by targeting BACH1 with specific siRNA in prostate cancer

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Introduction: Cancer lethality is mainly caused by metastasis. Therefore, understanding the nature of the genes involved in this process has become a priority. We hypothesized that BACH1 may be mediating metastatic behavior of prostate cancer and knockdown of BACH1 would reduce this property of prostate cancer. **Materials and Methods:** BACH1 was knocked down using siRNA in androgen insensitive prostate cancer DU145 cells and as confirmed by real-time PCR and the rule of BACH1 in migration of DU145 cells was examined by scratch wound healing assay. **Results**: In the DU145 cells after transfection with specific siRNA, BACH1 expression was remarkably reduced in mRNA level. More importantly, in this study it was shown that BACH1 mediated the metastatic pathway, as by knockdown the BACH1 and we can significantly prevent migration of DU145 cells. **Conclusion**: The results suggested that the BACH1 specific siRNA significantly prevented migration of DU145 cells and therefore could be considered as a potent therapy for metastatic form of prostate cancer.

10801O

A simple method for the generation of insulin producing cells from bone marrow-derived mesenchymal stem cells

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Introduction: Selective autoimmune destruction of the insulin-producing beta cells is the hallmark of the type 1 diabetes mellitus. Cell therapy has attracted the attention of researchers in recent years. Therefore, in the present study the capacity of a simple and inexpensive method for induction of insulin producing cells (IPCs) from bone marrow-derived MSCs has been investigated. **Materials and methods:** MSCs from Sprague Dawley rats were characterized and their differentiation into islet-like cells were compared in four different culture conditions. In the HG group, MSCs were cultured in L-DMEM media containing 450mg/l glucose and %10 fetal calf serum (FCS). In HG-NA, 10 mmol/L nicotinamide was added to the culture media. In the SS group, dishes were coated with 1% agarose to prevent MSCs attachments and facilitate their clustering. In SC group, cells were trypsinized before transferring into a 6 well plates. Aldehyde fuschine staining, immunocytochemistry and real time PCR were used to check differentiation into IPcs. **Results**: SS condition showed the best result for differentiation of MSCs towards IPCs. They formed clusters which resembled pancreatic islet-like cells. Aldehyde fuschine staining confirmed that in SS group the majority of cells were IPCs compared to the other groups ($p<0.0001$). Immunohistochemistry using a serum containing anti-insulin antibody confirmed the presence of insulin granules in SS group. Overexpression of INS-1 ($p=0.0001$) and PDX1 ($p<0.0004$) genes in SS group compared to MSC, HG, NA and SC groups was also confirmed. **Conclusion:** Culture of MSCs in SS condition is a simple and cost-effective method for IPCs induction. Further studies are required to show the in vivo efficacy of produced IPCs in the treatment of T1D. **Key words:** Type 1 diabetes**,** Mesenchymal stem cells (MSCs), insulin producing cells (IPCs).

10848O

Suppression of PTPN22 gene by siRNA induces apoptosis in T cell leukemia cell line (Jurkat) through the Akt signaling pathway

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Introduction: T cell acute lymphoblastic leukemia (T-ALL) is one of the most frequent malignancies related to T cells. The innovation of new treatment modalities to overcome ineffective therapies of tumor cells may be a potential source of improved therapies. The PTPN22 gene encodes the Lyp protein tyrosine phosphatase. Overexpression of PTPN22 gene was observed in hematopoietic malignancies including CLL and ALL. The aim of this study was to investigate the effect of specific PTPN22 siRNA, on the induction of apoptosis in Jurkat cells, and evaluation of apoptosis signaling pathways. **Materials and methods:** Jurkat cells were transfected by specific PTPN22 siRNA. Relative PTPN22 mRNA expression was measured via Quantitative Real-time PCR. Western blot analysis was performed to determine the protein levels of PTPN22. The cytotoxic effects of PTPN22 siRNA were determined using MTT assay. Apoptosis was quantified using Anexin V/PI assay. **Results:** In the Jurkat cells, PTPN22 siRNA effectively reduced PTPN22 expression in both mRNA and protein levels. Moreover, siRNA transfection had effects on Jurkat cells viability. More importantly, in this study it was shown that PTPN22 positively regulated the anti-apoptotic AKT kinase, which provides a powerful survival signal to T-ALL cells. **Conclusion:** The results suggested that the PTPN22 specific siRNA effectively decreases T-cell acute Leukemia cells viability, induces apoptosis in this cell line and therefore could be considered as a potent adjuvant in T-ALL therapy. **Keywords:** PTPN22, siRNA, Jurkat, apoptosis, Akt

10900O

Transgenic Mice Bone Marrow-Derived Mesenchymal Stem Cells Attenuate Bleomycin-Induced Pulmonary Fibrosis

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Introduction: Pulmonary fibrosis (PF) is a chronic and progressive form of interstitial lung disease. Mesenchymal stem cell (MSC) based therapy is a novel approach with high therapeutic potential for the treatment of lung diseases. Recent evidence suggested that mesenchymal stem cells (MSCs) can down modulate bleomycin-induced lung injury. This work examined whether intratracheal or Intravenous transplantation of transgenic mice bone marrow-derived MSCs can attenuate lung injury in mice. **Materials and methods:** PF was induced in Eight-week old male C57BL/6 mice by intratracheally administration of bleomycin (4U/kg) in 50 µL sterile PBS. Transgenic mice BM-MSCs were isolated and the expression of the GFP gene was confirmed using invert fluorescent microscopy. 10 days after the bleomycin injection, 1×10^5 MSCs in 50 µL PBS were injected in the PF mice through intratracheal or intravenous. On day 14 after stem cell transplantation, animals were sacrificed and lung was removed to assess MPO activity and pathological changes (hematoxylin/eosin and Masson's trichrome staining). **Results:** MPO activity was significantly increased in mice administrated with only bleomycin (PF group). However, transgenic mice bone marrow-derived MSCs transplantation, especially intratracheal, resulted in significant reduction of MPO activity as compared to the PF group. Also, intratracheal transplantation of this cells alleviates histological changes. **Conclusion:** Stem cell therapy using transgenic mice bone marrow-derived MSCs reduces inflammatory and fibrotic effects in experimental model of pulmonary fibrosis. **Keywords:** Pulmonary fibrosis**,** BM-MSCs**,** Transgenic**.**

11033O

MiR-21 and miR-200 expression profile in human breast cancer associated with metastatic related genes

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Introduction: Breast cancer is the most common cancers diagnosed among women in, worldwide. miR-21 and miR-200 are known as regulators of the epithelial–mesenchymal transition (EMT), however, their role in controlling the transition between tumoral and normal tissues is not well understood. **Materials and methods:** The expression of miR-21, miR200, MMP1, MMP9, MMP13, CXCR4, vimentin and E-cadherin in tumoral breast cancer tissues and normal marginal tissues was investigated using a quantitative Real Time PCR in 24 patients with grade 3-4 of breast cancer. miR-21, MMP1, MMP9, MMP13, vimentin and CXCR4 up-regulated greater than one fold and, miR-200 and E-cadherin down regulated significantly in tumoral tissues compared to normal marginal tissues. **Results:** Among the 24 breast cancer cases, high level expression of miR-21 was significantly correlated with MMP1, MMP9, MMP13, CXCR4 ($P < 0.05$, Fisher's exact text), and miR-200 was notably associated with vimentin and E-cadherin ($P < 0.05$, Fisher's exact text). This study could identify the differentiated miR-21, miR-200 and related gene expression in breast cancer and revealed that miR-21 up regulation and miR-200 down regulation in grade 3-4 of breast cancer. **Conclusion:** The results suggested miR-21 and miR-200 are key regulators in advanced clinical stage of breast cancer and they could be used as a diagnostic, prognostic and therapeutic markers in advanced breast cancer. **Key words:** MiR-21, MiR-200, Breast cancer, EMT

11131O

Efficient induction of immune tolerance by co-delivery of Mesenchymal stem cells and MOG-pulsed dendritic cells in experimental autoimmune encephalomyelitis

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Introduction: Mesenchymal stem cells are widely used as a general immuno-modulatory agent for treatment of multiple sclerosis. Adipose derived–Mesenchymal stem cells (AD-MSCs) are unique stem cells, which have been representing a promising cell-based therapeutic approach for autoimmune diseases, particularly MS and its related animal model, the experimental autoimmune encephalomyelitis (EAE) in this study simultaneous co-administration of AD-MSCs+DC-MOG through iv injection was evaluated for prevention of EAE. **Materials and methods:** AD-MSC was obtained from adipose tissue of female C57BL/6 mice . Three-lineage differentiation and stem cell capacity and marker of them by flow-cytometry was approved. Also, dendritic cells were confirmed for surface

markers e.g CD11c after differentiation from bone marrow in presence of IL-4 and GM-CSF by flow-cytometry. Then, MOG-pulsed dendritic cells, with MSC through IV was injected to EAE mouse. **Results:** The results showed that MSCs-+DC-MOG treated group has a dramatically augmented induced Treg cells (P-value <0.001) and IL-10 and decreased IL-17 compared to other groups. Altogether, the results indicated that applying these two cells simultaneously could improve quality of EAE prevention and played a critical role in down-regulating immune responses such as cell proliferation, inflammatory cytokines production e.g. IL-17 and IFN-γ and increase anti-inflammatory cytokine like TGF-β and IL-10 and their related transcription factors. **Conclusion:** The up- regulation expression of Foxp3 in MSCs-DC-MOG treated group could know as a valuable evidence supporting these cell based on preventive therapies. Considering the pivotal role of AD-MSCs as a safe cell based therapy, with general immuno-modulating properties as well as regenerating dendritic cell, was an Ag -specific tolerogenic in the immune system. Thus, it could be regarded as a future candidate to be used in clinic. **Keywords**: Adipose-derived mesenchymal stem cells (AD-MSCs), Dendritic cell (DCs), Treg and Experimental autoimmune encephalomyelitis (EAE)

Poster Presentations:

7514P

Suppression of HMGA2 Induces Apoptosis and Cell Cycle Arrest in Human Colorectal Carcinoma

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Introduction: Over expression of HMGA2, known as a small non-histone chromosomal protein, is associated with various tumors progression, including colorectal cancers. The aim of this study was to investigate the effect of a specific HMGA2 siRNA on apoptosis and cell cycle of HCT-116 (colorectal carcinoma cells). **Material and Methods:** The cells were transfected with siRNAs using transfection reagent. The cytotoxic effects of HMGA2 siRNA, on the colorectal carcinoma cells were determined using MTT assay. Relative HMGA2 mRNA and protein levels were measured by QRT- PCR and Western blotting, respectively. Apoptosis was measured by TUNEL test based on labeling of DNA strand breaks. Cell cycle was assessed by FACS, using PI DNA staining. Results: HMGA2 siRNA significantly reduced both mRNA and protein expression levels in 48 hours after transfection and dose-dependent manner in the colorectal carcinoma cells. It was also showed that the silence of HMGA2 led to the induction of apoptosis and arrest cell cycle in G2/M phases of interphase in HCT-116 cells in vitro. **Conclusions:** These results proposed that HMGA2 might play an important role in the progression of colorectal carcinoma, and be a potential therapeutic target for trigger apoptosis and arrest cell cycle in colorectal carcinoma. **Key words:** HMGA2 (High mobility group A2), small interference RNA (siRNA), colorectal carcinoma, apoptosis, cell cycle

7518P

BACH1 blockade by siRNA hinders migration of HT-29 colon cancer cells

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Introduction: Metastasis to distant organs is a hallmark of many tumor cells. BACH1 is a transcriptional factor its overexpression promotes the migration of cancer cells. BACH1 expression and its target genes are intimately associated with the metastasis possibility of clinical samples, and BACH1 reduction leads to meaningful depletion in experimental metastasis. **Materials and Methods**: To further delineate the role of BACH1 in colon cancer, its expression was inhibited using the siRNA method. Then, the wound healing assay was performed to evaluate the migration of HT-29 cells before and after the knockdown. **Results**: Quantitative RT-PCR analysis revealed that the expression levels of BACH1 mRNA in HT-29 cells were significantly declined after transfection. The results indicated that BACH1 suppression in HT-29 CRC cells prominently impeded cell migration. **Conclusion**: Collectively, these results suggested that BACH1 may function as an oncogenic driver in colon cancer and may represent as a potential target of gene therapy for CRC treatment. **Key words**: BACH1, siRNA, knockdown, colorectal, cancer, metastasis

7556P

siRNA-mediated silencing of MDR1 reverses the resistance to oxaliplatin in SW480/OxR colon cancer cells

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Introduction: MDR1 overexpression is one form of the multidrug resistance (MDR) phenotype, which can be acquired by patients initially responsive to chemotherapy. Development of multidrug resistance (MDR) is an almost universal phenomenon in patients with colon cancer. Overexpression of the *MDR1* gene and corresponding P-glycoprotein (Pgp) is one of the best known MDR mechanisms. Small interfering RNAs (siRNAs) were shown to be powerful tools for such a purpose. **Materials & Methods:** The aim of this study was to investigate the effect of a specific MDR1 small interference RNA (siRNA) on sensitivity of oxaliplatin-resistant SW480 human colon cancer cell line (SW480/OxR) to the chemotherapeutic drug oxaliplatin. Resistant cells were subsequently transfected with specific MDR1 siRNA. Relative MDR1 mRNA expression was measured by Quantitative real-time PCR. Western blot analysis was performed to determine the protein levels of P-gp. The cytotoxic effects of oxaliplatin and MDR1 siRNA, alone and in combination were assessed using MTT and the number of apoptotic cells was determined with the TUNEL assay. **Results:** The data demonstrated that RNA interference could down regulate MDR1 gene expression and reduce the P-gp level, and partially reverse the drug resistance in SW480/OxR cells in vitro. Therefore, the results could suggest that MDR1 silencing may be a potent adjuvant in human colon chemotherapy. **Conclusion:** In this study, we demonstrated that a MDR1 siRNA can sensitize SW480 colon cancer cells to the first line chemotherapeutic agent oxaliplatin.

7582P

Nanobody-based targeting of lentiviral vectors to VEGFR2-expressing cells for cancer targeted-gene therapy

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Introduction: Nanobodies derived from Camelidae heavy-chain antibodies, due to small size as well as high stability and affinity, represent a new and powerful means for cancer targeted-gene therapy. Vascular endothelial growth factor receptor-2 (VEGFR2), the key mediator of angiogenesis, is up-regulated in tumor associated endothelial cells, and hence, providing an ideal target for cancer targeted gene therapy approaches. Herein, the generation of a chimeric glycoprotein bearing a nanobody specific for VEGFR2 was described and used this envelope to produce a targeted lentiviral vector. **Materials and methods:** Sequence of a VEGFR2-specific nanobody was cloned into natural binding site of sindbis virus glycoprotein, recognized as a proper envelope for pseudotyping of LVs, to construct chimeric Sindbis-Nb. Lentiviral vectors (LVs) were pseudotyped with pSindbis-Nb (and pSindbis alone as control). The specificity and functionality of the incorporated nanobody was assessed via virus-capture ELISA. Finally, selective infectivity of Sindbis-Nb pseudotyped LVs was analyzed by transduction experiments on VEGFR2 expressing (293/KDR) cells and non-target control (293T) cells. **Results:** Western Blot analyses of LVs pseudotyped with Sindbis-Nb revealed a single band around the expected size (66.3kDa) corresponding to the incorporated fragment (Sindbis-Nb). Capture ELISA demonstrated 8.5-fold increase in attachment of Sindbis-Nb containing LVs to VEGFR2 in comparison to Sindbis pseudotyped LVs ($P \leq 0.0001$). Flow cytometric analyses indicated the selective and efficient transduction of 293/KDR cells (30%) by the employed VEGFR2-Nb compared to 293T cells (1-2%) **Conclusion:** Results of this study indicated the potency of VEGFR2-Nb pseudotyped LVs for cancer-targeted gene therapy in VEGFR2-expressing tumor cells.

7615P

Evaluation of Tat penetrating peptide for *in vitro* transfection of HIV-1 Nef protein

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Introduction: Development of an effective vaccine against HIV-1 infection is a main concern in worldwide. HIV-1 regulatory protein, Nef, is an attractive vaccine target because it is involved in viral pathogenesis, is expressed early in the viral life cycle and harbors many T and B cell epitopes. The HIV-1 Tat protein when fused with heterologous proteins or peptides, can traverse biological membranes in a process called “protein transduction,” delivering its cargo into cells. Among different vaccine strategies, protein-based vaccines are capable of generating CD8+ T cell responses in vaccinated animals and humans. Thus, determination of the best delivery system for protein transfection is one of the most important concerns. In current study, the potential of Tat transduction domain was evaluated to transfer HIV-1 Nef protein into cells. **Materials and Methods:** A fusion protein was produced named Tat-Nef and transfected it in mammalian cell line. At first, the Tat-Nef gene was cloned in PET-26b bacterial expression vector and expressed in bacterial BL21 (DE3) strain. The Tat-Nef expression was detected by SDS-PAGE and western blotting using Anti-Nef antibody. Then, the recombinant proteins were purified using affinity chromatography. The transfection efficiency of Tat-Nef protein was evaluated in comparison with Nef protein in

HEK-293T cell lines. **Results:** Our results showed a clear band of ~ 726 bp for Tat-Nef in agarose gel. In addition, a ~27 kDa band of Tat-Nef protein was revealed in SDS-PAGE and western blot analysis using Nef monoclonal antibody. The results of transfection indicated a proper band (~27 kDa) for Tat-Nef protein delivered in HEK 293T as compared to Nef protein and un-transfected cell line without the use of carrier. **Conclusion:** The efficient delivery of Nef protein *in vitro* supports the potential of Tat penetrating peptide as a potent carrier for the next use in animal model. **Keywords:** Cell penetrating peptide, HIV-1, Tat peptide, Nef protein, Bacterial expression system, cell transfection

9798P

Apoptotic effect of specific siRNA against HMGA2 on prostatic Adenocarcinoma (PC3)

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Introduction: Prostate cancer is one of the main reasons of death between men. Although there are many methods of treatment for this cancer, many advanced prostate cancer patients still died of the postoperative recurrence and metastasis of disease. Therefore, the innovation of new treatment modalities to overcome these ineffective therapies of tumor cells may be a potential source of improved therapies. Over expression of HMGA2 gene was observed in many human malignancies such as colorectal cancer, thyroid, pancreatic carcinoma, lung cancers. The aim of this study was to investigate the effect of specific HMGA2 small interfering RNAs (siRNAs), on the viability, induction of apoptosis of PC3 cells. **Materials and Methods:** siRNA transfection was performed with liposome approach. The cytotoxic effects of siRNA were determined using MTT assay on the PC3 cells. Apoptosis was quantified using TUNEL assay. **Results:** Transfection with siRNA significantly suppressed the expression of HMGA2 gene in dose dependent manner after 48 hours, resulting in spontaneous apoptosis. Moreover, siRNA transfection had effects on prostate cancer cells viability. **Conclusion:** The results suggested that the HMGA2 specific siRNA effectively decrease prostate cancer cells viability and induce apoptosis in this cell line and therefore can be considered as a potent adjuvant in prostate cancer therapy.

10916P

miR-7 and chemical transfection for producing Islet Like Cell Clusters

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Introduction: Functional islet cell replacement is a promising approach for treatment of type 1 diabetes; however, it is limited by a shortage of pancreas donors. Recent studies have demonstrated direct reprogramming of fibroblasts into different types of somatic cell. The pluripotent mesenchymal stromal cells (MSCs) in adult placenta offer are attractive source of stem cells for generation of surrogate beta-cells. Here, we demonstrated that *miR-7*, a class of small non-coding RNAs, promotes beta cell differentiation of MSCs. **Materials and Methods**: Human placental

decidua basalis (PDB-MSCs) cells were cultivated from full term human placenta. The imuno-phenotype of isolated cells was checked for CD90, CD105, CD44, CD133 and CD34 markers. The PDB-MSCs (P3) was separately and chemically transfected with miR-7. The qRT-PCR results revealed the expressions of PDX1, KIR6.2, NKX6.1, PAX4, NGN3, GLUT2, insulin, Glucagon and OCT4 genes on the fourth and seventh days after chemical transfection. On the sixth day, the potency of the clusters in response to glucose challenge was tested. **Results**: Flow cytometry analysis confirmed that more than 90% of cells were CD90+, CD105+, CD44+ and negative for CD133 and CD34. Morphological changes were followed from the second day, and cell clusters were formed on sixth day. Islet like clusters showed a deep red color with Dithizone. The expression of pancreatic specific transcription factors were remarkably increased during the four days after transfection and significantly increased on the seventh day. The clusters were positive for NGN3 and insulin proteins and in response to different glucose concentration (2.8 mM and 16.7mM) the C- peptide and insulin secretion were increased. **Conclusions:** These results suggested a therapeutic potential for miR-7 as a suitable way for islet cell regeneration. **Keywords:** Diabetes, Pancreas, beta-cell, PDB-MSCs, miR-7

10919P

Electroporation method with special microRNAs for Producing Islet like Cell Clusters

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Introduction: Islet transplantation is considered as an ultimate option for the treatment of type I diabetes. Human induced pluripotent stem cells (hiPSCs) have raised the possibility that patient-specific insulin-secreting cells might be derived from somatic cells through cell fate reprogramming. MicroRNAs (miRNAs) are key players in different stages of pancreatic development, miR-375 and miR-186have high expression level during pancreatic islet development in human. A novel method was presented that over-expression of miR-7 and miR-186 promotes pancreatic differentiation in hiPSCs. **Materials and Methods**: The hiPSCs colonies were transfected with hsa-miR-375 and had-miR-186 separately by electroporation method. Total RNA was extracted 24 and 48 hours after transfection. DTZ was used to identify the existence of the beta cells. The gene expressions of insulin, NGN3, GLUT2, PDX1, Glucagon, and OCT4 were then evaluated through Real- time qPCR. On the third day, the potency of the clusters was assessed in response to high glucose levels. Besides, the presence of insulin and NGN3 proteins was investigated by immunocytochemistry. **Results:** Morphological changes were observed on the first day after the physical transfection and cell clusters were formed on the second day. The expression of pancreatic specific transcription factors was increased on the first day and they had significantly increased on the second day. The ILCs were positive for insulin and NGN3 proteins in the immunocytochemistry. **Conclusion:** miR-375and miR-186 and transcription factor network are important in pancreatic endocrine differentiation. Physical transfection with miR-375 and miR-186 can differentiate human iPS cells into functional ILCs in a short time. **Keywords:** Diabetes, Pancreas, beta-cell, Human induced pluripotent stem cells, miR-375, miR-186

11023P

Long-term culture of umbilical cord vein mesenchymal stem cells attenuate bleomycin-induced pulmonary fibrosis in mice

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Introduction: In recent studies, mesenchymal stem cells (MSCs) have increasingly been employed to treat various diseases such as pulmonary fibrosis. MSCs are at low frequency in tissues and their in vitro expansion is necessary to obtain sufficient numbers for therapeutic applications. The aim of this study was, therfore, to investigate the effects of long term culture of human umbilical cord vein MSCs (UCV-MSCs) on pulmonary fibrosis (PF) in mice. **Materials and Methods:** MSCs were isolated from human umbilical cord vein and cultured to passage 18. In C57BL/6 mice, 15 min after belomycin instillation, passages 0, 4, 8, 12, and 18 (long-term culture) of UCV-MSCs were transpanted intratracheally. Mice were weighted every 5 days and were euthanized on the 21st day. For histopathological examination, the lung sections were stained with Hematoxylin-Eosin (H&E) and Masson-Trichrome methods. The mRNA expression of TGF-β1, α-SMA, and COL1A1 in lung tissues were assessed using RT-PCR. For cell tracking, human cytochrome b DNA was detected in mice lung tissues by PCR. **Results:** The weight of mice receiving long term culture of UCV-MSCs increased more than other mice. Also, transplantation of of UCV-MSCs led to increase in the alveolar space ($P<0.05$) and decreased connective tissue ($P<0.05$) and collagen deposition of lung tissues. The mRNA expression of TGF-β1, α-SMA, and COL1A1 decreased in this group too. **Conclusion:** Transplantation of long-term culture of UCV-MSCs reduced the expression of genes involved in PF and finally diminished lung fibrosis in mice. **Keywords:** Transplantation, Pulmonary fibrosis, Bleomycin, Mesenchymal stemcell (MSC), Long-term culture.

11101P

Micro RNA 34a and Let-7a expression profile in human breast cancer associated with apoptotic expression genes

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Introduction: Breast cancer is the most common cause of cancer-related death among women in the whole world. MiR-34a and let-7a are well known tumor suppressors. miR-34a and Let-7a participate in the regulation of apoptosis, invasion and other cellular functions. **Materials and Methods:** In this study, the expression MiR-34a, let-7a and the apoptosis pathway genes such as Bcl-2, Caspase-3 and P53 were evaluated using Quantitative Real-Time PCR in normal margine tissue and tumoral tissue in 24 samples which collected from breast cancer patient who were in the advanced stage (3-4 stage). **Results:** MiR-34a, let-7a, Caspase-3 and P53 expression were reduced and Bcl-2 expression was increased within tumoral tissues in comparison with normal margin tissues. P53 expression directly or indirectly was correlated with MiR-34a, let-7a, Bcl-2 and Caspase-3 expression. In this study, it was found that

MiR-34a and let-7a expression are reduced in the tumoral tissues. A down-regulation of these two molecules is correlated with the expression of genes associated with apoptosis. **Conclusion:** These results suggested that due to the correlation of MiR-34a and let-7a with apoptotic and anti-apoptotic pathways these molecules could be participated as regulators in advanced clinical stages of breast cancer and they were considered as markers for diagnostic, prognostic and targeted therapy. **Key words:** miR-34a, let-7a, Bcl-2, Caspase-3, P53

11148P

The herbal medicine Ajwain (Carum copticum seed) to inhibit proliferation of lung cancer cell lines (A549) by inducing apoptosis

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Introduction: Medical plants have been intensively studied as a source of antitumor compounds. The antitumor effects of the ajawan medicinal plant extract is not studied on the A549 lung cancer cell lines. In the present study, cytotoxic effects of the ajawan extract were investigated on viability of A549 lung cancer cell lines. **Material & methods**: The cytotoxic effects of ajawan on A549 lung cancer cell lines were studied using MTT assay, Trypan blue staining, and DNA fragmentation assay was conducted at selected concentrations of the plant extract. **Results**: According to the findings, the ajawan medicinal plant extract (stems and leaves) can alter cells morphology. So the ajawan extract inhibits cell growth albeit in a time and dose- dependent manner and results in degradation of chromosomal DNA. **Conclusion**: The data well established the anti-proliferative effect of ajawan extract, and clearly showed that the plant extract can induce apoptosis in vitro, but the mechanism of its activities has remained unclear. **Key words**: Ajawan, lung cancer, A549, apoptosis, proliferation

11267P

MicroRNA-330 replacement effect on growth and metastasis inhibition in melanoma cancer cell lines

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Introduction: Melanoma is the most serious type of skin cancer. In cancer, miRNAs act as oncogene or tumor suppressor. One of the tumor suppressor miRNA decreasing in Melanoma, is miR-330, as a result of this decreased expression, the expression level of MMP-1, e-cadherin, vimentin, CXCR4 genes get dys-regulated. Several therapeutic approaches used for the treatment of melanoma, one of them is restoring normal levels of miRNA expression in cancer cells, which is performed by miRNA replacement therapy techniques. **Materials and Methods:** Plasmids containing MiR-330 were transformed to the bacteria and proliferated inside the bacteria. Extracted plasmids were imported into melanoma cells by liposomes. Expression levels of miR-330 and MMP-1, E-cadherin, vimentin and CXCR4 genes after replacement of miR-330 in melanoma cells were evaluated by

Quantitative real-time. The proliferation of cancer cells were determined by MTT assay after replacement of miR-330 in melanoma cells. The migration of cancer cells were evaluated after miR-330 replacement by wound healing assay method. **Results:** The expression levels of miR-330 and MMP-1, E-cadherin, vimentin and CXCR4 genes were increased after miR-330 replacement into melanoma cells. The proliferation of cancer cells were decreased after replacement of miR-330 in melanoma cells. The migration of melanoma cells were reduced after miR-330 replacement. **Conclusion:** Since the replacement of miR-330 in melanoma decreases proliferation and metastasis and increases apoptosis, the miRNA can be introduced as a therapeutic target for melanoma, and proposed a new method called microRNA Replacement therapy. **Keywords:** miR-330, Melanoma Cancer, Migration, microRNA Replacement Therapy, Apoptosis

11270P

MiR-145 replacement effect on growth and migration inhibition in lung cancer cell line

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Introduction: Lung cancer is one of the most common malignancies in the world. MicroRNAs are types of RNA that have two subgroups: suppressor and oncogene. Mir-145 is one of tumor suppressors its expression in lung cancer is diminished. As a result, this diminishes the miRNA expression order in expression of CXCR4, E-cadherin, vimentin, MMP-1 genes. These genes have a part in immigration and metastasis. By replacement of Mir-145 in lung cancer cells, the level of Mir-145 and CXCR4, E-cadherin, vimentin, MMP-1 genes get neutralized, therefore it can be used as cancer treatment. **Materials and Methods:** Bacterial plasmids containing miR-145 were entered into lung cancer cells with liposomes. After replacement of miR-145 in cancer cells expression levels of miR-145 and MMP-1, E-cadherin, vimentin and CXCR4 genes were measured by Quantitative real-time. The proliferation of cancer cells was specified after replacement of miR-145 in cancer cells by MTT assay .The immigration of cancer cells was measured by Wound healing assay method after replacement. **Results:** After replacement of miR-145 into lung cancer cells the expression level of miR-145 and MMP-1, E-cadherin, vimentin and CXCR4 genes were increased and also the proliferation level of cancer cells were decreased after replacement of Mir-145. After miR-145 replacement the immigration of lung cancer cells were decreased. **Conclusion:** Replacing miRNA in cancer cells through transfecting intended miRNA gene by a vector, can normalized the expression of miR-145 in lung cancer cells and adjust the expression of target genes. **Keywords:** Lung Cancer, miR-145, microRNA Replacement Therapy, immigration

11271P

MiR-143 replacement effect on growth and migration inhibition in prostate cancer cell line

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Introduction: Metastatic prostate cancer is a leading cause of cancer-related death in men. Cancer stem cells (CSCs) are involved in tumor progression and metastasis, including in prostate cancer. There is an obvious and

urgent need for effective cancer stem cells specific therapies in metastatic prostate cancer. Down-regulation of miR-143 has been reported in a number of cancers. However, miR-143 expression was reduced in human prostate cancer. The goal of this study was to identify MiR-143 replacement effect on growth and migration inhibition in prostate cancer cell lines. **Materials and methods:** The expression of miR-143 and E-cadherin, vimentin, CXCR4 genes after the replacement of miR-143 in prostate cancer cells was evaluated by Quantitative real-time. Next inhibition of cancer cell growth after the replacement of miR-143 in prostate cancer cells was studied by MTT technique. Finally, inhibition of cancer cell migration after replacing miR-143 was checked by wound healing technique. **Results:** After Replacement of miR-143, the expression level of miR-143 was increased and the proliferation and migration capability were reduced in prostate cancer cell lines. **Conclusions:** These data demonstrated, for the first time, that expression of miR-143 and E-cadherin, vimentin, CXCR4 genes after the replacement of miR-143 in prostate cancer cells were increased. **Keywords:** miR-143, Prostate cancer, Cancer stem cells, replacement, immigration

Diagnostic Methods in Immunology

Poster Presentations:

7519P

Heat shock cognate 71 kDa protein auto-antibody as a candidate tumor biomarker for detection of colorectal cancer in stage III and IV

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Introduction: Colorectal cancer (CRC) is one of the most commonly cancers that is the result of uncontrolled growth of tumor cells in large and rectal intestine. In the advanced stages of CRC, tumor cells have the ability of invading or spreading in other parts of the body. Heat shock cognate 71 kDa protein (Hsc70) is a chaperone protein that is involved in various cellular function such as signal transduction, apoptosis, protein homeostasis, and cell growth and differentiation. It has been shown that the overexpression of Hsc70 is related to the chemo resistance in cancer cells. Also stressed human cells such as myocardium, erythro-leukemic and neural cells can release Hsc70. In this study we revealed the auto-antibody in CRC patient's sera against SW48 cell line proteome by high-throughput techniques such as 2-dimensional electrophoresis (2-DE) coupled to mass spectrometry (MS) methods. **Material and Methods:** Electrophoresis of Sw48 cell line protein extraction in 2D gels and transfer onto PVDF membrane for blotting with sera of CRC patients (including 20 serum in each stage) and normal subjects (20 normal serums) were conducted. Reactive spots in 2D blots were picked up from stained 2D gels and were identified by MALDI-TOF/TOF MS. **Results:** Analysis of proteins using MS suggested that Hsc70 is a target which can stimulate an antibody response with CRC patient's sera from stage III and IV disease, while sera from stage I and II of CRC patients and normal sera did not react with Hsc70. **Conclusion:** The results of this study showed that the presence of auto-antibody against Hsc70 in CRC patients' sera could be used for early detection and monitoring of disease progression but additional study is necessary to confirm.

7603P

Allele Frequency of HLA-DQ2 and HLA-DQ8 in celiac disease with new simple method of Real-time PCR in Iranian population

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Introduction: The presence of HLA-DQ2 and HLA-DQ8 alleles in the infected individuals is one of the important genetic factors in the development of celiac disease (CD). Many molecular techniques are available to determine these alleles, but these methods have many steps that make them difficult to use, therefore the aim of this study was to set up a simple and quick Real-time PCR based on SYBR ® Green method to determine the HLA-DQ alleles in patients with CD. **Materials and Methods:** To determine the HLA-DQ alleles and evaluation of Real-time PCR using SYBR ® Green technique, DNA of those patients whose disease was confirmed using serology and pathology. Then, the specific primers were used to examine HLA-DQ2 and HLA-DQ8 alleles and the results were compared with commercially kits. **Results:** The presence of HLA-DQ2 and HLA-DQ8 alleles were determined with sensitivity and specificity of 80 and 100 percentage, respectively and in comparison with low resolution commercially kits, the results of this method were more efficient. As well as, frequency of DQ2 and DQ8 were respectively in patients 77 and 29 percentage. 96 percentage of patients were also carriers of DQ2 and DQ8. **Conclusions:** Real-time PCR using SYBR® Green method has good efficiency in identifying the HLA-DQ2 and HLA-DQ8 alleles, and in comparing with conventional HLA-typing techniques in Iran, is faster, easier and has high sensitivity and specificity to distinguish these alleles. The high prevalence of DQ2 allele, confirmed the results of other studies in Iran. **Keywords:** Celiac disease, Real-time PCR technique, HLA-typing.

7608P

The Role of Brain-derived neurotrophic factor in Alzheimer's disease; A systematic review

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Introduction: Brain-derived neurotrophic factor (BDNF) has an important role in the development and plasticity of the nervous system. BDNF is one of the neurotrophic factors which level has changed in Alzheimer's disease. Alzheimer is a progressive disorder of neural degeneration that leads to dementia. The purpose of this study was to explore the role of BDNF in the development of Alzheimer's disease. **Method:** In this systematic review, 575 articles were found with Immunomodulatory, Alzheimer's disease, BDNF, Immunotherapy and Immune response key words in Pubmed, ScienceDirect, Scopus databases and Google Scholar search engine. This study included 1997-2015 time interval. We selected the articles that reported their results in phase *III* of clinical trials. Finally, 86 articles were chosen. **Results:** According to our observations, in the early stage of Alzheimer's disease, the BDNF level is increasing as a compensatory repair of neurons and contribute to the destruction of amyloid plaques in the brain. Notably, the level of this factor will be decreased with Alzheimer's development and this decline leads to the

accumulation of amyloid plaques and leading to the progressive degeneration in Alzheimer's-affected brain. **Conclusion:** Results of this study showed BDNF increases in biological fluids in the early stages of Alzheimer's disease. We think that the progression of the disease could be understood by measuring the amount of BDNF and it could be a candidate for early detection and therapeutic monitoring in Alzheimer's disease. **Key words:** Immunomodulatory, Alzheimer's disease, BDNF, Immunotherapy, Immune response.

7624P

Investigation of the 16SrRNA genes relationship in *Helicobacter pylori, Campylobacter* and *Bifidobacteria* in patients with positive stool antigen against *Helicobacter pylori*

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Introduction: *Helicobacter pylori*, has been known as the first carcinogenic bacteria and is one of the most common human pathogens, so that more than half of the world population are infected with these bacteria. The present study was carried out and designed to investigate the relationship of 16SrRNA genes in *Helicobacter pylori*, *Campylobacter* and *Bifidobacteria* in patients with celiac disease and positive stool antigen response (HPSA) against *Helicobacter pylori*. **Materials and methods:** Stool samples were collected from both case and control groups (80 cases) and after DNA extraction from the samples, fecal PCR was performed to amplify the 16SrRNA genes of *Helicobacter pylori*, *Bifidobacteria* and *Campylobacter*. Also HPSA test was carried out by Immunoassay using Acon kit. **Results:** 23 subjects (57.5%) of the patient group and 17 subjects (42.5%) of the control group were positive for HPSA. Also, 16SrRNA gene PCR results in *Helicobacter pylori*, *Bifidobacteria* and *Campylobacter* in HPSA positive patient group were 86.5%, 26.8% and 8.69%. The results of control group were 100%, 100% and 5.88, respectively. **Conclusion:** There was evident and strong relationship between fecal PCR of *H. pylori* with fecal test results, and the prevalence of these genes had no particular relevance with the incidence of celiac disease. However prevalence of *H. pylori* in patients group was more than control group. Also, release of 16SrRNA genes for Campylobacter was higher in case group than control group. **Keywords:** HPSA, Helicobacter pylori, Bifidobacteria, Campylobacter, 16SrRNA

7678P

Recombinant multi-epitop antigen of Helicobacter pylori : a promising antigen for serologic diagnosis

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Introduction: *Helicobacter pylori* (*H. pylori*) is the main cause of gastric cancer. Some diagnostic tests are available for detection of *H. pylori*. Invasive tests are performed *by* biopsy specimens including histology, culture, rapid urease test and molecular methods. Urea breathing test, stool antigen test and serology are non-invasive tests. Detection of H.pylori infection via serological methods is the easiest approach in diagnosing the infection. Several virulence genes have been identified in *H. pylori* and the proteins expressed by some of them, like UreB, VacA, HpaA, CagA, HspB, FlaA, FlaB have been investigated as diagnostic indicators of *H.pylori* infection. In some commercial diagnostic kits, a mixture of critical antigens is used because it provides higher specificity and sensitivity in comparison with using a single antigen. This study aimed at constructing a gene that encodes multi-immuno-dominant epitopes of flaA and UreB of H.pylori, expression, purification and antigenicity evaluation of recombinant multi epitope protein (rFlaA-UreB) as a promising diagnostic marker. **Materials and methods:** The antigenic regions of flaA and ureB genes was detected by bioinformatics methods, amplified and join together through PCR by special primers containing linker sequence and cloned into the pET -32a. After expression and purification, the diagnostic performance of rFlaA-UreB was evaluated by IgG enzyme-linked immunosorbent assay (ELISA) and Western Blot using human sera infected with H.pylori. **Results:** The data indicated that rflaA-UreB was recognized by all patients' sera and its sensitivity and specificity were high. **Conclusion:** This recombinant protein has close antigenic properties in the natural forms of these antigens, so it seems to be a promising antigen for serologic diagnosis of Helicobacter pylori. **Key word:** FlaA, Helicobacter pylori, Polymerase Chain Reaction, UreB

7699P

APAAP complex formation and its application in Immunohistochemistry

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Introduction: Bovine Intestinal Alkaline Phosphatase (BIAP) has many applications in immunoassay due to its numerous advantages. Types, amounts, and locations of antigens were identified in their original positions in immunohistochemistry method using specific labeled antibodies. This study aimed at producing a complex of monoclonal anti-alkaline phosphatase antibody produced by $A_1G_9G_3$ hybridoma clones and alkaline phosphatase enzyme (ALP) and applying it for immunohistochemical staining by using a three-layer method. **Materials and Methods**: Anti-cytokeratin was added after removing paraffin from appendix tissue incisions by their xylolization and retrieval using microwave. In the second phase, polyclonal antibody of anti-mouse antibodies was added. In the third phase, a complex with different amounts of ALP enzyme and a fixed amount of monoclonal antibody were added. Finally, staining of the tissues was performed by adding a substrate of Fast Red Th. Naphtol As.Mx Phosphate and Levamisole. **Results:** Appropriate levels of antigen and antibody were obtained with respect to immunohistochemistry staining results for monoclonal antibody secreting $A_1G_9G_3$ colon with concentrations of 1, 2, 4, and 8 micrograms per liter of the antigen (ALP). **Conclusion**: With respect to the economic aspects of the test, concentration of 4 µg/ml of ALP enzyme with concentration of 2.4 µg/ml of monoclonal antibody was the most appropriate concentration for producing APAAP complex using $A_1G_9G_3$ colon produced in the Immunology Laboratory of Tarbiat Modares University and using in a immunohistochemical diagnostic kit. **Keywords**: Immunohistochemistry, APAAP, Alkaline phosphatase, Monoclonal antibody, Hybridoma

11183P

Immunophenotypic Features of Leukemic Cells from Patients with Acute Myeloid Leukemia: a Report from North of Iran

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Introduction: Immunophenotyping by flow cytometry is the most powerful tool to assign the lineage of leukemic cells which is very critical in diagnosis of hematopoietic malignancies. Since the geographical heterogeneity of acute myeloid leukemia (AML) has been reported in previous studies, in the current study the immunophenotypic profile of AML patients from north of Iran was studied. **Materials and methods:** Bone marrow aspirates collected from 34 AML patients (aged 5-87, 19 males and 15 females) have been enrolled. French American British (FAB) classification was considered for all cases. A panel of nineteen monoclonal antibodies specific for CD2, CD7, CD10, CD11a, CD11b, CD13, CD14, CD16, CD18, CD19, CD20, CD33, CD34, CD45, CD56, CD64, CD117, HLA-DR and MPO molecules were applied for all patients to clearly define the lineage of leukemic cells by flow cytometry. **Results:** Based on the FAB criteria, AML patients were classified into seven subtypes, including M0 (n=2), M1 (n=8), M2 (n=10), M3 (n=6), M4 (n=3), M5 (n=3) and M6 (n=2). There were no diagnosed cases as M7 subtype. CD11a (27/31), CD13 (29/34), CD33 (25/34), CD34 (17/31), CD45 (27/29), CD117 (21/31), MPO (19/31) and HLA-DR (24/33) were the most expressed molecules. CD2 (5/29) and CD7 (5/31) were the most expressed T-cell associated aberrant markers. CD19 was also displayed in one patient. **Conclusion:** Our results indicated that the overall immunophenotypic profile of our AML patients is parallel to the previous published studies and it might be used for diagnosis, minimal residual disease and prognosis. **Keywords:** Immunophenotyping, Acute Myeloid Leukemia, Flow Cytometry

11252P

The Soluble form of stem cells marker, AA4.1, elevated in serum of patients with chronic obstructive pulmonary disease and associated with the disease stage

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Introduction: C1qRp was characterized as a hematopoietic stem cell marker, calling AA4.1, and has been recently shown to release from cell surface of inflammatory cells. We have recently found that soluble (s) AA4.1 is elevated in the serum of patients with rheumatoid arthritis. The aim of this research was to determine the level of sAA4.1 in the serum of patients with chronic obstructive pulmonary disease (COPD) in which inflammation plays a crucial role. **Method and materials**: 90 patients with COPD and 80 normal age and sex-matched individuals were participated in this study. Patients were diagnosed at Pulmonary Clinic, Tohid Hospital, Sanandaj, Iran. Serum samples were collected and kept at −80°C until analyzing by ELISA. All the questionnaires were prepared by co-worker physician. **Results**: The mean age of the patients and control participants were 59±9 and 57±10,

respectively. We observed that the serum level of AA4.1 in patients with COPD was significantly elevated compared with normal controls. Interestingly, we observed that the level of AA4.1 was: (i) associated with the stage of the disease; (ii) remarkably higher in smoking patients than non-smoking and (iii) much higher in male patients with COPD. However, it was similar in both genders in controls individuals. **Conclusions**: For the first time, we demonstrated that AA4.1 is higher in patients with COPD than normal individuals and that AA4.1 could be used as biomarker for diagnosis of COPD.

12365P

Adenosine deaminase activity in chronic lymphocytic leukemia and healthy subjects

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Introduction: B cell chronic lymphocytic leukemia is one of the most frequent hematologic malignancies in the world. Cellular surface CD markers and serum Beta-2-microglobulin may be used as a prognostic tool in CLL patients. In the present study serum adenosine deaminase was introduced as a diagnostic marker in CLL. **Materials and Methods:** Blood samples were collected from B-CLL and healthy subjects. White blood cells, red blood cells, platelets count and blood Erythrocyte sedimentation rate were recorded and serum Beta-2-microglobulin, Lactate dehydrogenase and total ADA enzyme activity were determined. **Results:** Serum ADA activity was significantly higher in patients group than controls. ADA had a significant and direct correlation with B2M, WBC, LDH and ESR. However, there wasn't any relation between ADA and the stages of disease. Diagnostic cut-off, sensitivity and specificity of the serum ADA test were 27.97 U/L, 91% and 94%, respectively. **Conclusion:** The higher ADA activity in patients group and its correlation with CLL markers was observed in this study. High diagnostic value of serum ADA in this study suggested that it might be considered as a useful screening tool among the other markers in CLL. **Keywords:** Adenosine deaminase, Beta-2-microglobulin, Chronic lymphocytic leukemia

12416P

Development of Inhibition Enzyme-Linked Immunosorbent Assay (ELISA) for Cardiac Troponin I Using Immuno-dominant Epitopes as Competitor

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Introduction: Human Cardiac troponin I is a gold marker for early diagnostic of myocardial infarctions. Development of inhibition enzyme-linked immunosorbent assay (ELISA) of cardiac troponin I using its four immuno-dominant epitopes as competitor to evaluate and assess their diagnostic performance. Four immune-dominant epitopes were predicted and their structure was determined using in silico analyses. Then, a 1

nanosecond MD run was exerted on each epitope. **Materials and methods:** The pET-32a-Troponin I vector was transformed to E.Coli Bl21. Expression was optimized by altering IPTG and temperature. Purified troponin I was detected and confirmed with SDS-PAGE and western blotting. Sensitivity of Insilco-selected peptides was evaluated with the developed polyclonal antibody-based ELISA. The structure of four predicted epitopes were modeled and MD runs were successfully performed to achieve a more reliable structure. **Result:** Recombinant troponin I is optimally expressed with 1 mM IPTG and at 18oC; purification was best with 250 mM imidazole, employing nickel column. The competitive performance of immuno-dominant peptides was monitored with the developed polyclonal antibody-based indirect competitive ELISA; a half-maximal inhibitory concentration (IC50) of 0.49 (µg/ml) and detection limit of 0.037(µg/ml) were achieved for recombinant cardiac troponin I. The competitive ELISA determined sensitivity levels of 0.306, 0.141, 0.960, and 0.155 (µg/ml), respectively, for each peptide as competitor. **Discussion:** Various troponin I epitopes have different sensitivity scales to be used for competitive ELISA design. **Keywords:** Cardiac troponin I. Competitive ELISA. Immunodominant epitopes. Polyclonal antibody. Protein expression.

Exercise & Aging Immunology

Poster Presentations:

10933P

The Effect of an Acute Exercise on IgA and TNF-α Salivary Levels in Inactive Men

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Introduction: There are numerous unanswered questions in relation with the effect of acute exercise and changes in the immune system and inflammation. So the aim of this study was the survey effect of an acute exercise session on IgA and TNF-α salivary levels in inactive adult men. **Materials and Methods**: A total of 12 inactive healthy men (age 30-40yrs) after completing the health questionnaire participated in the study. Volunteers salivary samples were collected in three time before exercise test (GXT), immediately and 3 hours after the exercise protocol for determination of IgA and TNF-α salivary levels. ELISA method was used for measuring IgA (Diameterkit) and TNF-α (AviBion kit). Repeated measure test for analysis of data by using SPSS (v.22) software was performed in a significant level of P <0.05**. Results**: The results of TNF-α levels of salivary, immediately and three hours after the GXT exercise had no significant increase compared to basal levels (sig=0.102). Also the levels of salivary IgA concentrations immediately and 3 hours after the activity showed no significant increase and decrease compared to pre-exercise (sig=0.633). **Conclusion:** The results showed a session of incremental exercise and activity has no significant effect on the response of salivary inflammatory indices in inactive adult men.

10939P

Immune and Inflammatory Markers of Active Adult Men in Response to Short-term Physical Stress: RelationshipWith Albumin and Lactate Salivary

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Introduction : Exercise as a physiological stress has a significant effect on changes of immune and inflammatory system. So the aim of this study was to examine the response of albumin and lactate salivary as a marker of immune and inflammatory to acute exercise. **Materials and Methods:** The volunteers who present the study consisted of 12 active healthy men) 50-60 minutes of physical activity, 3days a week) with average age of 35±5. After completing health questionnaires, the volunteers participated in the study. Volunteers salivary samples were collected before (T1), immediately (T2) and 3 hours after performing the incremental exercise test (T3).To determine the amounts of albumin and lactate salivary, East Biofarm and Glory Bioscience kits in the order and ELISA method were used. The analysis of data was performed by using SPSS software) v.22). **Results:** No significant increase was observed in albumin salivary concentrations at T2 and T3 compared to T1(sig=0.414). Also there were no significant changes in lactate salivary levels in adult men at T2 and T3 compared to T1(sig=0.522). **Conclusion:**Our results showed that albumin and lactate salivary had no changes. This is due to the adaptation responses of inflamatory and immunne markers of physical activity in adult active men.

11029P

High Intensity of Interval Training Changes Inflammatory Cytokine Gene Expression: TGF-α as an Injury and Cells Stimulation Reflector Marker

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Introduction: TGF-α is one of the most important cytokines that plays great role in showing alveolar macrophage activity. The aim of the present study was to investigate the high intensity interval training changes of inflammatory cytokine gene expression: TGF-α as an injury and cell stimulation reflector index**. Materials and Methods**: Twenty-four healthy middle-aged men were volunteered in this study and randomly divided into control (n=12) and exercise (n=12) groups. Exercise group performed HIIT training (30 min/day, 3 days/week at 60-90% of heart rate reserve) for 8 weeks. Participants' blood samples, body composition, VO2 max and transforming growth factor-α gene expression were measured 24 hours before and after the training program. Bio Easy Master Mix Kit and Real-time PCR method was used for TGF-α gene expression. The data have been analyzed by independent t-test with SPSS software. **Results**: After 8 week exercise training, there was a significant decrease in TGF-α gene expression ($p<0.001$) in the exercise group compared to the control group. **Conclusion**: The present study in middle-aged men supportsed a prevention role of exercise training on reducing the TGF-α gene expression as a most important inflammatory cytokine that can result the inflammation and macrophages activity reduction, reduction of injury and cell stimulation that finally leads to the improvement of immune system.

Immunodeficiency

Poster Presentations:

6512P

Effect of esteradiol treated mesenchymal stem cell in amiliorating animal model of multiple sclerosis

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Introduction: Preliminary studies revealed that mesenchymal stem cells (MSCs) therapy is a worthwhile strategy to down-regulate pathogenic immune responses in multiple sclerosis (MS).Nevertheless, insufficient implantation of cells to damaged brain and spinal cord has limited their potential therapeutic effects. There is evidence that estradiol (E2) enhances homing of stem cells. This study was conducted to investigate the therapeutic effects of E2 treated MSCs in experimental autoimmune encephalomyelitis as an animal model of MS. **Materials and Methods:** EAE was induced in Wistar rats by guinea pig spinal cord homogenates and complete Freund's adjuvant. Therapies were initiated at day 12 post immunization when the mice developed a disability score with 2×106 of E2 treated MSCs or MSCs without treatment. After day 33, the mice were sacrificed and the effects of cell therapy were investigated. **Results:** Clinical scores, leukocyte infiltration and lymphocyte proliferation were significantly decreased in EAE mice receiving E2 treated MSC more prominent than EAE mice receiving MSCs without treatment. Furthermore, Body weight was significantly improved in EAE mice receiving E2 treated MSC more prominent than EAE mice receiving MSCs without treatment. **Conclusion**: The findings indicated that conditioning of MSCs with E2 may be as a useful approach to control MS. **Keywords:** Mesenchymal stem cell, estradiol, Multiple sclerosis, experimental autoimmune encephalitis.

10850P

Genetic diagnosis of Hereditary Angioedema disease in Iran (three cases report in a family)

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Introduction: Hereditary Angioedema (HAE) is an autosomal dominant disease with deficiency of C1 inhibitor protein. The heterozygous mutation in SERPING1 gene causes low level of C1inhibitor (HAE type I) and can be resulted in normal level of dysfunction C1 inhibitor (HAE type II). C1 inhibitor (C1INH) is a serpin-type protease inhibitor that controls the complement, fibrinolytic, kinin and coagulation pathway. The aim of this study was genetic diagnosis of three relative patients with HAE type I. **Materials and Methods**: Three relative patients who had family history of angioedema were entered this study based on low levels of C4 and C1-inhibitor. They had Clinical phenotype of edema in the face, upper and lower limbs, laryngeal and abdominal pain that lead to hospitalization. These patients refferd to Immunology, Asthma & Allergy Research institute (IAARI). Blood samples were obtained from the patients. Subsequently, DNA were extracted from PBMCs and polymerase chain reaction (PCR) was accomplished for exon 2 to 8 of SERPING1 gene and PCR products were subjected to sequencing analyses. **Results**: A heterozygous mutation was found in the exon 5 SERPING1 gene which leads to frameshift variant (c.727delC). This mutation was reported previously. **Conclusion**: This study is the first genetic report of HAE patients in Iran. This disease is life threatening and if edema occurs in laryngeal possibly, will lead to death. Therefore, genetic diagnosis in the families with HAE patients could be helpful for early diagnosis of other family members.

11173P

Blood Cells and Fibroblasts Count, Kidney and Spleen Weight in C57bl /6 Nude, Wild and Dexamethasone Treated Mice

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Introduction: Thymus, a lympho-epithelial organ, plays an important allostatic role both in mice and human. Reportedly thymus produces the different groups of T lymphocytes. However the effects of thymus on non-lymphoid organs remain to be uncovered. In this study we investigated the effect of thymus on some hematological parameters, lymphoid organs (spleen) and non-lymphoid organs (kidneys and skin). **Materials and Methods:** 15 mice (C57BL/6) placed equally in 3 groups of male wild, female nude, and male dexamethasone-injected (a daily Dexamethasone dose of4mg/kg were intraperitoneally injected for 7 days) were compared. Hematological indices were measured, and after removing the organs they were weighed. Also, tissues were stained with H&E, and skin fibroblasts were cultured and data were analyzed by ANOVA test. **Results**: The weight of spleens ($P<0.04$) and right kidneys ($P<0.01$), number of WBCs and fibroblastin nude mice were more than wild mice. Neutrophil to lymphocyte ratio ($P<0.03$) in Dexamethasone injected mice group were more than the other groups, Nude and wild mice groups in the second and third places respectively. **Conclusions:** It seems that the condition of nude mice group's immune system lies between the two other groups. Contrary to expectations, there was no significant differences in number of leukocytes and lymphocytes between normal and nude mice. Furthermore, thymus may have influence on skin fibroblasts, as well as on size of kidney and spleen.

11207P

The percentage of Th1 lymphocytes in patients with hyper IgE syndrome

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Introduction: Hyper-IgE Syndrome (HIES) or Job's syndrome is a primary immunodeficiency syndrome with an increased susceptibility to some pathogens, including Staphylococcus aureus and Candida albicans. Some Cells, cytokines and genes are involved in pathogenesis of this disease, which among them, Th1 and IFN-γ can be pointed out. **Materials and methods**: Five patients with hyper IgE syndrome and 6 healthy controls without any history of allergy diseases or clinical laboratory findings for allergy were enrolled in this study and informed consents were obtained. Peripheral blood mononuclear cells were isolated via ficoll gradient centrifugation. After separation of monocytes, lymphocytes were stimulated by PMA and Ionomycin, cultured for 12 hours and then were stained with anti-CD3-PE-Cy5 and anti-IFN-γ-PE antibodies. Cells were assessed by BD FACS Calibur, and analyzed by Flowjo software (7.6.1 version). **Results:** The average age of patients and control were (8.62 (_-^+) 2.8 (and (6.6 (_-^+) 2.7) years, respectively and the average of Th1 cells percentage in patients was (12.6(_-^+) 7.2) and in control group (18(_-^+) 7.24), which showed a significant reduction in the numbers of Th1 cells in patients compared to the control group (P <0.001). **Conclusion:** Hyper-IgE syndrome is a complex multisystem disease, which may be the result of an altered Th1/Th2 cytokine profile towards a Th2 bias. This reduction in numbers of Th1 lymphocytes may increase the susceptibility to fungal and intracellular bacterial infections. **Key words:** HIES, Th1, Job's syndrome, flow cytometry

12330P

Study of unknown chronic granulomatous disease among Lak ethnic group lived in Northern region of Iran

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Introduction: Chronic granulomatous disease (CGD) is an uncommon inherited immunodeficiency disorder caused by severe respiratory burst defects. Patients with CGD, were commonly diagnosed in infancy or childhood with frequent severe bacterial or fungal infections. Overall survival in CGD cases has changed extremely over the last decade using prophylactic antibiotics and antifungal. Therefore, the diagnosis of CGD is crucial to prolong the survival by prescribing prophylactic antibiotics and antifungals. **Materials and Methods**: In order to diagnosis the unknown CGD cases in a suspected population, this population-based study was conducted in 190 individuals that

live in eight villages located in Northern part of Iran. Blood samples were obtained and the NBT test was performed. **Results:** The results indicated that 3.5% of cases had severe respiratory burst defect, 28.7% of cases were carrier and the remaining (67.8%) were normal. The highest percentage of CGD cases was seen in Eslam-Abad (30.8%) and Pool (13.3%) villages. **Conclusion:** The highest CGD carriers were detected in 80%, 69.2% and 40% of samples of Pool, Eslam-Abad and King Villages, respectively. This study suggested that CGD cases and CGD carrier subjects are frequent in some villages in northern part of Iran. Further studies with larger sample size are needed in this area.

12502P

Kawasaki disease in children hospital of Tabriz

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Introduction: Kawasaki disease (KD), a systemic vasculitis of unknown etiology with an increasing incidence in childhood, can cause the acquired heart disease. This study aimed to describe the prevalence of clinical manifestations and laboratory findings of KD patients referred to children hospital of Tabriz during 1993 to 2013. **Materials and Methods**: This descriptive case series study, performed on 82 KD patients referred to children hospital of Tabriz during 1993 to 2013. Diagnostic criteria were fever, skin rash, bilateral noninfectious conjunctivitis, desquamation of extremities and alterations in the mouth and pharyngeal mucosa. Age, sex, clinical and para-clinical changes of the patients were evaluated. **Results**: Among 82 cases, 51 (62.1%) were males and 31 (37.8%) females. The majority of patients (79.3%) were younger than 5 years old. The age range was from 3.5 months to 11 years. Fever was seen in 100% of cases, conjunctivitis in 73.1% (60), skin rash in 70.7% (58) and neck lymphadenopathy in 32.9% (27) of cases. Moreover, the laboratory findings included leukocytosis in 42.6% of cases, thrombocytosis in 43.9%, abnormal ESR in 95.1 % and cardiac sequels in 29.2% of cases. **Conclusion**: To prevent cardiac sequels of KD disease, diagnostic work up suggested in children with prolonged fever unresponsive to antibiotics. **Keywords:** Tabriz, Kawasaki disease, Children, Coronary artery

12503P

A case of Nuclear factor kappaB essential modulator-deficient with repeated infection and conical teeth without anhidrosis andother signs of ectodermal dysplasia.

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Introduction: Impaired ability to signal and activate specific gene transcription through nuclear factor kappa B (NF kappa B) has been directly linked to immunodeficiency. Hypomorphic mutations in the gene encoding NF kappa B essential modulator (NEMO), located on the X chromosome, impair NF kappa B function and lead to ectodermal dysplasia with immunodeficiency (ED-ID) with increased susceptibility to pyogenic bacteria, viruses and nonpathogenic mycobacterial infections. **Materials and Methods**: The case is an 8 y/o boy who presented with

disseminated BCGeitis in age of 9 y/o that responded to four anti mycobacterial regimens drugs. In later stages of life, he had repeated pneumonia and one time aspergillous pneumonia. In physical examination he had conical teeth, without any sign and symptoms of anhidrosis and ectodermal dysplasia. **Results**: In immunologic work up, he had lack of antibodies against carbohydrates. Other immunologic work up was normal. Because of presence of conical teeth and also history of disseminated BCG infection, we suspected to nuclear factor kappa B, essential modulator mutation with immunodeficiency disorder (NEMO-ID). In genetic analysis, the NEMO mutation was identified. **Conclusion:** This case demonstrated that patients with NEMO mutations can present with an immunodeficiency without ectodermal dysplasia. An investigation of NEMO should thus be undertaken in every boy with disseminated BCG infection and conical teeth.

12504P

Malignant external otitis as a first clinical presentation of leukocyte adhesion deficiency 1 disease in infancy

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Introduction: 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. Here, an infant was described with LAD1 who initially presented with malignant external otitis which is rare in this age. **Case presentation:** An 8 month-old female infant presented with pussy discharge from left ear canal and admitted in otolaryngology ward. She received antibiotics for external otitis caused by pseudomonas aeroginosa and also tympanostomy and mastoidectomy were conducted. Because of severity of infection, history of delayed cord separation and detection of neutrophilia in peripheral blood, we suspected to LAD 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 LAD.

Immunodermatology

Poster Presentations:

12418P

Treatment of pemphigus vulgaris, an immunobullous disorder

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Introduction: Pemphigus vulgaris is a common immunobullous disorder that without treatment is fatal in almost all cases. So many therapeutic modalities has been suggested. In this article we will note some systematic reviews about them. Objective is to evaluate the efficacy, steroid-sparing effect, and safety of available treatment modalities **Methods and Material:** PubMed, the Cochrane Central Register of Controlled Trials and the ClinicalTrials.gov registry and reference lists were searched for randomized controlled trials of any treatment modality for pemphigus vulgaris. **Results:** Based on five meta-analyses, each pooling the data of two to three trials, Azathioprine had a steroid-sparing effect but did not increase remission rate. Mycophenolate mofetil induced sustained remission more quickly than did placebo and delayed time to relapse but did not have a steroid-sparing effect or favorable remission rate. Cyclophosphamide had a steroid-sparing effect, though less than azathioprine, but did not affect the remission rate or time-to-disease control. Intravenous immunoglobulin had more favorable short-term efficacy than did placebo. Topical epidermal growth factor hastened lesion healing. Another meta- analysis revealed that mycophenolate is more effective in achieving disease control than azathioprine. There was evidence of a steroid-sparing benefit of azathioprine and cyclophosphamide compared to glucocorticoids alone. **Conclusion:** Cyclophosphamide and azathioprine have more steroid sparing effect than mycophenolate in the treatment of pemphigus vulgaris.

Immunogenetics

Oral Presentations:

10783O

Association between TRAF3IP2 rs33980500 polymorphism and Ejection fraction in acute myocardial infarction

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Introduction: Cardiac output or ejection fraction (EF) is one of the most important prognostic factors in heart diseases. As such, an EF less than 35% increases the risk of sudden cardiac arrest and death. Inflammatory chemokines including CXCL1 is elevated in patients with low cardiac output. Due to the role of TRAF3IP2 in CXCL1 expression; we investigated TRAF3IP2-rs33980500 variation in acute myocardial infarction (AMI). **Material and Methods:** All confirmed AMI patients (n=159) referring to the hospitals of Shiraz University of Medical Sciences in a one-year period were included. The TRAF3IP2-rs33980500 variation was identified using PCR-RFLP. Control individuals (n=201) were recruited among healthy age/sex matched blood donors. **Results:** The percentages of homozygote CC and TT genotypes in patients were 80.5% and 2.5% vs. 81.1% and 1% in controls. The frequencies of heterozygote individuals were 17% and 17.9% in patients and controls, respectively. There was no association between the TRAF3IP2-rs33980500 variations and AMI (p=0.53). There was, however, a difference between left ventricular EF at the time of admission of different genotypes. In this regard, patients with LVEF<35 had a significantly lower frequency of CC genotype and higher frequency of TT genotype (p=0.04). **Conclusion:** The results indicated an inverse effect of the mutated variant on the prognosis of AMI. The C to T alteration resulting in the substitution of Asp to Asn that increases the activity of TRAF2/TRAF5 pathway. Therefore, by enhancing the stability of CXCL1, neutrophils recruitment to the ischemic lesion is accelerated which exacerbates the disease. **Keywords:** Myocardial infarction, Single nucleotide polymorphisms, TRAF3IP2, Ejection Fraction

10798O

Association of leptin and adiponectin genetic variants on susceptibility to Multiple Sclerosis in Iranian patients

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Introduction: Adipocytokines such as leptin and adiponectin represent a link between metabolism, nutritional status and immune responses. The aim of the present study was to investigate the association between single nucleotide polymorphisms (SNPs) in the genes of leptin (2548A/G and 19G/A) and adiponectin (+276G/T and -11377G/C) with multiple sclerosis (MS) in Iranian patients. **Material and Methods:** Four SNPs were genotyped in 300 patients and 300 healthy individuals using PCR-RFLP. Sera levels of leptin and adiponectin were measured using ELISA kits. **Results:** G allele of 19G/A was significantly more frequent in MS patients (p=0.02). +276 G/T polymorphism did not show any significant association with disease susceptibility, though after gender categorization, the frequency of T allele was more significant in male patients than controls (p=0.01). Haplotype analysis revealed that GA (p=0.04) and GG (p=0.017) haplotypes at *LEP* gene were associated with the development of MS. Moreover, while the sera levels of leptin were not different between patients and controls, adiponection levels were significantly higher in healthy controls (p< 0.001). **Conclusion:** G allele of 19G/A might increase leptin gene transcription in MS patients, thereby predispose G allele carriers to MS. In addition, given the fact that adiponectin down-regulates innate and adaptive immune responses, higher levels of this adipokine in healthy controls may show the significant role of adiponectin in resistance to MS development. **Keywords:** Single Nucleotide Polymorphism**,** Leptin, Adiponectin, Multiple sclerosis.

11035O

Interleukin 7 receptor alpha gene variants correlated with gene expression in patients with relapsing-remitting multiple sclerosis

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Introduction: The association of numerous single nucleotide polymorphisms (SNPs) of IL-7RA gene (e.g. rs s6897932) with multiple sclerosis (MS) has been documented in various populations. This study aimed at evaluating the genotype distributions of two SNPs, rs6897932 and rs201084372, and functional association of rs6897932 in relation with IL-7RA gene expression in a group of Iranian relapsing-remitting MS (RRMS) patients. **Material and Methods:** A total 100 RRMS patients as well as 100 ethnic-matched healthy controls were recruited in this study. Genotyping for both SNPs in IL7RA gene and relative quantification of mRNA expression for both isoforms of IL-7Rα were performed for all RRMS patients and healthy controls. **Results:** Higher significant frequencies of T allele and TT genotype for rs6897932 (C/T) were observed in patients than controls (P=0.006). Also, higher frequencies of T allele TT and TG genotypes as well as lower frequencies of G allele and GG genotypes for rs201084372 (G/A) were found in patients versus controls (P<0.0001). A decreased level of mRNA expression for membrane-bound IL-7Rα (mbIL-7Rα) and increased level of mRNA for soluble IL-7Rα (sIL-7Rα) were observed in patients versus controls (P=0.005 and P=0.002, respectively). Additionally, a significant decreased level of mRNA expression for mbIL-7Rα (P=0.01) and increased level of mRNA for sIL-7Rα (P=0.008) were observed in those RRMS patients

carrying TT+CT genotypes compared to healthy controls with those genotypes. **Conclusion:** Elucidation of higher levels of mRNA expression for sIL-7Rα isoform in patients carrying IL7R*TT genotype is a remarkable difference compared to previous studies with regard to genotype-induced effects of IL-7Rα expression in multiple sclerosis. Further investigations are needed to find out the exact role of these SNPs in IL7RA gene and their functional relevance in predisposing of MS or protection against disease. **Keywords:** Multiple Sclerosis; IL7RA gene, Polymorphism, Expression

11113O

Analysis of interleukin-1β (3954C/T) genetic polymorphism and its association with gastric cancer

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Introduction: Cytokines as the potent mediator of immune system, play a critical role in biological activities such as inflammation processes. Inflammation triggering factor in many diseases, one of the pro-inflammatory cytokines is IL-1. Recent studies confirmed that interleukin-1β (IL-1β) is highly genetic polymorphic, and a few of the genetic polymorphisms of this cytokine is associated with an increased risk of pathogenesis in gastric cancer.The current study was conducted to assess the influence of IL-1β+3954 genotypes in the risk of gastric cancer in Iranian population. **Material and methods:** Genomic DNA was isolated from endoscopic fresh biopsy samples in 49 gastric cancer patients and peripheral blood of 53 healthy volunteers. The polymorphism of IL-1β C/T was studied with a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. **Results:** In both patients and control group, the genotype frequencies of IL-1β+3954 gene were in Hardy–Weinberg equilibrium. Based on obtained results, the frequencies of IL-1β+3954A1A1, A1A2 and A2A2 genotypes in healthy subjects were 26.4, 66 and 7.6 %, respectively. Also, in gastric cancer patients, A1A1, A1A2 and A2A2 were observed with 4.1, 51 and 44.9% ($p<0.05$), respectively. **Conclusion:** Our results showed a significant association between IL-1B+3954genotypes with the risk of gastric cancer disease in Iranian population. However, further studies with a larger sample size are needed to confirm these results. **Keywords:** Genetic polymorphism, Gastric cancer, Inflammation, Iran, Interleukin-1β

11191O

Activating KIR receptors associated with the risk of bladder cancer development

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Introduction: Natural killer cells (NK) are key component of innate immune system which create the first line of defense against tumor and infection. NK cell responses are regulated through a family of 16 killer immunoglobulin like receptors (KIRs). The aim of the current study was to survey the allelic diversity of KIR receptors in bladder cancer patients. **Material and methods:** 12۱bladder cancer patients and 120 healthy normal controls were included in this study. Genomic DNA of all study subjects was extracted by using a QIAamp blood kit. Sequence specific polymerase chain reaction (PCR-SSP) method was used to characterize the presence or absence of 16 KIR genes. **Results:** KIR2DL2 occurred less frequently in bladder cancer patients (p value: 0.01, Odds Ratio: 2.01, CI: 1.184-3.413). Due to the strong linkage disequilibrium between KIR2DL2 and KIR2DS2, KIR2DS2 is also less frequent in patients (p value: 0. 5, Odds Ratio: 0.5,0.3398-0.9728). On the other side KIR2DS5 was more frequently in patients in comparison with the control group (p value: 0.046, Odds Ratio: 1.70, CI: 1.017-2.855). Furthermore the T4 gene cluster containing more activating KIR genes occurred strongly more frequent in patients; the difference was statically significant (p value: 0.0002, Odds Ratio: 3.85, CI: 1.91-7.76). KIR2DS5 was significantly increased in advanced stage patients than the control group (p-value: 0.029, Odds Ratio: 2.91, CI: 1.17-7.23). **Conclusion:** excessive and chronic NK cell responses in tumor microenvironment may cause persistent inflammation; which increase the risk of cancer development.

11192O

NK cell receptors diversity in non-melanoma skin cancer patients: a case-control study

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Introduction: The innate immune system against malignancies is mainly orchestrated by NK cells and which carry out killing mechanisms by using their receptors including killer immunoglobulin like receptors or KIRs. This study was designed to determine the diversity of killer immunoglobulin like receptors (KIR) in non-melanoma types of skin cancer. **Material and methods:** 163 subjects with skin cancer and 149 healthy subjects formed the studied groups. DNA was extracted from blood samples using QIAamp mini kit, then sequence specific polymerase chain reaction (SSP-PCR)-typing was performed to detect the presence or absence of 16 KIR genes. **Results:** KIR3DL1 was increased in total cancer group and in BCC compared to healthy controls (p-value= 0.002 and 0.0006 respectively). 2DS4 was also increased in BCC and total patients compared to healthy controls (p-value=0.04 and 0.025 respectively). The only allele which frequency was significantly different between BCC and SCC patients was 2DP1 (P-value=0.04). The frequency of AA genotype was significantly higher in patients compared to controls (p=0.024, OR=1.94, %95CI=1.09-3.44). A decreased frequency of CxTx cluster detected in BCC, SCC and in total patients compared to controls (p-value=0.01, 0.01 and 0.002 respectively). **Conclusion:** Increasing 3DL1 may lead to inhibition of NK cells activity and therefore supporting the malignant cells to escape from the immunosurvillance in which NK cells have an eminent role. In addition, 2DS4 an activating KIR molecule probably by inducing a persistent chronic inflammatory in tumor niche, may contribute to the initiation of malignancy.

11318O

Association of PD1.5 C/T, but not PD1.3G/A, with malignant and benign brain tumors

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Introduction: Programmed death-1 (PD-1) (CD279) is a negative regulatory molecule of the immune system. The aim of this study was to assess the association of two single nucleotide polymorphisms in PD-1 gene, PD-1.5(+7785C/T–rs2227981) and PD-1.3 (+7146G/A- rs11568821), with benign and malignant brain tumors. **Material and Methods:** In this case-control study, 152 patients with brain tumor (96 patients with benign and 56 patients with malignant brain tumor) and 150 healthy controls were included. PCR-RFLP was performed for genotyping PD-1 gene polymorphisms in these positions. **Results:** The frequencies of CT genotype and T allele of PD1.5C/T polymorphisms were higher in patients ($P< 0.05$). In contrast, the frequency of PD1.3G/A genotypes and alleles in patients showed no significant difference between patients and control groups ($P> 0.05$). Patients were then divided into those with malignant, and those with benign tumors. Results revealed a significant difference between either malignant brain tumors or benign brain tumor and control group in position PD-1.5C/T ($P<0.05$), but not in position PD1.3G/A. The GC haplotype was the most frequent haplotype in the whole group of patients and controls, and GT haplotype frequency was significantly different between patients and control group (Bonferroni corrected $P<0.0125$). **Conclusion:** For the first time, results of this study showed that PD-1.5 C/T polymorphism is associated with the risk of brain tumor. Further investigations are required to disclose the consequence of PD-1.5C/T polymorphism on the immune response in brain tumors.

Poster Presentations:

3467P

Association between Genetic Polymorphisms of IL-33, IL1R1, with levels of IL-33 in asthma and multiple sclerosis diseases

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Introduction: Recent evidence suggested that the IL-33/IL1RL1 axis plays a critical role in autoimmune and inflammatory disorders. However, its mechanistic role in these diseases have not been clearly defined. The aim of the present study was to investigate the frequency of rs1342326 polymorphism of IL-33 gene /rs10204137 polymorphism of IL1RL1 gene and serum levels of IL-33 in Multiple sclerosis (MS), asthma and healthy subjects In Isfahan Province. **Material and methods:** Patients with asthma, MS (140) and healthy controls (72) were collected.

Different genotypes T> G and G> A polymorphisms using the HRM (Feldan Germany) Real Time PCR techniques were studied. Serum level of IL-33 by Elisa method (BOSTER BIOLOGICAL TECHNOLOGY Co,CA) was measured. The results were analyzed by SPSS software version 16**. Results:** The present study showed that there was no relationship between the frequency of polymorphism rs10204137 of IL1RL1 gene and susceptibility to MS /asthma. There was no significant correlation between the frequency of rs1342326 polymorphism of IL-33 gene in healthy subjects with asthma and MS subjects. Serum levels of IL-33 was higher in MS and asthma patients compared to healthy subjects. **Conclusion:** Serum levels of IL-33 in the two patient groups increased substantially compared to the control.

3473P

Association of RS56066773 Polymorphism in the 3'-UTR of FOXP3 gene with rheumatoid arthritis

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Introduction: Rheumatoid arthritis is an autoimmune disease that affects about 1% of the world's population. Treg cell function is impaired in patients with rheumatoid arthritis.Factor FOXP3 is a key regulator of the development and performance Treg cells. Among the factors involved on FOXP3 expression are microRNAs (MIR) which bind to 3´UTR. A nucleotide substitution in the sequence of the target site of microRNA can affect the regulation of microRNA. RS56066773 polymorphism in the 3´UTR of gene FOXP3 can relate with rheumatoid arthritis through the target gene. This study investigated the relationship between RS56066773 polymorphism with rheumatoid arthritis. **Material and Methods:** In this case-control study 98 RA patients were recruited from Emam Ali rheumatology clinic and 124 healthy individuals (without the negative history of autoimmune diseases) served as control. RS56066773 polymorphism in the 3´UTR of FOXP3 gene were investigated with Polymerase Chain Reaction (PCR) Restricted Fragment Length Polymorphism (RFLP). The data were analyzed using SPSS. **Results:** In the study population, the frequency of the A/G inRS56066773 was 3.1 and 1.6% in patients and control respectively. **Conclusion:** Although no significant relationship between polymorphisms and arthritis was found, but previous studies have been established the relationship between polymorphisms in the microRNA target site with a number of diseases. Therefore necessary examination other than FOXP3 gene 3'UTR polymorphisms is required. **Keywords:** Polymorphism, SNP, MicroRNA, Rheumatoid arthritis

7541P

Investigation of rs1044243 polymorphisms in ALCAM gene and the risk of multiple scrlosis

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory disease which destroys myelin cells of the nervous system (CNS), particularly in developed societies. Although the cause is not clear, but genetic factors are discussed.Activated leukocyte cell adhesion molecule (ALCAM) is a molecule involved in leukocyte migration across the blood–brain barrier which is a key stage in multiple sclerosis (MS) pathogenesis. The aim of this study was to investigate the association between rs1044243 gene polymorphism and risk of MS in the city. **Material and Methods:** 100 patients with MS and 100 healthy people were studied. DNA extraction was performed using kit method. PCR method was used for gene amplification and then RseI digestive enzymes for polymorphism detection. The data collected were analyzed using software spss ver18.**Results:** In this study, the frequency of Tallel polymorphism rs1044243 in healthy subjects and patients with high blood pressure, %8and %5.5 respectively, and there was no significant difference between groups (OR= 1.494; 95%CI= 0.675-3.305; P =0.426) The frequency distribution of genotypes of the rs1044243 polymorphism in healthy subjects and patients, were respectively genotype TT %5, %3(P= 0.465) and genotype TC %6, %5(P=0.730) and genotype CC %89,%92 (P=Refrence). The CT and TT genotype distribution were not significantly different between the two groups. **Conclusion:** Despite the Association of ALCAM gene rs1044243 polymorphism with hypertension in some studies in out of Iran, findings of this study indicated that there is no association in jahrom's population. In discussing why the findings in a number of different societies or agree with the findings in our study, this should be considered in addition to the difference in terms of selection and control patients in different studies, factors such as ethnicity, race, diet, environmental factors, etc. **Keywords:** polymorphism rs1044243, gene ALCAM, Multiple sclerosis

7715P

Computational analysis on the possible role of miR-301a in Th17 differentiation

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Introduction: T helper TH17 cells have been proposed to represent a new CD4+ T cell lineage that is important for development of autoimmune diseases. STAT3 is a master regulator of TH17 cell, acting at multiple levels in vivo, including TH17 T cell differentiation and cytokine production, as well as induction of RORγt and the IL-23R. miRNAs are a new group of non-coding RNAs which take part in post-transcriptional regulation of gene expression by attaching to 3'UTR of their target mRNAs and inhibition of their translation. The aim of this study was to evaluate the expression of mir-301a in patients with rheumatoid arthritis by bioinformatics methods. **Material and Methods:** Using bioinformatic database designed to predict miRNA-mRNA interaction with various algorithms (such as Target Scan), possible inhibitory effects of miR-301a on positive and negative regulators of Th17 differentiation discovered were analyzed by now. **Results:** The results revealed that miR-301a contributed to the development of the T-helper type 17 subset via targeting the IL-6/23–STAT3 pathway. These results were consistent with that of previous studies which have reported the up-regulation of miR-301a during in vitro Th17 differentiation. **Conclusion:** According to the results miR-301a could have an inducing role in Th17 differentiation. However, in vivo and in vitro experiments are needed to confirm the computational analysis. **Keywords:** miR-301a, Th17 subset, Differentiation, Autoimmune disease

9714P

Bioinformatics targeting of programmed cell death protein-1 ligands

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Introduction: Programmed cell death protein-1 (PD-1) is one of the immune checkpoint molecules, which suppresses T lymphocytes responses by its inhibitory function. Anti PD-1/ PD-Ls antibodies can prevent PD1/PDLs interactions, but adverse events of antibodies require considering other alternatives. So it is speculating to consider extracellular domain of PD-1 that probably could be effective. Binding of this domain to PD-Ls blocks membranous PD-1/ligands interactions. Bioinformatics study and gene designing of this domain was the main aim of the present study. **Material and Methods:** Bioinformatics databases (e.g. NCBI) and software (e.g. signalP) were applied to design the human PD-1 N-terminal domain expressing gene. This gene was synthesized in PUC57 plasmid, and then isolated from plasmid by XbaI and EcorI restriction enzymes. Isolated gene was ligated into the pcDNA3.1hygro+ plasmid by T4DNA ligase enzyme and afterward chemically transformed into the competent TOP10cells using CaCl2. Ampicillin selection and colony PCR methods were used to confirm the transformation. Transformed cells with ampicillin resistance gene survived in growth media with ampicillin and formed colonies. Colony PCR method was performed for each colony using designed forward and reverse primers. Agarose gel 1% was used to reveal the PCR product. **Results:** PCR product band (600bp) of each colony demonstrated the correct ligation of insert into plasmid. Confirmed colonies are TOP10 cells that contain pcDNA3.1hygro-PD-1 extracellular domain expressing gene. **Conclusion:** In this study, suitable bioinformatics design of anti-PD-Ls was achieved and this construction was applied for PD-1. This preliminary achievement is encouraged for step forward.

9718P

IL-27 -964 A>G polymorphism has effective role in risk of type1 diabetes

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Introduction: Interleukin 27 (IL-27) is a newly discovered cytokine consisting of two subunits, the Epstein-Barr virus-induced gene3 (EBI3) and p28. This cytokine can promote both anti- and pro-inflammatory immune responses, as well as Th1 differentiation. IL-27 has been linked to the activation of CD8+ T cells and promotion of 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 the p28 gene -964 A>G polymorphism in Type 1 diabetes mellitus (T1D) compared to healthy control group. **Material and Methods:** DNA was extracted from blood samples of 110 T1D patients and 302 sex, age and ethnically matched healthy controls. Flanking region of -964 position of the IL-27 p28 subunit encompassing 468 bp nucleotides was amplified by PCR and analyzed by restriction fragment length polymorphism (PCR-RFLP).**Results:** the significant differences were found in allele and genotype frequencies of IL-27 -964 A>G polymorphism between type 1 diabetes and controls (p=0.028). 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

indicated that carriers of IL-27 -964 A allele may have an increased susceptibility to type 1 diabetes. **Keywords:** interleukin-27 gene, single nucleotide polymorphism, PCR-RFLP.

9772P

The frequency of CCR5Δ32 allele in Iranian normal population; a systematic review

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Introduction: The CC chemokine receptor 5 (CCR5) is a specific modulator of the immune response.The CCR5 gene is a map of chromosome 3p21.3. Several variations may influence resistance to infectious and immune system diseases. The 32-base pair deletion in the C–C CCR5gene (CCR5-delta32mutation) is known as a protective allele against immune system disease such as Asthma, Multiple Sclerosis and HIV-1. This mutation results in resistance against HIV by producing a truncated antigen which down-modulates the expression of the receptor on the immune cell surface. This study was carried out to determine the frequency of CCR5Δ32allele in normal sample of Iranian population by studying the related articles**. Material and Methods:** The related and available data for this topic were achieved by searching for the terms "CCR5Δ32" OR" Rs333 ", " Iranian " OR "Iran", " Frequency " OR " Contribution ", using ISI Web of Knowledge, Science direct, Medline/PubMed , Scopus, Scholar, SID and Magiran search engines .**Results:** Based on the articles, frequency of CCR5Δ32 among the normal Iranian population with different ethnic status was 0.0216. **Conclusion:** Low frequency of CCR5Δ32 allele in normal Iranian population is higher and genetically susceptible to HIV-1 virus infection in Iranians during exposure to this virus. And also, low frequency of this mutant allele in Iranians suggested that there was similar frequency with East Asians and lower frequency compared to European population. **Keywords:** HIV-1, C-C chemokine receptor type 5,frequency, Iranian.

9785P

Evaluation of Gene Expression in peripheral blood and serum levels of OX40 in Multiple sclerosis patients.

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Introduction: Multiple sclerosis (MS) is an autoimmune disease of human central nervous system (CNS). T cells play critical role in MS. OX40 is a member of the tumor necrosis factor (TNF) receptor family. Ligation of OX40 is crucial for clonal expansion of antigen-specific T-cells, survival and generation of T-cell memory. Previous studies have reported that OX40 activated T cell in autoimmune diseases. Thus in this study we investigated serum levels and gene expression of OX40 as a paraclinical marker in MS patient in compared with healthy control group .**Materials and Methods:** In this research, 40 new case of MS patients and 40 healthy people as control group were investigated. After extracting RNA from peripheral blood and cDNA synthesis, We examined gene expression using Real-time PCR technique and Serum level of soluble OX40 was measured by commercially available ELISA. To compare the gene expression between the two groups used Mann-Whitney test. **Results:** Evidence did not provide significant correlation between OX40 gene expression and MS disease (P= 0.1). As well as Soluble OX40 serum level of MS patients was not significantly different. **Conclusion:** According to the results of current study, expression of OX40 gene as an inflammatory factor in peripheral blood, also serum levels of OX40 could not be considered as paraclinical marker of this disease. **Key words:** Multiple sclerosis, OX40, Gene expression, Serum level

10787P

Lack of association between NFκB-94 ins/del ATTG and Acute Myocardial Infarction

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Introduction: NFκB is well appreciated as a molecule in the heart immune activation. Increasing evidence suggested a link between inflammation, immune system and cardiac pathology. One of the links is the IL-17 pathway which recently was showed to be activated in the atherosclerosis. Not surprisingly, the pathway uses NFκB as one of the key transcription factors. In this study, the correlation between NFκB-94 ins/del ATTG variation and acute myocardial infarction (AMI) was investigated. **Material and methods:** All confirmed AMI patients (n=157) referring to the affiliated hospitals of Shiraz University of Medical Sciences in a one year period were included in this study. DNA was extracted from venous blood and the genetic variation in the NFκB was identified using PCR-RFLP method. Control individuals (n=197) were recruited among healthy blood donors of the same age range and gender. **Results:** The percentage of ins/ins homozygote genotype was 46.5% in patients vs. 47.2% in controls. The frequencies of heterozygote individuals were 42% and 42.1% in patients and controls, respectively. The percentage of del/del homozygote genotype was 11.5% in patients vs. 10.7% in controls. There was no correlation between NFκB-94 ins/del ATTG variations and AMI in our study population (p=0.97). Also there was no correlation between left ventricular ejection fraction at the time of admission as well as diastolic and systolic blood pressures with NFκB-94 ins/del ATTG genotypes .**Conclusion:** Contrary to the reports from East Asia, no association found between existed NFκB SNP and AMI, which emphasizes the differences between these populations. **Keywords:** Myocardial infarction, Single nucleotide polymorphisms, NFκB-94 ins/del ATTG

10796P

Association of genetic variants of leptin and adiponectin genes with susceptibility to scleroderma

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Introduction: Systemic scleroderma (SSc) is a systemic connective tissue disease characterized by vascular damage and fibrosis. Due to the fact that leptin and adiponectin can affect the susceptibility to SSc through Th1/Th17 deviation, in the present study, the association of leptin and adiponectin gene polymorphisms with susceptibility to scleroderma and its clinical manifestations were investigated. **Material and methods:** 198 sclerodermic patients and 223 healthy subjects matched for age and ethnicity enrolled in this study. Analyses of leptin-2548 G/A (rs7799039) polymorphism and two polymorphisms in the adiponectin gene (-11377 C/G or rs266729 and +276T/G or rs1501299) were conducted by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. **Results:** The frequency of rs7799039 GG genotype in leptin gene (p=0.049) as well as G allele frequency (p=0.01) were significantly decreased in patients compared to controls. Allelic and genotype frequencies for the adiponectin polymorphisms (rs266729 and rs1501299) were not different between patients and controls. However, for rs266729 polymorphism, a significant increase in C allele frequency was detected in patients with scleroderma compared to those in controls (97.2% and 92.4% respectively; p=0.03). **Conclusion:** Due to the fact that G allele of rs7799039 polymorphism is associated with lower production of leptin, decreased frequency of this allele in patients indicated the higher ability of leptin production in SSc patients. Accordingly, the genetic make-up of patients at leptin locus makes them more prone for Th1/Th17 development. Given the fact that systemic scleroderma can be considered as a Th1/Th17-dependent disease, the lower frequency of G allele (or the increased frequency of high leptin producer an allele) of rs7799039 polymorphism in patients can explain one of the genetic mechanisms that might be involved in scleroderma pathogenesis. **Keywords:** Systemic sclerosis, Leptin, Adiponectin, Single nucleotide polymorphism.

10806P

Comparison of sera levels and gene polymorphisms of interleukin-33 between patients with relapsing-remitting multiple sclerosis and normal controls

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Introduction: IL-33 is a member of the IL-1 cytokine gene family and as an alarmin involved in innate immune responses to helminthic infections. Recent studies in the intracellular expression of IL-33 showed increase in multiple sclerosis (MS). Of interest, IL-33 might attenuate the severity of MS through suppression of IL-17 and

IFN-γ production. Therefore, to clarify the role of IL-33 in MS pathogenesis, in the present study the sera levels as well as the alleles and genotypes frequencies of IL-33 gene polymorphisms (rs1157505 and rs7044343) were compared between patients suffer from relapsing-remitting MS (RR-MS) and normal controls. **Material and Methods:** IL-33 polymorphisms were checked in 338 MS patients and 366 healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The sera levels of IL-33 were determined in 43 patients and 43 controls by human IL-33 ELISA kit. **Results:** IL-33 serum level was not significantly different between patients and controls (75.9±25.1 pg/ml and 74.2±13.6 pg/ml, respectively; p=0.18). The frequency of alleles and genotypes frequencies for rs1157505 and rs7044343 polymorphisms have not shown any significant differences between MS patients and controls. **Conclusion:** Although the results of the present study did not show an increase in the patient's sera levels of IL-33, according to the reports indicating an increased expression of IL-33 receptors in the CNS of MS patients, determining the IL-33 levels in the cerebrospinal fluid is recommended. In addition, lack of association between polymorphisms of IL-33 gene and MS might indicate that susceptibility to MS is not affected by host genetic make-up at IL-33 locus. **Keywords:** Multiple Sclerosis, Interleukine-17, interleukine-33, polymorphism.

10815P

Gene-environment interactions study in pemphigus disease: a network-based approach

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Introduction: Pemphigus is a rare chronic skin disease characterized by autoantibodies against structural proteins in the dermal- epidermal junction that cause blister and erosion on skin or mucous membranes (mouth, nose, throat, eyes, and genitals). Here, by using bioinformatics techniques, we identified a link between the chemicals and pemphigus candidate genes. **Material and methods:** Initially, to find pemphigus-linked genes, the GeneCards database was selected and also, chemicals associated with the genes were mined from the Comparative Toxicogenomics Database (CTD). Then, chemical-protein interactions network was built to explore known and predicted interactions of chemicals and genes by using STITCH 4 database. **Results:** In this study, 11 chemical substances were identified as interacting with 66 pemphigus genes. The network construction by using the STITCH 4 database predicted the highly significant and top scoring results, containing 6 genes and 4chemicals.The genes(FOS, EGF, EIF4EBP1, RPS6KB1, MDM2 and CDKN1A) and the chemicals (rapamycin, gefitinib, 6-mercaptopuri) contribute to decreaseing inflammation and L-arginine enhancing inflammation. **Conclusion:** To generate and analysis a chemical-gene interactions network helped us to identify thehighly significantgenes and chemicals link to pemphigus and might be helpful for the development of novel therapies. **Keywords:** Pemphigus, gene, environment, gene-environment interaction

10826P

Analysis of HLA-DRB1*15 haplotype in part of Iranian MS patients

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Introduction: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that is characterized by inflammation, demyelination, axon loss and affecting young adults. The cause of MS is unknown; however, susceptibility to MS is thought to be as a result of complex interactions between genes and environmental factors. During the last decades, due to the clinical and pathophysiological complexities of the disease, diagnostic biomarkers for MS as well as other diseases are required and this is questionable. Genetic studies showed that genes encoding major histocompatibility complex antigens (HLA) particularly class II is one of the biomarkers, related to increased susceptibility to the disease. Based on different studies, the association between HLA alleles and MS disease susceptibility is controversial. The aim of this study was to determine the presence of HLA-DRB1*15 haplotype in a population of patients with multiple sclerosis in Iran. **Material and Methods:** In this study, the presence of DRB1*15 allele was investigated in 70 Iranian multiple sclerosis patients compared with 70 healthy individuals. HLA typing for this allele was performed by manual polymerase chain reaction (PCR) amplification with allele-specific primers (PCR-ASP) method. **Results:** The results suggested that, compared to healthy controls, the frequencies of HLA-DRB1*15 were significantly higher in MS patients with different geographic area in Iran (35.7% vs. 15.7%, PV=0.007). **Conclusion:** Using a highly sensitive allele-specific PCR method, it is shown that DRB1*15 allele confers increased susceptibility in Iranian MS patients and this could be as a biomarker for earlier prognosis of MS disease.

10869P

Association of the TLR2-196 to -174 del/ins gene polymorphism with risk of peptic ulcer in Babol, north of Iran

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Introduction: Most polymorphisms that occur in TLR-2 are associated with gastrointestinal disorders such as peptic ulcer. Hence, in current study, association of TLR2-196 to -174 del / ins polymorphism with risk of peptic ulcer in north of Iran was evaluated. **Material and Methods:** This case-control study included 45 patients as cases that were referred to endoscopy, and their peptic ulcer disease was confirmed, while 45 people without peptic ulcer were included as control group. DNA was extracted from the biopsy samples and using allele-specific PCR, TLR2-196 to -174 del/ins gene polymorphism were assessed. **Results:** Patients with peptic ulcer was consisted of 70% gastric ulcers, 22% duodenal ulcers and 8% gastric+ duodenal ulcers. The ins/ins, del/del and ins/del genotype frequencies in patients with peptic ulcer were 46.6%, 53.4% and 0%, respectively, while frequencies of the same genotypes in the control group were 57.7%, 40.0% and 2.3%, respectively. More analysis confirmed that del/del genotype in

patients with peptic ulcer significantly higher than the control group, while genotypes ins/ins and ins/del were less frequent in patients. Intra-group analysis showed that the del/del genotype also significantly increased the risks of developing duodenal ulcers. **Conclusion:** The findings showed that del/del genotype in TLR2-196 to -174 del/ins gene polymorphism had direct association with the risk of peptic ulcer and people with this genotype had a higher chance of developing duodenal ulcers.

10887P

A study on the relevance between Glutathione S-transferase polymorphism and the severity stage of Mustard lung.

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Introduction: Mustard gas exposed individuals represent different stages of an inflammatory and oxidative stress related response (also known as Mustard lung). Polymorphous enzyme Glutathione S- transferase plays an important role in local detoxification of alveoli. The aim of current study is to explore the hypothesis that genetic polymorphism of glutathione S- transferase family is associated with severity of mustard lung. **Material and Methods:** Blood samples were taken from 185 sulfur mustard exposed patients and 67 unexposed subjects. Multiplex PCR were conducted to genotype GSTM1 and GSTT1 deletion. To determine the polymorphism of GSTP1 in exon 5 (Ile105Val) and exon 6 (Ala114Val), RFLP-PCR method was performed. **Results:** The frequency of GSTM1 homozygous deletion was significantly higher in severe and very severe patients compared with mild and moderate subjects (p-value= 0.013). Variation in frequency among three groups for homozygous GSTM1 deletion were also significant. There were no significant association among GSTT1 and GSTP1 polymorphisms and severity of mustard lung. **Conclusion:** Genetic polymorphism in GSTM1 affects the severity of mustard lung and is a risk factor for Mustard gas related inflammatory response.

10955P

AT2R -1332 G: A Polymorphisms and diabetic nephropathy in patients with type 2 diabete mellitus in Kermanshah

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Introduction: Diabete mellitus is one of the most common endocrine disease and recognized as an important public health problem which is prevalent throughout the world. The rennin-angiotensin system (RAS) plays a central role in the regulation of sodium metabolism, blood pressure, renal hemodynamics, and is activated by hyperglycemia.

Material and Methods: In a case-control study, 70 individuals with type 2 diabetes mellitus (T2DM) including normo-, micro- and macro-albuminuria patients and 112 healthy subjects from the Kermanshah province were studied. DNA was extracted by phenol - chloroform and was studied to investigate the association between the angiotensin type 2 receptor (AT2R) -1332 G:A variants with the risk of T2DM and its complications. The genotypes of the AT2R were detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. **Results:** Analysis of AT2R -1332 G:A polymorphism indicated the absence of association between this polymorphism with T2DM and diabetic nephropathy. In females with diabetic nephropathy a significantly higher frequency of AA genotype (50%) was detected compared to those without nephropathy (13.3%, p=0.015). The presence of A allele of AT2R was associated with significantly (p=0.029) increased risk of coronary artery disease (CAD) in diabetic patients without nephropathy. **Conclusion:** Our study indicated an association between the AT2R -1332 G:A polymorphism and the risk of diabetic nephropathy only in females. Also, the A allele was associated with the risk of CAD in those diabetic patients without nephropathy. **Keywords:** Type 2 diabetes mellitus,AT2R -1332 G:A,Polymorphism,Diabetic nephropathy

10964P

Arg677Trp and Arg753Gln polymorphisms in TLR2gene in patients with smear-positive pulmonary tuberculosis in Golestan province

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Introduction: The receptors of TLRs, especially TLR 2 play an important role in the immune response in patients with tuberculosis.The prevalence of TB is 38.15 per 100 000 population in Golestan province where tuberculosis is most prevalent compared to the other provinces.The aim of this study was to detect Arg677Trp and Arg753Gln polymorphismsin TLR2 in patients with tuberculosis in Golestan province. **Material and Methods:** A total of 130 blood samples of patients with sputum smear-positive TB and 130 blood samples from healthy individuals were collected. Two polymorphisms of the TLR2 gene" Arg677Trp and Arg753Gln" were detected by PCR- (SSCP) - and sequencing methods.**Results:** DNA sequencing data for Arg677Trp polymorphism showed that the duplicated sequence is a pseudo-gene which is the same with the mutant sequence(C> T) as the Arg677Trp polymorphism cannot be considered conclusive. Arg753Gln polymorphism was not found in our samples but a deletion in nucleotide G in the position 808 was found in 15 out of 18 samples from patients. **Conclusion:** Arg753Gln polymorphism is not involved in susceptibility to the tuberculosis and the polymorphism in the nucleotide 808 position should be investigated in further studies. The presence of pseudo-gene in some genes including TLR2 requires careful analysis of data in these type of studies.

10990P

Association of the TLR-4 +3725 G/C gene polymorphisms with risk of peptic ulcer in north of Iran

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Introduction: Most polymorphisms that occur in TLR-4 are associated with gastrointestinal disorders such as gastritis, peptic ulcer and gastric cancer. Since, there was a high rate of peptic ulcer in north of Iran. So in this study, TLR-4 +3725 G/C gene polymorphisms with risk of peptic ulcer was evaluated. **Material and Methods:** This case-control study included 45 patients as cases that were referred to endoscopy, and their peptic ulcer disease was confirmed, while 45 people without peptic ulcer were included as control group. DNA was extracted from the biopsy samples and using PCR-CTPP the TLR2 TLR-4 +3725 G/C gene polymorphisms were assessed. **Results:** Patients with peptic ulcer were consisted of 70% gastric ulcers, 22% duodenal ulcers and 8% gastric+ duodenal ulcers. The G/G ، G/C ، C/C genotype frequencies in patients with peptic ulcer was 14.6%, 85.4% and 0%, respectively, while frequencies of the same genotypes in the control group was 63.6%, 36.4% and 0%, respectively. Therefore, the G/G genotype in patients with peptic was significantly higher than the control group, while genotype G/C was less frequent in patients. More analysis suggested that the G/G genotype also significantly increased the risks of developing duodenal ulcers compared with gastric ulcer. **Conclusion:** The findings revealed that genotype G/C ، C/C had direct association with the risk of peptic ulcer and people carrying these genotypes had chance to develop duodenal ulcers. The findings showed that G/G genotype in TLR-4 +3725 G/C gene polymorphisms had direct association with the risk of peptic ulcer and people with this genotype had a higher chance of developing duodenal ulcers.

11015P

Isolation and purification of HLA-DR antigen from Daudi cell line by immuno-affinity chromatography

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Introduction: HLA-DR antigens are products of HLA-D region of the human major histocompatibility locus on the short arm of the 6th chromosome whose importance in immunological reaction has been demonstrated. HLA-DR antigens can be isolated from the cell surface by utilizing affinity chromatography. This technique applied antibodies attached to an insoluble support to purify antigens from a solution. The aim of this study was to obtain proper amounts of HLA-DR antigens for immunologic studies. **Material and Methods:** HLA-DR antigens were solubilized from the membrane of an immortalized B cell line (Human Burkitt's Lymphoma Daudi) cells using a lysis buffer included EDTA and NP40, pH 7.5 with 100mM PMSF (a serine protease inhibitor). After incubation for 2 hour, they were dialyzed by dialysis tubing, cut off 3000KD. Afterwards, they were purified by affinity chromatography using anti-HLA-DR antibodies that were covalently bound to a cyanogen bromide-activated-Sepharose4B column. The purified protein was concentrated and their specificity was confirmed using enzyme-linked immuno-sorbent assay (ELISA). **Results:** ELISA assay revealed the HLA specificity of the purified antigen that was bound to an immunoassay plate using an anti-HLA-DR antibody. The purified protein showed the antigenic feature of HLA-DR. This result was obtained in the presence of proper controls and unrelated proteins (HCG).**Conclusion:** This study revealed that a membrane antigen like HLA-DR can be solubilized and subsequently purified by affinity chromatography without loss of antigenic feature.

11026P

Distribution of HLA-A and HLA-B alleles in Lak population of Iran

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Introduction: Anthropological studies based on highly polymorphic Human leukocyte antigen (HLA) genes provide useful information for bone marrow donor registry, forensic medicine, disease association studies, as well as designing peptide vaccines against tumors, and infectious or autoimmune diseases. The aim of this study was to determine the HLA-A and HLA-B allele frequencies in 100 unrelated Lak individuals from Lorestan province of Iran. Finally, the results were compared with that of previously described in Iranian population. **Material and Methods:** Commercial HLA-Type kits from BAG (Lich, Germany) company were used for determination of the HLA-A and HLA-B allele frequencies in genomic DNA, based on PCR-SSP assay. Differences between populations in the distribution of HLA-A and HLA-B alleles were estimated by chi-squared test with Yate's correction. **Results:** The most frequent HLA-A alleles were *24 (20%), *02 (18%), *03 (12%) and *11 (10%) while HLA-B*35 (24%), *51 (16%), *18 (6%) and *38 (6%) were the most frequent alleles in Lak population. HLA-A*66 (1%), *74(1%) and HLA-B*48 (1%), *55(1%) were the least observed frequencies in Lak population. **Conclusion:** The results based on HLA-A and HLA-B allele frequencies showed that Lak population possesses the previously reported general features of the Lur and Kurd populations but still with unique, decreased or increased frequencies of several alleles. **Keywords:** HLA class I, Lak population, Iran, PCR-SSP.

11206P

Association of CD247 and CD226 Gene Single Nucleotide Polymorphisms with Systemic Sclerosis in Iranian Population

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Introduction: Systemic sclerosis (SSc) is a chronic autoimmune disease distinguished by inflammation, vasculopathy and skin fibrosis. Among various genes involving in SSc and immune dysregulation, *CD226* and *CD247* also participate in the regulation of T cells.Some studies have established *CD226* and *CD247*gene polymorphisms as SSc risk factors. The aim of this study was to investigate the association of *CD226* (rs763361) and *CD247* (rs2056626) gene polymorphisms in Iranian SSc population. **Material and Methods:** Blood samples were collected from 455 SSc patients and 455 healthy sex, race and age matched individuals. Specific TaqMan genotyping (allelic discriminations) was performed by Real-Time PCR ABI system. **Results:** No significant difference was observed between patients and controls with regard to the both G/T alleles and GT, GG and TT genotypes of *CD247* polymorphism. Moreover, none of the C/T alleles and TT,CT and CC genotypes of *CD226* polymorphism had significantly different frequency between SSc patients and control group. **Conclusion:** Considering the lack of association, *CD226* and *CD247* gene polymorphisms seem to have no role in the etiopathology of the disease in Iranian SSc population and emphasizing on involvement of genetic diversity in SSc proneness. **Keywords:** *CD226,CD247*, gene polymorphism, systemic sclerosis

11208P

Down regulation of miR-143 and up regulation of MMP13 in colorectal cancer compared to normal colon mucosa

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Introduction: Colorectal cancer (CRC) is the third lethal cancer worldwide. Genetic variants have an obvious effect on colorectal cancer progression. New studies approved that microRNA-143 (miR-143) has been down regulated in specific types of cancer, including, bladder, oral squamous cell, nasopharyngeal, lymphoma and prostate cancer. In this study, the expression level of mir143 and Matrix Metalloproteinase 13 (MMP13) a gene that regulated by mir143, were evaluated. **Material and Methods:** In this study the expression level of the target genes in 45 tumor and 45 normal samples were compared. Trizol was used for total RNA extraction then extracted RNA was used in order to cDNA synthesis and at the end, target gene expression levels were measured by quantitative real time PCR. **Results:** Results of the present study demonstrated that miR-143 expression level was decreased in our tumoral samples in comparison to normal tissues and the data showed over expression of MMP13 in tumoral tissues. **Conclusion:**miR-143 may function as a novel tumor suppressor gene in colorectal cancer . miR-143 and MMP13 hopefully, could serve as a potential biomarkers and therapeutic target towards colorectal cancer.
Keywords:microRNA-143, MMP13, colorectal cancer

11219P

Analysis of MMP 13 gene expression in esophageal squamous cell carcinoma patients

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Introduction: ESCC is the second most common cancer in Iran. In this cancer clinical symptoms emerge in advanced stages of disease and treatment is very difficult in this level. Therefore survival rate in esophageal cancer is lower than other solid tumors. So finding a diagnostic marker is really efficacious in diagnosing disease more rapidly. In this study, MMP13 tested as a diagnostic marker. MMP13 has been reported as an angiogenesis and metastatic factor in ESCC. Due to its performance and previous studies, it is expected that MMP13-expression significantly increase in Iranian ESCC patients. **Material and Methods:** In this study 50 patients were tested (fresh tissue) by quantitative real-time PCR, with 50 tumor samples and 50 samples of normal tissues from same patients. The **Results:** Results of MMP13 real-time PCR demonstrated 3 level of expression in patients: 28 patients (56%) with MMP13 overexpression, 20 patients with few changes in MMP13 expression (40% unchanged) and 2 patients (4%) with under-expression. Despite of the high percentage of overexpression in patients, the statistical analysis between the expression level of MMP13 and tumor location, depth of invasion, stage, N, and grade, no significant relationships were found. **Conclusion:** ESCC patients were necessarily all in similar stages of disease (biopsy needed patients).So type of sampling could be a reason that why in this study no significant relationship was found between MMP13-experission and clinic-pathological parameters; while ELISA and serum of patients who were

tested in previous studies showed significant results. Consequently it is suggested that future researches performed on the same patients, ELISA and real-time PCR simultaneously, led to more comprehensive results. **Keywords:** MMP13, ESCC, real-time PCR, esophageal cancer

11238P

Evaluation of mirlet_7a and BACH1 genes expression in colorectal cancer and normal tissues

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Introduction: Colorectal cancer is the third most common cancer and the fourth leading cause of death among cancers in the world. Colorectal cancer is the development of cancer in the colon or rectum. BACH1 is one of the most important genes in DNA. Due to the contribution of this gene in regulatory pathways, BACH1 is believed to be associated with various cancers. To the extent that, the altered expression of this gene is a destabilizing factor of the cell. On the other hand, the microRNAlet-7a is shown to be one of the most important regulators of BACH 1protein translation. **Material and Methods:** In this study we compared the expression levels of BACH1 and microRNAlet-7ain 45tumor and 45normal samples. Trizol was used for total RNA extraction. Then, extracted RNA was used in order to cDNA synthesis and eventually, expression levels of target genes were assessed by quantitative real time PCR. **Results:** Expression of miRlet_7a was significantly decreased in tumoral samples in comparison to normal tissues ($p<0.001$). Instead, there was assigned efficient increase in the expression of BACH1 mRNA in tumoral tissues compared to normal samples ($p<0.001$).**Conclusion:**MiR-let7a may function as a novel tumor suppressor gene in colorectal cancer. Furthermore, miR-let7a and BACH1 hopefully, could serve as a potential biomarkers and therapeutic targets in colorectal cancer. **Keywords:**microRNAlet7a, BACH 1, CRC

11264P

Cd95l in multiple sclerosis patients: results from a case-control study

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Introduction: Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS). Elimination of autoreactive T cells by activation induced cell-death (AICD) is considered to be one major process in MS. The aim of this investigation was to evaluate expression level of FasL in whole blood from patients with Relapsing-Remitting (RR) form of MS, and to survey the association of FasL expression with risk, EDSS and duration of the disease. **Material and Methods:** FasL expression was compared between 50 RR-MS patients and 50 healthy controls by TaqMan Real time PCR technique. Albeit, there was an expression decrease. No statistically significant difference was found between total RR-MS patients and controls. **Results:** However, the results showed a clear association between FasL expression of females especially older than

40 years with risk of the disease (p= 0.04, 95% CI= 0.387-1.14; p= 0.003, 95% CI= 0.139-3.12, respectively). Moreover, there was no significant correlation between EDSS and duration of the disease and FasL expression. This finding makes a valuable question that what the principal concept is for this significant association between FasL expression and risk of RR-MS in females who are older than 40 years. In this study, we failed to draw an exact expression–phenotype correlation which may be due to limited statistical confirmation as a result of the small sample size and needs more investigation. **Conclusion:** These findings may possibly reflect differences in the pathogenic mechanisms associated with the failure of AICD observed in this group of MS patients. **Keywords:** multiple sclerosis, expression, CD95L

11280P

Molecular analysis of TRAF5 gene polymorphisms (rs10863888 and rs7514863) in patients with Behcet's Disease in Azeri population of northwest Iran.

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Introduction: Behcet's disease (BD) is a systemic inflammatory and autoimmune disease with unknown causes in which environmental factors in association with genetic factors are predisposing individuals with this disease. TNF Receptor-Associated Factor 5 (TRAF5) has been shown to be associated with autoimmune disease. The current study sought to investigate the potential association of TRAF5 with Behcet's disease in Iranian Azeri Turkish patients. **Material and Methods:** Two TRAF5 SNPs were analyzed in 50 Behcet's patients and 52 healthy controls by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Numerous variables were evaluated, including age, sex distribution, and clinical and laboratory observations. **Results:** The genotype and allele frequency obtained from the chi-square test done for Two SNPs (rs7514863, rs10863888) of TRAF5. In patients and healthy controls were similar and according to p- value the order 0.088, 0.65. No association was found between rs10863888, rs7514863 and Behcet's disease. **Conclusion:** The studies of SNPs in TRAF5 gene with Behçet's disease in different populations suggested various results. The study in Iranian Azeri Turkish patients revealed that TRAF5 is not involved in the development of Behcet's disease. Further stratified analysis according to the laboratory observations suggested that rs10863888, rs7514863/TRAF5 may not play a role in the development of Behcet's disease. This Results could be due of diversity of genetic background among different populations.

11321P

Investigation of programmed cell death-1(PD-1) gene variations in positions of PD1.3 (+7146) G/A and PD1.5 (+7785 C/T) in patients with non-small cell lung cancer (NSCLC)

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Introduction: Programmed Death-1 (PD-1) is an immune inhibitory molecule expressed by lymphocytes. We aimed to investigate whether polymorphisms in positions PD1.3 G/A (+7146) and PD1.5 C/T (+7785) are associated with susceptibility to non-small cell lung cancer (NSCLC) in Iranian population. **Material and Methods:** Two hundred and six patients with NSCLC and 173 age/sex matched healthy controls were enrolled. Genomic DNA was extracted from fresh whole blood samples. PCR-RFLP technique was performed for genotyping. Statistical analysis was performed by SPSS and Arlequin software packages. P-value less than 0.05 was considered statistically significant. **Results:** Genotypes distribution among both patients and controls were in agreement with Hardy–Weinberg equilibrium. The frequencies of GG, GA and AA genotypes were respectively 171/206(83.0%), 31/206 (15.0%), 4/206 (1.9%) in patients, and 144/173 (83.2%), 26/173 (15.0%), 3/173 (1.7%) in controls (p=0.988). The frequencies of CC, CT and TT genotypes were respectively 78/206 (37.9%), 100/206 (52.9%), 28/206 (53.8%) in patients and 60/173 (34.7%), 89/206 (51.4%), 24/173 (13.9%) in controls (p=0.80). There was no significant difference in allele frequencies, nor in haplotype frequencies, between patient and control groups (Pv>0.05). **Conclusion:** The data collectively suggested no association between single nucleotide polymorphisms at positions PD-1.3 and PD-1.5 in PD-1gene with susceptibility to NSCLC in Iranian population.

12366P

Different frequencies and effects of ABCB1 T3435C polymorphism on clinical and laboratory features of B cell chronic lymphocytic leukemia in Kurdish patients

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Introduction: Finding the effects of gene polymorphism on cancer pathogenesis is very desirable. The ATP-binding cassette is involved in drug metabolism, and the polymorphism of this gene may be an important risk factor in B cell chronic lymphocytic leukemia (B-CLL) or progression and/or response to chemotherapy agents**.** For the first time, the present study was aimed to evaluate the probable effects of ABCB1 T3435C polymorphism on clinical and laboratory features of Kurdish patients with B-CLL. **Material and Methods:** This descriptive analytical case-control study was performed on 50 B-CLL patients and 100 healthy subjects. Serum levels of beta-2-microglobulin (B2M) and lactate dehydrogenase (LDH) and blood WBC, RBC, Plt and ESR were measured. The T3435C polymorphism of the ABCB1 gene was determined by PCR-RFLP. **Results:** Concentration of serum and blood markers was significantly higher in the malignant group than in the benign subjects. The CC genotype had the highest frequency (66 %) in the patient group. There are no significant differences between the genotypes and type of treatment. The results demonstrate the high frequency of C allele of ABCB1 T3435C in B-CLL patients with Kurdish ethnicity. **Conclusion:** We also showed that this polymorphism is a significant risk factor in B-CLL. However, the effect of this polymorphism on clinical and laboratory characteristics of B-CLL patients was not significant. **Keywords:** B-CLL. ABCB1. T3435C polymorphism. B2M, LDH**.**

12382P

PD-1 gene polymorphisms in patients with squamous cell carcinomas of head and neck

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Introduction: Head and neck squamous cell carcinomas (HNSCCs) are the most common cancers of head and neck, and the sixth most common malignancy worldwide. Programmed cell death 1 (PD1) is an immune inhibitory molecule which delivers negative signals to, and suppresses activated B and T cells, as well as monocytes. This study aimed to investigate the association of PD-1 gene polymorphisms in positions PD1.3 (rs11568821), PD1.5 (rs2227981) and PD1.9 (rs2227982) with susceptibility to HNSCCs. **Material and Methods:** A hundred and fifty patients pathologically confirmed to suffer from HNSCCs (mean age: 60.66 ± 14.20) and 150 age-sex matched healthy controls (mean age: 60.19 ± 13.80) were recruited. Genomic DNA was extracted from white blood cells using salting out method, and restricted fragment length polymorphisms (RFLP)-PCR was performed for genotyping. **Results:** The minor allele frequency in positions PD1.3, PD1.5 and PD1.9 was 0.12, 0.34 and 0.01 in patients and 0.14, 0.32 and 0.01 among controls, respectively. No significant differences were observed in the frequencies of genotypes, alleles and haplotypes between patients and controls. The inherited genotypes were not associated with clinicopathological characteristics in HNSCC patients. Haplotype analysis revealed that the frequencies of GCC/GCT, GCC/GCC and GCT/GCC haplotypes are higher in patients than control group. Conversely, GCT/GCT, GCT/ACC, GCT/ACT and ACC/GCT occurred with significant higher frequencies in control group. **Conclusion:** The results suggested that the investigated *PD.1* gene polymorphisms are not solely associated with susceptibility to HNSCC, however; the haplotype combinations emerged from these three loci may render susceptibility to HNSCC.

12390P

MicroRNA-155 induces apoptosis in astrocytoma via targets caspase transcripts

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Introduction: Caspase plays crucial roles in induction of apoptosis. Previous studies suggested that micro-RNAs (miRNAs) are also candidate molecules in the modulation 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 astrocytoma cell line and its effects on the mRNA levels of caspase transcripts. **Material and Methods:** Astrocytoma were transfected with miR-155, as well as a scrambled sequence and mock, as controls, using Lipofectamine 2000 commercial kit. The expression of caspase transcripts were quantitated 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 Astrocytoma 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, Astrocytoma.

12505P

Evaluation of the CCNE2 Gene Polymorphism in Iranian Patients with breast cancer

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Introduction: Breast cancer, like other cancers, occurs because of an interaction between an environmental (external) factor and a genetically susceptible host. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin. In invasive breast cancers, cyclin E has been found to be overexpressed out of proportion to other markers of cell proliferation, suggesting that dysregulated expression of the gene may contribute to, rather than be a consequence of, increased cell division. Increased expression of cyclin E has been shown to correlate with poor grade and late stage lesions as well as negative estrogen receptor status. This study analyzed the association of CCNE2 (Cyclin E2) gene polymorphism with susceptibility of breast cancer. **Material and Methods:** We investigated the mRNA expression of CCNE2 in peripheral blood mononuclear cells of 53 patients with breast cancer and 53 healthy controls by Quantitative Real time PCR. **Results:** Expression of CCNE2 mRNA was significantly higher in breast cancer patients than in controls (p=0.001and p=0.01 respectively). **Conclusion:** Our data show that breast cancer is associated with an increased expression of CCNE2 mRNA. Up-regulation of CCNE2 may be important in breast cancer pathogenesis. **Keywords:** CCNE2, Polymorphism, breast cancer

12601P

Gene expression of interleukin -6 in Colonic cancer patients induced the cachexia syndrome

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Introduction: The gene expression of IL-6 and its production in the cancer cachexia patients is not well understood, in this study, we investigated the gene expression of IL-6 and it's production in colon cancer cachexia patients by using the Co-culture technique. **Material and Methods:** DNA microarray analysis and quantitative reverse transcription –PCR were used for IL-6 by Co culture of peripheral blood mononuclear cells (PBMCs) from non-cacheixa and cachectic patients diagnosed with colon cancer and normal samples, with two types of colon cancer cell lines (SW-480) and (LS-180).After 24hr and48hr culturing, RNA was extraced and then translated to e cDNA for RT-PCR in order to study the IL-6gene expression . GAPDH as a housekeeping gene used for internal control. **Results:** The results of IL-6gene expression showd it's significant over expression in colon cancer cachexia patients cell lines (LS-180) compared to colon cancer non-cachexia patients cell lines (SW-480) co-cultured with PBMCs and normal samples. **Conclusion:** Present results revealed that the level of IL-6 production in cancer cachexia patients are higher compared with the non-cachetic cancer patients, suggesting that elevated IL-6 in cancer cachexia patients may be directly linked with the mechanism of cachexia syndrome in cancer patients. **Keywords:**IL-6, Colon cancer cachexia, gene expression.

Immunohematology

Oral Presentations:

7581O

Long-term prophylaxis in patients with severe congenital factor XIII deficiency is not complicated with inhibitor formation

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Introduction: Iran with 473 patients has the highest global incidence of severe congenital factor XIII (FXIII) deficiency. Severely deficient patients require regular replacement therapies. Currently with development of FXIII concentrate, the risk of viral infections transmitted by fresh frozen plasma (FFP) and cryoprecipitate (CP) has been diminished, but the possibility of inhibitor development still remains as a challenging issue in the management of these patients. The aim of this study was to assess FXIII inhibitor development in Iranian patients with FXIII deficiency (FXIIID). **Material and Method**: This study enrolled 50 patients with severe congenital FXIIID who underwent long termo-prophylaxis with FXIII concentrate (Fibrogammin). Demographic data and clinical presentations of the patients were recorded. We evaluated plasma FXIII activity and FXIII inhibitor in day 28 after the last prophylaxis administration. The method for investigation of FXIII inhibitor was based on Bethesda assay. **Results:** The mean age of study population was 13.8 + 8.3 years. The minimum and maximum FXIII activity levels were 0 % and 4.5%, respectively. All the studied patients had a history of bleeding events, most frequently umbilical cord bleeding, and intracranial hemorrhage (ICH). Our investigations were indicative that all patients had severe form of FXIIID (FXIII activity<5%) without inhibitor development. **Conclusion:** Despite long term prophylaxis in the studied patients, none of them was detected to develop FXIII inhibitors. **Keywords:** Factor XIII deficiency, Inhibitor development, Replacement therapy

11211O

Human platelet-specific antigens frequencies among blood donors in Birjand- Iran

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Introduction: Human platelet antigens (HPAs) are antigenic determinants in platelet membrane glycoproteins. The frequencies of human platelet antigens (HPAs) vary among different populations, and are major determinant for the prevalence of HPA allo-immunization and its clinical associated entities following pregnancy or upon a transfusion. A total of 104 blood donor individuals from Birjand in East of Iran were studied for the frequency distribution of HPA-1,-2,-3,-4 and -5 systems. Material and Method: DNA extraction was performed from peripheral venous blood samples. DNA-based polymerase chain reaction with sequence-specific primers genotyping method was used for HPA genotyping. The HPA bands were visualized by using Gel Red-stained agarose gel, after electrophoresis. Genotypes 1a/1a, 1a/1b, and 1b/1b were assigned accordingly. **Results:** The frequencies obtained from blood donors were 0.95 and 0.05 for HPA-la and -Ib, 0.81, 0.19 for HPA-2a and -2b, 0.605 and 0.395 for HPA-3a and -3b and 1.00 and 0.00 for HPA-4a and -4b and 0.933 and 0.067 for HPA-5a and -5b. **Conclusion:** The HPA-1b, -4b and -5b homozygous donors were detected at low frequencies but HPA-2b frequency was higher than expected.

Poster Presentations:

7583P

ABO and Rh blood groups discrepancies among hospitals and blood transfusion center in southeast of Iran, an 3 years' experience

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Introduction: ABO and Rh bloodgrouping discrepancies are significant causes of transfusion related to morbidity and mortality. Most of these errors occurred because of deficiency in using standard blood grouping in hospitals. Thus, this study designed to determine the error rates among multi hospitals in Zahedan city, southeast of Iran. The prevalence of ABO and Rh blood grouping errors were also assessed in Iranian Blood Transfusion Organization (IBTO) of Zahedan city. **Material and Method**: During the period of the study, 80254 blood bags were sent to five hospitals. In hospitals, pre-transfusion blood grouping was carried out by slide method. Any sample with

discrepancy between IBTO and hospital laboratory was returned for more precise study to IBTO for detection of error by AABB standards protocol. **Results:** Out of 80254 samples, 420 ABO and Rh blood groups discrepancies were observed indicating a 0.5% error in pre-transfusion blood grouping among hospitals. Among these errors most common was A diminish blood groups that was diagnosed as O blood group (62 cases), while B blood group was diagnosed as O blood group in 41 cases. We also had some critical errors such as diagnosis of A blood group as B and vice versa which could endanger the patients'life. 20 ABO grouping errors were also observed in IBTO laboratory that revealed a 0.02% error. **Conclusion:** This high incidence of pre-transfusion ABO and Rh blood grouping emphasizes the necessity for using standard forward and reverse blood grouping in hospitals. **Keywords:** ABO, Rh, Blood grouping, Discrepancy

7657P

Study of anti-HLA platelet antibodies frequency in recipients of blood products patients in Taleghani and Imam Reza hospitals in Kermanshah

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Introduction: There are alloantibodies in patients with hematologic disorders after multiple blood transfusion. These antibodies are IgM and IgG and produce against allo-antigens of platelets like HLA antigen type I. Resistant platelets were detected in %2-%30 of patients with blood transfusion. The aim of this study was to determine the role of these alloantibodies in formation of unsuitable response to platelet transfusion. **Materials and Methods:** In this analytic-Cross Sectional Study anti-HLA platelet antibodies were detected by lympho-cytotoxicity method in serum of 18 patients that received blood products in Imam REZA and Taleghani hospitals then statistically analyzed according to volume and time of blood products transfusion. **Results:** Anti-HLA platelet antibodies were detected in 9 of 18 serum of patients with blood disorders and malignancies. The mean age of patients was 24.5 years. Anti-HLA antibodies were detected in 50% of patients. There were no significant relation between anti-HLA antibodies and FFP, platelet volume and time of transfusion (P>0.05). But a significant relation was seen between volume and time of pack cell transfusion and anti- HLA antibodies (P=0.03). **Conclusion:** Presence of anti-HLA platelet antibodies in patients with hematologic disorders receiving blood products leading to unsuitable response to platelet transfusion, could be prevented by the use of blood products without leukocytes.

9722P

A survey for reserved blood units with the number of cross matched and blood transfusion in Sant. Alzahra University Hospital

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Introduction: 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 who need blood transfusion. On the other hand, the hospital blood bank plays a key role in the management of the surgical patients. So it is essential to pay attention to cost benefit of blood cross match order**.** This paper discusses the interpretations of the CTR in sant. Alzahra general hospital for minimizing of the blood preparation for elective operative surgery. **Materials and Methods:** 683 blood bank cases were evaluated in the elective procedures in two departments in sant. Alzahra general hospital. 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 study is shown that in our elective operative surgery the needs were 2360 unit blood, which there were 1115 unit blood for Cross matching. In addition, the data shown that cross match transfusion ratio (CTR) was 1.9. **Conclusions:** Therefore, we could conclude that the utilize a new procedure in the hospital blood bank which play a key role in the management of the surgical patients; By using the new procedure it might be omit the reservation of blood and blood products for the surgeries. So it could be possible to decrease the ratio of CTR. **Keywords:** CTR, Transfusion, Cross- match, blood bank

9810P

Critical titres of ABO antibodies in O blood group donors

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Introduction: ABO Blood group antibodies naturally engender in normal individuals. However some incidents of intravascular haemolysis have been reported after transfusing the blood products, particularly apheresis platelets across a minor ABO incompatibility. A large volume of these antibodies in concentrates of apheresis platelets and other blood components, increased the risk of this complication. It, therefore, seems of interest to acquire a critical titres of anti-A and anti-B on the occurrence of this complication as well as on the measures that are taken to prevent it. **Materials and Methods:** 360O blood group donors were selected randomly and master two-fold serial dilution were performed for their plasma samples. Standard titration methods, as described in AABB, were implemented in this study and total antibody (IgM, IgG) in tubes and simply amounts of IgG in gel cards were determined following the detection of hem-agglutination by using both techniques in AHG phase. **Results:** Among all the samples been asserted, 29% contained high level of anti-A/B IgM and IgG with titre above 256.There were approximately 7% of cases found with the titre above 512 and only 5 cases (1%) detected as dangerous titre with the titre more than 1024. Also just 1 case was detected with the titre more than 2048 for anti-A both IgM and IgG. **Conclusion:** There is general awareness of the danger of haemolysis after the transfusion of products containing ABO-incompatible plasma, and screening for high titre ABO antibodies in blood component could be performed to efficiently reduce the risk of red blood cell destruction in recipient. **Keywords:** Antibody, Blood group, Transfusion.

10952P

The prevalence of cytomegalovirus, hepatitis B, hepatitis C and HIV infections among hemophilia - Sanandaj in 2015

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Introduction: Hemophilia patients due to impaired coagulation factor need to receive blood products exogenously to maintain their homeostasis. Therefore, there is always the concern that these patients suffer from blood-borne infections. According to the importance of viruses in infection diseases, prevention of conduction is important. So the aim of this study was to determine the prevalence of CMV virus, hepatitis B, hepatitis C and HIV among Kurdistans' hemophiliacs (Sanandaj) in 2015. **Materials and Methods:** In this study 121 patients were studied and their demographic information including sex, age, and background of blood transfusion were collected. Then, the specimens for the presence of HCVAb, HBsAb, HIVAb andCMVAb were evaluated by ELISA. HCV-Ab positive samples were confirmed by RIBA method and the results were analyzed using the software STATA-11. **Results:** From 121 patients, 95 subjects were tested for hepatitis C. The HCV-Ab, 22 patients (1/23%) positive and 13 (1/13%) were suspicious. Of the 88 participants in the CMV test, 70 patients (79/5 %) were positive for CMV-Ab.All tested patients in terms of HIV and HBV were sero-negative. **Conclusion:** Considering the role of primary prevention in modern medicine, not only the use of new and more sensitive laboratory methods, but also the use of virus inactivating coagulation factors are essential. Regarding these considerations, it is possible to prevent the disease and its complications. **Keywords**: Hemophilia, Cytomegalovirus, Hepatitis C, Hepatitis B, Sanandaj

11064P

CC Chemokines CCL2 and CCL5 are differentially expressed in patients with Sickle cell disease

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Introduction: Sickle Cell Disease (SCD) is amongst a group of genetic disorders resulting from a single base pair DNA mutation at the beta chain of hemoglobin. Chemokines play roles in the pathogenesis of inflammatory and infectious diseases. They are also involved in neovascularization processes to from new vascular networks. The aim of the present study was to measure the circulating CC chemokines CCL2 and CCL5 in the plasma of sickle cell patients. **Material and Method**: Present cross-sectional study was conducted in Rafsanjan Molecular Medicine Research Center. Peripheral blood was collected from 77 children with SCD and 70 controls. Serum was isolated and both CCL2 and CCL5 were examined using ELISA. **Results:** The findings of this study demonstrated that serum concentrations of both chemokines increased in SCD patients compared to controls. We also showed an increased level of these chemokines was also observed in patients suffering from pain crisis compared to control. **Conclusion:** Based on the results of the present study, it can probably be concluded that the balance between angiogenesis/angiostasis of CC chemokines is an important predictive factor for initiation of complications in SCD patients. The elevated levels of these chemokines may also be related to pain crisis complications in SCD.

Immunology & Nutrition

Oral Presentations:

11239O

PROBIOTICS: AN INNOVATIVE THERAPEUTIC STRATEGY FOR ALLERGIC DISEASE

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Introduction: Eczema, allergic rhinitis (AR), and asthma cause significant disease bundles worldwide. Atopic disorders are heterogeneous, resulting from complex interactions between environmental factors, including exposure to microbes, host genes, modulating innate and acquired immune responses, and mucosal and skin integrity. Allergies are inflammatory diseases depending on an abnormal activity of type 2 T helper (Th2), which is not regulated by the physiological anti-inflammatory system in allergic subjects. Several studies underlined the pivotal role of the gut micro biota in atopic diseases. Probiotics are live bacteria that colonize the gastrointestinal tract and impart beneficial effects for health. Evidence suggested that possible imbalances in intestinal micro biota composition may be implicated in the occurrence of allergic diseases. **Material and Methods**: Systematic search of MEDLINE, EMBASE, and Cochrane Library was conducted for all comparative studies since 2007 to 2015 without any limitations in languages. **Results**: Several studies underlined the pivotal role of the gut micro biota in atopic diseases, as it is able to stimulate the immune system, inducing a maturation of Th1 cells immune responses and

inhibiting the development of allergic Th2 responses and allergic antibody (IgE) production. Several studies highlighted the ability of probiotic bacteria to modulate mucosal immune responses, enhancing mucosal barrier functions, and inducing the production of anti-inflammatory cytokines. **Conclusions**: Food allergy rates have rapidly increased in both the developed and developing world, some food agents that modulate gut micro biota, such as probiotics, could be preventive and therapeutic agents in human allergic disease. **Key words**: probiotic, allergy, immune system

Poster Presentations:

3485P

Assessing the effect of 5-hydroxy methyl furfural on selected components of immune response in BALB/c mice immunized with ovalbumin

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Introduction: 5-Hydroxymethylfurfural (5-HMF) is one of the most important products of maillard reaction. In recent years, many profitable biological effects of this compound have been demonstrated. This study sought to elucidate anti-allergic effect of 5-HMF by investigating some selected components of immune response in BALB/c mice immunized with ovalbumin (OVA). **Materia and Methods:** Four groups of BALB/c mice (n=8 for each group) including: control, vehicle and two different dose of 5-HMF (188 and 750 mg/kg bw) treatment groups were studied. All groups except the controls, were immunized with ovalbumin on days 7 and 28. Serum level of total and OVA-specific IgE, interleukin 4(IL-4) and interferon gamma (IFN-γ) were measured in OVA-stimulated splenocytes using ELISA. **Results**: Immunized animals had increased level of serum total and OVA-specific antibodies as compared to control ($P<0.01$). It was found that OVA-induced increase in serum IgE and OVA-specific IgE were significantly suppressed in 5-HMF treatment groups ($P<0.05$). Moreover, IL-4 and IFN-γ were significantly reduced in a dose-independent manner compared to vehicle ($P<0.05$). **Conclusions:** 5-HMF inhibited up-regulation of serum total and OVA-specific IgE maybe through the suppression of Th2-type immune response in immunized BALB/c mice. These changes may reflect that 5-HMF could be a novel therapeutic approach for prevention of IgE-mediated allergic diseases.

10979P

Evaluation of the presence of antibodies reacting with pathogenic bacteria in camel milk

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Introduction: Camel's milk plays an important role in nutrition of human in different parts of the world and is assumed as therapeutic substance in traditional medicine. In this study, the presence of antibodies in camel milk against entero-pathogenic bacteria including *Escherichia coli , Salmonella typhi ,Shigella dysenteriae* and the bacteria involved in burning infections: *Pseudomonas aeruginosa*and *Staphylococcus aureus* was conducted. **Materials and methods**: Antigens were prepared from their bacterial pure cultures. Milk samples were collected and centrifuged for removing the fat. Then casein was precipitated by lactic acid and then, samples were prepared for reaction. Agglutination method was used in 96-well microtiter plate for evaluation of reaction between bacterial antigens and camel's milk samples in serial dilutions. **Results**: Evaluation of antibodies showed that milk samples had antibodies against pathogenic bacteria although the antibody concentration in the various samples was different. Antibody titers were viewed against *S.typhi,S.dysenteriae*and *P.aeroginosa* in the range of 1/4 – 1/16 and antibody titers against *E.coli* and*S.aureus*, respectively, in the range of 1/4 – 1/64 and 1/2 – 1/64 in various samples. **Conclusion**: In this study the presence of antibodies reacting with the bacterial antigens in camel's milk was detected. The presence of antibodies against pathogenic bacteria in camel's milk may be useful for some beneficial uses of camel milk in traditional and complementary medicine, but it is suggested to improve research about it. **Keywords**: Camel'smilk, Agglutination, E.coli

11121P

The prevalence of zinc deficiency this element in the blood serum-based test in Jahrom University of Medical Sciences

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Introduction: Zinc is an essential mineral that occurs naturally in available food and food supplements. It is a building block of more than 300 enzymes and plays an important role in the immune system's natural growth and development. Zinc deficiency can reduce learning. Due to lack of storage in the body, it is necessary daily intake. Given the high prevalence of zinc deficiency in developing countries, this study aimed at determining the prevalence of zinc deficiency in the blood serum-based test of Jahrom University of Medical Sciences**. Materials and Methods:** In this cross-sectional study, 66 volunteers were randomly selected. To complete the consent form and demographic information related to their tips. Then, 5 ml blood was received and quickly centrifuged to separate serum and by atomic absorption spectrophotometer, serum zinc level was measured. The results of descriptive statistics were analyzed by using the software. SPSS **Results:** In this study, the concentration of less than 70 mcg /

ml of zinc deficiency was considered. According to the analysis of the study data Zinc deficiency was not observed. A significant correlation between the family, home, mother's education, age and sex and zinc deficiency was not detected. But a significant relationship was observed between education level of the father and Zinc deficiency. None of those reports have underlying disease. **Conclusion:** Given to our population who are medical students, the use of nutrients and methods of using them are properly trained and families also have a higher level of awareness and education.On the other hand, the student's food and food professionals (university's cafeteria) are designed. According to our study population, and the location, there was no detected problem due to zinc deficiency.

12500P

The association between serum thyroid hormones, interleukin-23 (IL-23), transforming growth factor β (TGF β) and vascular endothelial growth factor (VEGF) in patients with Hashimoto's thyroiditis

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Introduction: Hashimoto's thyroiditis is an autoimmune disorder and is the most common cause of hypothyroidism. Recently, the studies have shown that T-helper cell imbalance specifically Th-17 and Treg cells play a major role in the pathogenesis of the disease. The aim of the current study is to evaluate the association between serum interleukin-23, TGF-β and VEGF with thyroid hormones in patients with Hashimoto's thyroiditis. **Materials and methods:** Forty patients with Hashimoto's thyroiditis aged between 22-50 years old participated in the current study. Serum concentrations of IL-23, TGF-β, VEGF, thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) were assessed by ELISA method and their association was evaluated with partial correlations analysis. **Results**: Significantly positive association between serum IL-23 and TSH ($r = 0.37$, $P = 0.018$), VEGF ($r = 0.62$, $P < 0.001$) and TGF-β ($r = 0.63$, $P < 0.001$) was found. These associations remained significant even after adjusting for the confounding effects of BMI and physical activity. Other parameters were not associated with each other. **Conclusions:** In the current study IL-23 identified as a positive indicator of thyroid stimulating hormone and was in positive association with growth factors involved in the pathogenesis of Hashimoto's thyroiditis. Further interventional studies are warranted to reduce this cytokine level and other related parameters in treatment of Hashimoto's thyroiditis. **Key words:** Hashimoto's thyroiditis, IL-23, TGF-β, VEGF

12501P

The association between serum thyroid hormones, lipid profile, glycemic status and Nesfatin-1 in patients with Hashimoto's thyroiditis

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Introduction: lipoprotein metabolism, the aim of the current study was to evaluate the association between serum thyroid hormones, lipid profile, glycemic status and Nesfatin-1 in patients with Hashimoto's thyroiditis. **Materials and methods:** Forty patients aged between 22-50 years old, with Hashimoto's thyroiditis, participated in the current

study. Serum concentrations of lipid profile, fasting blood sugar, insulin, thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4) and Nesfatin-1 were assessed by ELISA method and their association was evaluated with partial correlations analysis. **Results:** In the current study, serum TSH was in negative association with HDL (r = - 0.4, P= 0.01) while serum T3 was in negative associations with TG (r = -0.41, P = 0.009) and serum LDL (r= -0.34, P 0.03). There was also a negative association between serum Nesfatin-1 and TG concentrations (r = -0.31, P = 0.04). **Conclusions:** The findings of the current study confirm the associations between lipid profile, Nesfatin-1 and thyroid hormones in patients with Hashimoto's thyroiditis. Further studies are needed to confirm these associations and underlying mechanisms. **Key words:** Hashimoto's thyroiditis, lipid profile, glycemic status, Nesfatin-1

Immunology in Reproductive Medicine

Oral Presentations:

7538O

Uterine Natural Killer Cell and Human Leukocyte Antigen-G1 and Human Leukocyte Antigen-G5 Expression in Vaginal Discharge of Threatened-Abortion Women

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Introduction : The immuno-tolerant human leukocyte antigen-G (HLA-G) molecules have a major role in fetal-maternal tolerance during pregnancy. Interaction between these molecules and uterine natural killer (uNK) cells inhibitory receptors prevent NK cell invasion against fetus trophoblast cells. The aim of this study was to evaluate the percentages of uNK cells and HLA-G1 and HLA-G5 isoforms expression in vaginal discharge of threatened-abortion women in comparison with control. **Material and Methods:**In a case-control study, 30 threatened-abortion women were investigated with bleeding or spotting less than 20 weeks of pregnancy as compared to 30 normal pregnant women. uNK cells percentage was assessed by flow cytometry. Furthermore, HLA-G1 and HLA-G5 isoforms expression were evaluated by Real-Time PCR in these groups. **Results:** The results of this study showed that threatened-abortion women had increased uNK cells and decreased T cells percentage in vaginal discharge in comparison with normal pregnant women ($p=0.01$,$p=0.003$, resp.). In addition, HLA-G1 isoform had lower expression in threatened-abortion women in comparison with control group ($p=0.0001$). **Conclusion:**The increase of uNK cells level with the decrease of HLA-G expression in vaginal discharge of threatened-abortion pregnant women is an indicator of mother's immune dysregulation. It is concluded that HLA-G expression level with uNK cells percentage could be determined as a diagnostic marker for threatened-abortion women.**Keyword:** threatened-abortion, HLA-G, uNK cell

7602O

The study of cell proliferation, TNF-α cytokine production and expression of CD8 marker in peripheral blood lymphocytes of women with polycystic ovary syndrome (PCOS) by co-culture with breast tumor cell lines (MDA-468, MCF-7)

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Introduction: Polycystic ovarian Syndrome (PCOS) is the most prevalent endocrinology disorder in women. Probability of malignancy for example in breast cancer is doubtful in some epidemiological studies.The state of systemic inflammatory cytokines especially TNF-α in the patients is the main reason for immunological disturbance including insulin resistance and metabolic syndrome. In this research, the ability of antitumor immunity by peripheral mononuclear leukocyte of these patients was studied and evaluated in experimental approach with co-culture system between effectors and target tumor cell lines. **Material & methods:** 50 samples of isolated PBMNCs from peripheral blood samples (patient and health group) were examined by density gradient of ficoll. MDA-468 and MCF-7 tumor cell lines were incubated as two target cells and cultured adjacent to mononuclear cells in trans well incubation system. In two time intervals (48 and 72 hours) after co-culture, the proliferation rate of effectors cells was evaluated by BrdU technique. Assessment of produced TNF-α Cytokine in culture supernatant was performed by ELISA method. Determination of CD3+CD8+ lymphocyte has been rendered by flow cytometer. **Results:** Proliferating response of effector cells by stimulation with tumor cell lines had significant differences between two groups (p=0.05) in 72 hours and was continued. Production level of the cytokine from MDA-468 co-culture in patient samples was observed by significant increasing (p=0.031), but no differences were observed in two time intervals. **Conclusion:** The obtained differences between 2 groups of samples in antitumor cell responses could be emerged and manifested for indicate evidence from systemic inflammatory state in PCOS patient or glucose metabolic disorders. **Key Words:** Polycystic ovarian syndrome, Breast cancer, co-culture, TNF-α, CD8+

9780O

The study of cell proliferation, TNF-α cytokine production and expression of CD8 marker in peripheral blood lymphocytes of women with polycystic ovary syndrome (PCOS) by co-culture with ovarian tumor cell lines (SKOV3,A2780)

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Introduction: Polycystic ovarian syndrome (PCOS) is a pro-inflammatory state that underpins the development of metabolic aberration and ovarian dysfunction in the disorder. Chronic inflammation and increased levels of androgens in this group of patients and their impact on the immune system, may be able to disrupt the antitumor

activity and thus increased the risk of developing malignancies including ovarian cancer. **Material & methods:** Peripheral blood mononuclear cells of 50 patients with PCOS and healthy samples were purified by Ficoll density gradient centrifugation. Then, the cell proliferation, concentrations of cytokines TNF-α and percentage of CD_3^+ CD_8^+lymphocytes in two-time (48 and 72 hours) after co-culture of ovarian tumor cell lines (SKOV3 and A2780) with PBMC in indirect contact of trans-well system were measured.**Results:** Proliferative response of executive cells during stimulation with tumor cell lines despite lower average in the control group, and the difference was statistically significant only at 72 hours compared to 48 hours in both groups ($P < 0.01$). The production of TNF-α in co-culture of A2780 cell lines significantly increased in the group of patients and in time compared to the control group ($P < 0.05$). Also, determination of the percentage of the population of cytotoxic lymphocytes, showed no significant difference. **Conclusion:** The low level of chronic inflammation in patients with PCOS, was approved by increased proliferative response of effector cells and secreted TNF-α levels compared to healthy individuals. However, an increased risk of cancers in patients with PCOS, requires an examination of anti-tumor responses with higher sample volume. **Keywords:** polycystic ovarian syndrome; chronic inflammation; ovarian cancer; co-culture

10855O

The study of TNF-α cytokine production ،expression of CD8marker and proliferation of peripheral blood lymphocyte of multiparous women compared with nulliparous women by Co-culture with MDA-231 and MCF-7 breast tumor cell lines

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Introduction: Breast cancer is the second most common cancers in the world. Many factors are involved in its incidence. Epidemiological studies showed that breast cancer in multiparous women is less common. One of hypothesis in this regard is hormonal changes and more importantly, Microchimerism phenomenon during pregnancy. In this study, a co-culture model was designed and lymphocytes proliferation of multiparous and nulliparous women, concentrations of TNF-α cytokine and percentage of cytotoxic lymphocytes in exposure to breast tumor cell lines were measured. **Material & methods:** Peripheral blood mononuclear cells of 48 multiparous and nulliparous women were purified by Ficoll density gradient centrifugation. Then, by co-culture system and indirect contact with 0.4µm trans-wells pore size, breast tumor cell lines MDA-231 and MCF-7 were exposed to mononuclear cells. Cell proliferation by BrdU method, concentrations of TNF-α cytokine from the culture supernatant by ELISA method and percentage of lymphocytes CD_3^+ CD_8 by flow cytometry were evaluated two times (48 and 72 hours) after co-culture . **Results:** Effector cells proliferative response during stimulation with tumor cell lines between multiparous and nulliparous women, was significant ($P<0.04$). The mean of lymphocyte proliferation 48h after co-culture in both cell lines were statistically significant ($P < 0.04$). The production of TNFα in co-culture of tumor cell lines two times (48h, 72h) were significant ($P<0.05$). But the percentage of the cytotoxic lymphocytes (CD3 + CD8 +) showed no significant difference between the two groups. **Conclusion:** Increasing the lymphocyte proliferation and concentration of TNFα cytokine during co-culture, particularly in multiparous women showed that multiple pregnancy can provoke anti-tumor response and resistance to the development of cancer. By considering the findings of this study, it is possible that immune system of multiparous women due to multiple chimerism resists better than nulliparous against breast cancer. However, the conclusion about relationship between breast cancer and parity requires an examination of additional anti-tumor responses with higher sample volume. **Keywords:** breast cancer; multiparous; nulliparous; lymphocyte proliferation; cytokine

10873O

Evaluation of apoptosis and angiogenesis in ectopic and eutopic stromal cells ofpatients with endometriosis

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Introduction: Endometriosis is a chronic, painful, and inflammatory disease characterized by the growth of endometrial tissue outside the uterine cavity. Increased angiogenesis and resistance to apoptosis have been suggested to be involved in pathogenesis and development of endometriosis. The objective of this study was to examine the apoptosis potential and angiogenesis contribution of eutopic (EuESCs) and ectopic (EESCs) endometrial stromal cells in patients with endometriosis compared to endometrial stromal cells from non-endometriotic controls (CESCs). **Material and Methods:** Stromal cells were isolated though enzymatic digestion of ectopic and eutopic endometrial tissues from 10 laparoscopically-confirmed endometriotic patients and characterized by flow cytometry. Endometrial stromal cells of 10 non-endometriotic patients were served as control. Following the cell characterization by immuno-fluorescent staining and flow cytometry using a panel of 13 monoclonal antibodies, the total RNA was isolated from the cultured cells, and analyzed for expression of genes involved in apoptosis (Bcl-2, Bcl-xL, Bax and Caspase-3) and angiogenesis[vascular endothelial growth factor (VEFF-A)]byReal-time PCR. **Results:** Endometrial stromal cells from all three sources expressed markers associated with mesenchymal origin (CD9, CD10, CD29, CD44, CD73, CD105, Vimentin, and Nestin), but failed to express markers of other origins (CD34, CD38, CD45, CD133, and Cytokeratin). EuESCs exhibited a significantly lower Caspase-3 gene expression compared to CESCs or EESCs ($p<0.01$). Although, Bax gene showed increased expression in EESCs compared to EuESCs, and CESCs, the difference was not statistically significant. Significantly higher gene expression levels of Bcl-2 and Bcl-xL were found in EESCs compared to EuESCs and CESCs ($p<0.01$). VEGF gene expression by EESCs and EuESCs were significantly higher compared to those of CESCs ($p<0.001$).**Conclusion:** The findings suggested reduced propensity of apoptosis and increased angiogenesis potential of EESCs, which may be involved in pathogenesis of endometriosis. **Key words:** Endometriosis, Apoptosis, Angiogenesis, Gene expression

11229O

MSC administration inducing CD4+CD25+FoxP3+ regulatory T cells and reducing fetal rejection in the abortion prone mouse model

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Introduction: Recurrent spontaneous abortion is one of the most common complications of pregnancy with a prevalence of 2-5% among pregnant women. Regulatory T cells emerge in the last recent years as key players in allowing fetal survival within the maternal uterus. MSCs have been shown to modulate immune responses by the de novo induction and expansion of $CD4^+CD25^+FoxP3^+$ Treg cells. **Material and methods:** The MSCs were derived from the abdominal fat of CBA/J mice, and their phenotype and polypotency were determined by flow cytometry and differentiation tests. In the day 4 of gestation MSCs was administered to abortion-prone mice through i.p rote.

On day 14 of the gestation, spleens and reginal lymph nodes of pregnant mice were collected and the percentage of $CD4^+CD25^+$ $FoxP3^+$ was analyzed by flow cytometry. The abortion rate was also determined in MSCs treated and untreated groups. **Results:** The abortion rate diminished significantely in MSCs-treated group compared to untreated mice, as expected (Mean: 6.87% vs. 29.82%, p=0. 0019). The frequency of Tregs in the lymph node of MSCs-treated mice was also increased significantely. **Conclusion:** Treg cells have been proposed to be important players during murine and human pregnancies. Here, it was shown for the first time that adoptive transfer of MSCs protects against fetal rejection in abortion-prone mice. These data suggested that high levels of $CD4^+CD25^+Foxp3^+$ Tregs induced by MSCs administration is one of the key players in reduction of the abortion rate in the abortion prone mouse model.

11284O

Compression of the Effect of Acute and Chronic Psychological Stress on the Rat's Seminiferous Tubules Germ Cell's Apoptosis

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Introduction: Recent studies have shown that apoptosis is involved in psychological stress responses and could affect male fertility. The aim of the present study was to investigate the effects of acute and chronic psychological stress on the rat's seminiferous tubules germ cell's apoptosis. **Material and methods:** Forty two adult Wistar male rats were randomly divided into seven groups of 6 animals. In the test groups, rats were exposed to stress for 1 day (T1), 3 days (T3), and 6 days (T6). In the sham groups, rats were placed on the same waterless platform for 1 day (s1), 3 days (s3) and 6 days (s6). Rats of control group received no intervention. Acute stress was defined as 1 day and 3 days stress, and chronic stress was defined as 6 days stress. Testis tissues were prepared for TUNEL assay for detection of apoptosis .**Results:** There was a significant enhancement in the serum corticosterone in all tests (T1:3.92±0/22, T3: 14.57±0/09, T6: 15.26±0/5 ng/ml) and sham (S1:16.32±0/27 S3: 17.76±0/15 s6: 18.3±0/1 ng/ml) groups in both acute and chronic stress when compared to the control (10.13±0/24 ng/ml) group ($P<0.05$). Also, apoptotic cells were significantly increased in all tests (T1:10±0/58, T2:14±0/45, T3: 16±0/57) and sham (S1:14±0/55, S2:19±0/76, S3: 19±0/61) groups when compared to the control (8±0/61) group ($P<0.05$). **Conclusion:** Based on the results, acute and chronic psychological stress increased the rat's seminiferous tubules germ cell's apoptosis and it is notable that stress and apoptosis of sham groups was more severe than tests group.

Poster Presentations:

3474P

Comparison of the result of Interlukin-6 in maternal serum with cervico-vaginal fluid in preterm labor

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Introduction: Preterm birth is a common problem in obstetrical field. Although in recent years morbidity and mortality of premature infants has decreased, less improvement in prediction and prevention of preterm delivery has been achieved. Cytokines such as (Interlukin-6) IL-6 and IL-8 in women with preterm labor are used to predict of preterm delivery. The purpose of this study was to measure maternal serum IL-6 in mothers with preterm uterine contractions and compare it with cervico-vaginal IL-6 in the same women. **Material and Methods:** In this cross-sectional study, IL-6 in serum and cervico-vaginal fluid of 86 women with preterm uterine contractions were measured. All participants had an intact membrane. IL-6 was measured by using the ELISA method. All participants were followed up until delivery. Statistical analysis was conducted using U-Man Whitney, Chi-Square and Kendall's tests. **Results:** In this study, the median (Quartile25, Quartile75) of IL-6 in cervico-vaginal fluid was higher than maternal serum IL-6. There was a statically significant difference in the median of serum and cervico-vaginal IL-6 in preterm labor ($P<0.0001$). There was no significant correlation between serum and cervico-vaginl IL-6 ($r=0.048$, $P=0.548$). **Conclusion:** It was found that maternal serum IL-6 is not a suitable biomarker for predicting preterm delivery. **Key words:** cervico-vaginal fluid, maternal serum, interlukin-6, preterm labor

7688P

Different expression of IL-6 and TNF-α in placenta from women complicated with pre-eclampsia compared with healthy pregnant women

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Introduction: Pre-eclampsia (PE) is one of the most complex and life-threatening pregnancy disorders and a major cause of mortality among mothers and fetuses in worldwide. It is well known that immune system has a major role in pregnancy. On the other hand, parturition and an inflammatory response are inseparable. In contrast to normal pregnancy, increased inflammatory responses established in pre-eclamptic pregnancy. The present study aimed to investigate the expression of IL-6 and TNF-α in placenta from women complicated with pre-eclampsia compared to healthy pregnant women. **Material and Methods:** The sample of the present study was undertaken on 30 primiparous pregnant women at third trimester of pregnancy. Fifteen out of 30 women were diagnosed with PE while the remaining 15 were healthy. Total RNA was extracted from 500 mg of all fetal and maternal parts of the

collected placentas using total RNA extraction kit. cDNA was synthesized. Quantitative real-time PCR (Q-PCR) was performed by using SYBR Green method to amplify IL-6 and TNF-α. Comparative CT and fold differentiation ($2^{-\Delta\Delta CT}$) methods were used to quantify the target gene. Unpaired student's T tests were used for quantification analysis of Q-PCR results. **Results:** Statistical analysis revealed that the expression of both cytokines is up-regulated in the pre-eclamptic women. IL-6 by 2.65 and TNF-α by 2.86 times showed over expression in maternal part of placenta from women with pre-eclampsia. Moreover in the fetal part of placenta the fold changes of IL-6 and TNF-α were 1.12 and 1.43 respectively. **Conclusion:** The findings of the current study showed that in pre-eclamptic patients, the maternal parts of the placentas actively participate in the induction of the exaggerated placental inflammatory status by production of high levels of TNF-α and IL-6. Therefore, it seems that over expressed TNF-α and IL-6 from both maternal and fetal parts work together to generate the increased inflammation in PE placentas.

7689P

Different expression of IL-6 and TNF-α in blood samples from women complicated with pre-eclampsia compared with healthy pregnant women

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Introduction: 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 in worldwide. It is well known that immune system has a major role in pregnancy. On the other hands parturition and an inflammatory response are inseparable. In contrast to normal pregnancy, increased inflammatory responses established in preeclamptic pregnancy. The present study aimed to investigate the expression of IL-6 and TNF-α in blood samples from women complicated with pre-eclampsia compared with healthy pregnant women. **Material and Methods:** The samples of the present study were undertaken on 30 primiparous pregnant women at third trimester of pregnancy. Fifteen out of 30 women were diagnosed with PE while the remaining 15 were healthy. Mononuclear cells were separated from cord and peripheral blood. RNA extracted and cDNA was synthesized. Quantitative real-time PCR (Q-PCR) was performed by using SYBR Green method to amplify IL-6 and TNF-α. Comparative CT and fold differentiation (2-ΔΔCT) methods were used for quantification of target gene. Unpaired student's T tests were used for quantification analysis of Q-PCR results. **Results:** Statistical analysis revealed that the expression of both cytokines is up regulated in blood samples of preeclamptic women. IL-6 by 6.66 and TNF-α by 3.23 times showed over expression in peripheral blood of women with preeclampsia. Moreover in the cord blood samples of preeclamptic women the fold changes of IL-6 and TNF-α were 13.04 and 2.15 respectively. **Conclusions:** The comparison of the cytokines levels between peripheral and cord bloods indicated that IL-6 in the peripheral blood and TNF- α in cord blood samples of preeclamptic patients were more over-expressed.

9723P

Immunologic Modifications in Pregnancy

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Introduction: Pregnancy is a special situation of immune homeostatic with modifications in the maternal immune system that leads to maternal tolerance to the fetal tissue with genetically of different pattern. These modifications are associated with the repression of cell-mediated immunity. Studies showed that during pregnancy, immune system cells such as T, B lymphocytes, NK cells, and granulocytes modify in function, phenotype, counts and production of soluble factors such as cytokines. For example, T cells have impaired proliferation and secretion of cytokines such as IL-2 (Interleukin-2) and IFN-γ (Interferon-γ). In contrast, the function of B lymphocytes is normal. The phenotypic markers of monocytes and granulocytes showed different expression patterns. The TCD4+ cells increase and TCD8+ decrease while NK cells differ in number. **Material and Methods:** This is a review study performed on original and review papers from databases of PubMed and Google Scholar that have been published from Feb 2011 to Dec 2015 in the fields of immunology of pregnancy, pregnancy effects on the immune system and immunological aspect of pregnancy. **Result:** During pregnancy major changes occur in the immune system of pregnant women that cause susceptibility to some infection and fetal survival. **Conclusion:** The fluctuation of maternal immune system is essential for the protection of fetus as an allograft tissue. **Key word:** Pregnancy, Modifications of immune system, Fetus

10805P

Association of IL-23R gene polymorphism (rs 10889677) with susceptibility to unexplained recurrent spontaneous abortion

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Introduction: Among other cytokines, IL-23 might be involved in the pathogenesis of recurrent spontaneous abortion (RSA) by induction of CD4+ Th17 cells. Therefore, in the present study association of an IL-23R gene polymorphism located in the 3′-UTR (rs10889677) and susceptibility to RSA was investigated. **Material and Methods:** In this study, the frequency of alleles and genotypes of IL-23R gene polymorphism (rs10889677) were compared between 174 patients with RSA and 154 age and sex matched normal females using Allele Specific PCR. **Results:** 21.26% of RSA patients carried the CC genotype (low IL-23R producing genotype) while 31.81% of normal healthy controls had this genotype. Thus, the percentage of this genotype was significantly higher in controls compared to those of patients (p=0.041). **Conclusion:** The results of the present study indicated that RSA patients have genetically more ability in expression of IL-23R compared to the normal females. Therefore, higher activity of Th17 cells and consequently more tendency to abortion in RSA patients might be explained according to the inheritance of high IL-23R producing polymorphisms in these patients. In addition, these results could be considered as a further evidence for the participation of Th17 cells in the pathogenesis of RSA. **Key words:** Recurrent spontaneous abortion, IL-23R, Polymorphism.

10866P

Evaluation of serum Peroxiredoxin3 and Peroxiredoxin4 auto antibodies in Recurrent Spontaneous Abortion patients

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Introduction: Recent studies showed the importance of immunological factors in pregnancy and the relationship between autoimmunity and Recurrent Spontaneous Abortion (RSA) has been recognized. Some surveys reported the presence of auto antibodies in recurrent miscarriage which can affect the placenta or fetus and result into abortion. Recently placental antigens, Peroxiredoxin 3 (Prx3) and Peroxiredoxin 4 (Prx4) were introduced and related autoantibodies have been detected in RSA patients. Because of antioxidant activity of these molecules and their 10855presence of autoantibodies in serum of RSA patients and quantitative assessment of a new autoimmune factor in this disorder. **Material and methods:** In this case-control study, 100 women with a history of at least three RSA and 32 women with at least two successful pregnancies as a control group were included. Serum level of Anti-Prx3 and Prx4 were assessed in these two groups with related designed ELISA method. Results were evaluated with independent sample t-test. **Results:** The data demonstrated that the level of anti-Prx4 is higher in RSA patients compared to normal controls significantly (P=0.004). However there was no statistically difference in the level of anti-Prx3 between RSA and healthy women (P=0.51). **Conclusion:** The results indicated that due to the high level of anti-Prx4 in women with RSA compared to controls, anti-Prx4 could be evaluated as a new autoantibody associated with RSA patients and by more studies it can be introduced as a diagnostic test for immunologic abortions.

10969P

Presence of antibody against placental HSP70 protein in serum of women afflicted with Multiple Sclerosis

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Introduction: Pregnancy is a unique biological phenomenon, in which mother's immune system adjusts itself to the embryo. Some pregnancy complications like recurrent miscarriage and pre-eclampsia are secondary to other diseases such as autoimmune diseases. Association between some autoantibodies including Anti-phospholipid antibodies and Angiotensin II type1 receptor autoantibody and pregnancy complications is clearly known. Multiple sclerosis (MS) is the most frequent autoimmune disease of central nervous system and about 2/3 of MS patients are women at childbearing ages. The presence of different antibodies in serum of MS patients have been reported. During pregnancy a diverse group of functional proteins are expressed by the placenta which are involved in processes like angiogenesis and immune system responses. Inappropriate responses of the mother's immune

system such as antibody production against these proteins, may lead to pregnancy-related complications. The aim of the present study was to investigate the probable placental antigens that may be targeted by antibodies in the sera of MS patients. **Method:** Total placental proteins were extracted from normal fresh placenta and separated using two dimensional gel electrophoresis (2-DE) technique. The separated proteins were transferred to PVDF membrane and blotted with sera from 20 women afflicted with MS, and compared with the membrane blotted using sera of 20 healthy women. Differentially blotted spot were picked from 2DE gels and identified by mass spectrometry and confirmed by western blot techniques.**Result:** The results indicated that MS women may produce antibody against placental HSP70.**Conclusion:**The presence of antibody against HSP70 may introduce a new autoimmune hypothesis in MS, which is needed to be tested in future experiments. Considering the role of HSP70 as a chaperone with anti-inflammatory and immune modulatory properties, the presence of antibody against HSP70 may interfere with its normal functions in pregnancy and lead to undesirable clinical pregnancy outcomes.**Keywords:** Multiple sclerosis, Placenta, Pregnancy, Auto antibody, HSP70

10975P

The serum levels of soluble sCD93 and TNF-α is higher in patients with Pre-eclampsia

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Introduction: Pre-eclampsia (PE) is a potentially dangerous pregnancy complication that characterized by high blood pressure. Although the etiology of PE is not well known many studies indicated that inflammation could play a crucial role in its pathobiology. sCD93 is a newly identified inflammatory mediator which recently was shown to excrete from cell surface of monocytes during inflammation. The aim of this study was to determine the levels of soluble CD93, and TNF-a in patients with PE compared to normal pregnant women (NPW).**Material and methods:** Blood samples were taken from 41 patients with PE and 41 age matched NPW that both approved by Gynecologist. CBC and common laboratory tests such SGOT, SGPT, Creatinine and Urea were performed for both groups. sCD93 levels were detected by ELISA. All the data were analyzed by SPSS 16.**Results:** The mean age of the patients and control participants were 27 years old. SGOT, SGPT, and creatinine were not significantly different between two groups. However, the levels of other laboratories test such as urea and platelets number were higher in control group. Interestingly, the levels of sCD93 and TNF-α were significantly elevated in the PE patients, indicating that these inflammatory biomarkers may play a role in the pathogenesis of PE. **Conclusion:** For the first time, it was demonstrated that sCD93 was elevated in serum of patients with PE, and sCD93 could be a biomarker for diagnosis of PE. However, we considered evaluating this biomarker in PE patients as well as in Eclampsia patients.

11031P

FOXP3 gene promoter polymorphism affects susceptibility to preeclampsia

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Introduction: Preeclampsia (PE) is a multifactorial pregnancy disorder that originates in the placenta and a major cause of maternal morbidity and mortality. Despite intense study, the pathophysiology of preeclampsia remains enigmatic. Recent studies have reported that regulatory T cells (Tregs) is linked with infertility, miscarriage and PE. It is well identified FOXP3/Scurfin, a member of forkhead/winged-helix proteins, is involved in development and function of Tregs. However, the association between PE and the Foxp3 gene polymorphism has not been sufficiently investigated. In this study, we hypothesized that polymorphisms of the foxp3 may be related to PE. **Material and Methods:** We assessed the relationship between four single-nucleotide polymorphisms (SNPs) in the Foxp3 genes with sequence-specific primers (PCR-SSP) in 81 PE patients and 90 age- matched controls. **Result:** We identified significant difference of rs4824747 GG genotype frequency between the PE and control groups. Women with GG genotype exhibited higher (OR = 0.24; P < 0.0001) risk of developing PE. None of the other investigated SNPs (rs2232365, rs3761547 and rs3761548) showed significant association with PE in our study. **Conclusion:** We suggest that foxp3 polymorphisms (rs4824747) be a potential contributor for the development of PE in Iranian women. Our results are suggestive of T allele to be protective against PE and G allele as predisposing in our population. **Key words:** FoxP3, polymorphism, regulatory T cell

11058P

Differential expression pattern of maternal and neonatal CXC chemokines during pre-eclampsia: An insight to the roles played by CXCL9, CXCL10 and CXCL12 in pre-eclampsia

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Introduction: Pre-eclampsia is a gestational complication characterized by sedimentation of fibrin in endothelium that activates the innate and adaptive immune responses. Pre-eclampsia is associated with hypertension, proteinuria, oedema and occurs during second or third trimesters of pregnancy. Biomolecules such as cytokines and chemokines are involved in inflammatory associated processes of pre-eclampsia. The main purpose of the present study was to determine the expression of CXC chemokines, CXCL9, CXCL10 and CXCL12 in pregnant women with and without pre-eclampsia. **Material and methods:** The serum protein levels of CXCL9, CXCL10 and CXCL12 were measured in cord and peripheral blood by using ELISA techniques. The placental tissue expression of chemokines was also analyzed by western and northern blotting. Demographic data were also obtained by questionnaire. **Results:** Findings of the present study revealed elevated levels of pro-inflammatory chemokines CXCL9 and CXCL10 in parallel with CXCL12 as homeostatic chemokine in pre-eclamptic mothers. It was also observed a similar pattern of chemokine's expression in both placental and serum levels of neonates from pre-eclampsia compared to control. **Conclusion:** According to the results presented, it could presumably be concluded that CXC chemokines are involved in pathogenesis of pre-eclampsia. This may possibly be related to their roles in several processes such as neovascularization, embryonic development and inflammatory responses that are mediated by pre-ecalmpsia.

11065P

Association of CD46 IVS1-1724 C>G single nucleotide polymorphism in Iranian women with spontaneous recurrent abortion (RSA)

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Introduction: There are several known and unknown factors for recurrent spontaneous abortion (RSA). Of them, complement regulatory protein CD46 plays pivotal role in preventing uncontrolled activation of complement and continuing successful pregnancy. The aim of this study was to investigate the possible association of CD46 IVS1-1724 C>G polymorphism with RSA in Iranian women .**Material and Methods:**141 women with RSA and 153 women with normal pregnancy were enrolled in this study. RSA was confirmed as at least three consecutive abortions without any known immunologic, pathologic and genetic reason. Genomic DNA was extracted and RFLP-PCR was performed using specific primer pair and Hind III restriction enzyme. Statistical analysis was conducted for genotype and allele frequency, and also for Odds Ratio (OR).**Results:** Statistical analysis showed no significant difference in genotype frequency between two RSA and normal groups. However, G allele was significantly more frequent in fertile women and represented as protective allele (p=0.04, OR=0.8). **Conclusion:** In contrary to similar studies in other two ethnic populations, this study showed no genotype difference in CD46 IVS1-1724 C>G SNP between RSA and fertile women. On the other hand, G allele has revealed as protective allele for RSA. CD46 polymorphisms may predict the outcome of pregnancy; however, more studies in different ethnic groups are needed.

11179P

The association between single nucleotide polymorphism in interleukin-27 gene and recurrent pregnancy loss in Iranian women

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Introduction: Recurrent pregnancy loss (RPL) has been defined as two or more miscarriages before 20th week of gestation. It seems that IL-27 may reduce inflammatory responses and affect the survival of the embryo during human pregnancy. *IL-27* polymorphisms may influence RPL by altering the levels or the activity of gene product. For the first time, the association of *IL-27* -964 A>G single nucleotide polymorphism (SNP) with RPL in Iranian women was studied. **Material and Methods:** A case-controlled study was performed on two groups consisting of 150 healthy women with at least one delivery (control group) and 150 women with two or more primary RPLs history (RPL group). The -964 A>G SNP in *IL-27* gene was determined by PCR-RFLP technique. Genotype and allele frequencies were compared using ^{2} tests between two groups. **Results:** There was no difference between the two groups regarding age of women (29±4.4 [control] vs. 30.84±5.2 years [case]). In the RPL group, the genotype frequencies of -964 A>G polymorphism were AG (49.3%), AA (40%), and GG (10.7%), and in the control group, they were AG (43.3%), AA (48.7%), and GG (8%). There was no significant difference between the genotypes of AA, AG, and GG in two groups (p=0.23). As the frequency of allele A was 64.7% in the RPL group and 70.3% in the control group, the difference in frequency of allele A in -964 A>G between two groups was not significant

(p=0.19). **Conclusion:** The findings indicated that SNP of -964 A>G in *IL-27* gene is not a risk factor for RPL in Iranian women. **Key Words:** Cytokine, IL-27, Inflammation, Polymorphism, Recurrent abortion

11193P

Frequency of null allele of HLA-G locus in subjects with recurrent miscarriage

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Introduction: Human leukocyte antigen-G (HLA-G) is a non-classical class I molecule highly expressed by extravillous cytotrophoblast cells. Due to a single base pair deletion, its function can be compensated by other isoforms. Investigating the frequency of the null allele in recurrent miscarriage subjects could thus be useful in understanding the relationship between the frequency of this allele and recurrent miscarriage in a given population. The aim of this study was to demonstrate the frequency of HLA-G*0105N null allele and its potential association with down-regulation of HLA-G in subjects with recurrent miscarriage. **Material and Methods:** Western blotting was used to assess the level of HLA-G protein expression. For investigating the frequency of HLA-G*0105N null allele in recurrent miscarriage subjects, PCR-RFLP method was used. Exon 3 of HLA-G gene was amplified by polymerase chain reaction (PCR). Subsequently, PpuM-1 enzyme was employed to digest the PCR products and fragments were analyzed using gel electrophoresis. **Results:** Digestion using restriction enzyme showed the presence of heterozygous HLA-G* 0105N null allele in 10% of the tested population. Western blotting results confirmed the decreased expression of HLA-G in the placental tissue of subjects with recurrent miscarriage compared to subjects who could give normal birth. **Conclusion:** The frequency of heterozygous HLA-G*0105N null allele is high to some extent in subjects with recurrent miscarriage. The mutation rate in subjects suggested that there is a significant association between recurrent miscarriage and frequency of mutations in this allele. **Key words:** Allele, HLA-G*0105N; recurrent miscarriage; RFLPs.

11283P

Role of Cytokines in male infertility related disorders

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Introduction: Infertility is defined as inability of couples to achieve pregnancy following one year of unprotected intercourse. By this criterion, infertility affects 13-18% of couples and male factor accounts for up to half of all the cases. In the male reproductive tract, the cytokines and other immune regulatory factors are mainly produced in the testis by somatic cells, including Leydig and Sertoli cells, and are involved in the regulation of spermatogenesis and

other testicular cell functions. **Material and Methods:** Male subjects were sexually abstinent for 3-5 days. Semen samples were obtained by masturbation. After complete semen liquefaction, volume, appearance, fluidity and basic sperm parameters were determined following the procedures described by the WHO Manual (WHO, 2010). The routine seminological analysis was carried out estimating sperm density, motility, viability and morphology. The seminal plasma samples were kept at –20˚C and cytokines and were determined using the quantitative ELISA method. Data were analyzed by using SPSS version 19. **Results:** Although some studies indicated the lack of any connection between the cytokine levels and semen quality, the results of this study showed a negative correlation between cytokine levels in the semen and standard semen quality parameters such as sperm concentration, motility, viability, morphology, and viscosity. **Conclusion:** The issue of the clinical significance in the detection of cytokines in the seminal plasma in the context of infertility is still open to debate. Undoubtedly, controlled prospective studies, including a large number of patients and a wide range of analyzed cytokines are urgently required to answer the question about the future of these novel biomarkers. **Key words:** Infertility, Cytokine, Semen, ELISA

Immunology of Environmental Pollution & Chemical Victims

Oral Presentations:

7604O

The investigation on the effect of environmentally relevant level of inorganic arsenic onantigen presenting and T-cell proliferation-inducing capacities of porcine dendritic cells

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Introduction: Arsenic is a chemical element with symbol As. This study aimed to determine the molecular aspects of immuno-toxic impacts of inorganic As on animals' pivotal antigen presenting cells, dendritic cells (DC).**Materials and Methods** The in vitro effects of environmentally relevant level of NaAsO2 (20ng/ml for 12-24 hr) on porcine monocyte-derived DC (MoDC) functions were evaluated using flow cytometry-based phagocytosis, markers CD80/86 and MHCII and co-stimulatory molecules CD40 and CD25 expression and □3H□methyl-thymidine pulse-labelled MoDCs- CD6+ T-cells co-cultured T-cell polarization, and ELISA-based cytokine secretion profile. **Results:** A low dose of iAs eventually reduced the phagocytic capacity of MoDC. Furthermore, though the protein expression of MoDC activation markers CD80/86 and MHCII and co-stimulatory molecules CD40 and CD25 only slightly changed, the T-cell polarization-inducing capacity of MoDC was remarkably diminished upon As treatment. Additionally, As induced a significantly higher IL-6 secretion by MoDC upon 12 h and 24 h incubation, while the IL-1β secretion was only significantly upregulated upon 12 h incubation. Secretion pattern of IL-8, TNFα and IL-10 in As-treated MoDC was almost similar to non-treated MoDC.**Conclusion**: Data of this study indicated that As can be immunosuppressive. Considering the broad roles of DC in immuno-biology, this finding also opens a new insight to understanding the molecular mechanisms and functional consequences of As in inducing immuno-dysregulation, immuno-toxicity, and thus infectious and non-infectious diseases in animals and humans. Further fundamental studies on the effect of low level of As on immune cells and molecules in animals and humans are in progress. **Key words**: Environmental arsenic, Cell markers, co-stimulatory molecules, Dendritic cells, Porcine, Immunotoxicity, T-cells

10836O

Evaluation of CXCL10/CXCR3 axis in lung tissue of sulfur mustard exposed individuals with Long-term pulmonary complications

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Introduction: Sulfur mustard (SM) as a chemical warfare agent is highly toxic to the lung but the pathogenesis of pulmonary complications induced by SM is not clearly understood. Chronic obstructive pulmonary disease (COPD) is a chronic pulmonary consequence after SM exposure. According to the last studies, inflammation has main role in SM complications. CXCL10/CXCR3 axis is recruitment of $CXCR3^+$ cells to inflammation site in COPD. The aim of this study was to determine the gene expression of CXCL10 (IP-10) and the percent of $CXCR3^+$ cells in the lung tissue of sulfur mustard exposed individuals with Long-term pulmonary complications compared to non-exposed patients. **Materials and Methods** this study has been conducted on 40 paraffin-embedded lung tissues of sulfur mustard exposed individuals and 15 paraffin-embedded lung tissues of unexposed patients as control group. The gene expression of CXCL10 was assisted by Real-time PCR and $CXCR3^+$cells were detected by IHC.**Results:** The percent of $CXCR3^+$ cells in lung tissue of chemical victims was significantly increased in compared to the control group but the CXCL10 gene expression in chemical victims was not significantly different compared to the control group. **Conclusion:** According to the result, it is concluded that the percent of $CXCR3^+$ cells could play a role in long–term pulmonary complication in SM exposed patient. It could be dependent on special inflammation condition in the lung of SM exposed and it is possible that other ligands of CXCR3 such as CXCL9 and CXCL11 have role in attracting $CXCR3^+$ cells to the lung.

11216O

Evaluation of Apoptosis in the LungTissue of Sulfur Mustard -Exposed Injuries

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Introduction: Exposure to Sulfur Mustard (SM) results in pulmonary complication that has been known to be the main cause of long-term disability and morbidity. Up to now the precise mechanisms of SM-induced lung complications is not identified. The aim of the current study was evaluation of apoptosis in lung tissue of SM exposed individuals. **Materials and Methods:** The study was performed on lung paraffin-embedded tissue specimens from 21 patients who suffer from pulmonary complications due to previous SM exposing and 9 unexposed patients who had undergone lung resections for another lung disease. Evaluation of apoptosis in paraffin-embedded lung tissue sections were performed using Terminal deoxynucleotidyl Transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay and the cleaved caspase-3 immunohistochemistry assay. **Results:** Both of TUNEL-positive apoptotic features and caspase-3 expression of specimens were significantly higher in the SM exposed group as compared to the control group. This result was demonstrating higher apoptosis rate in the SM exposed group. Furthermore, the majority of positive cells were alveolar epithelial cells in both methods. **Conclusion:** It seems that exposure to SM may be resulted in increased apoptosis in respiratory epithelium. More studies are needed to evaluate the role of apoptosis in SM induced lung complication to use the results in designing new and effective therapeutic protocols.

12419O

TLR4 expression in lung tissue of sulfur mustard exposed individual with long-term pulmonary complication

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Introduction: Toll like receptor (TLR4) is one of the molecules that mediate production of inflammatory mediators. Factors like reactive oxygen specie which arise following oxidative stress as an endogenous ligand can activate TLR4. Due to the fact that inflammatory mediators and oxidative stress factors have been changed in serum, sputum and Broncho-Alveolar Lavage (BAL) samples in sulfur mustard (SM) exposed victims with delayed complications, we have evaluated gene and surface protein expression of TLR4 in SM injured lung tissues. **Materials and Methods:** The study was approved in ethical committee of Shahed University. 28 paraffin embedded lung tissues from chemical victims and 10 paraffin embedded lung tissues from others with pulmonary complications without SM exposure as control were included. Tissue samples of control and exposed group were diagnosed by expert pathologists. Then, RNA samples were extracted from formalin fixed paraffin embedded lung tissues of chemical exposed and not exposed individuals and gene expression analysis has been detected through real-time PCR test. Moreover, surface protein of TLR4 has been evaluated by immunohistochemistry technique. **Results:** Majority of patients in exposed and control groups had constructive bronchiolitis. There was no significant difference between two groups in TLR4 expression, however, remarkably surface protein of TLR4 decreased in exposed group in compare to control ones. **Conclusion:** Difference between gene expression and the volume of surface protein of TLR4 can be due to internalization of protein or regulations in the gene expression level. However, further studies with more samples, a comparison with health group.

Poster Presentations:

7676P

Specific role of the monoclonal antibody for detection of Glomalin-Pb complex, a strategy for supplying and promoting of the food quality and security

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Introduction: Environmental contamination by toxic metals has harmful effects on organisms because of their non-degradability and high accumulation potential. Arbuscularmycorrhizal (AM) fungi occurs in different ecosystems around the world, including toxic metal contaminated soils. The use of AM fungi can be a valuable tool to enhance site-remediation processes. Glomalin known as a specific protein of AM fungi in the spore and hyphal wall, has especial efficiency in toxic metals sequestration. Immunoassay using monoclonal antibody 32B11 is already accurate and specific method to determine glomalin. **Material and methods**: An in vitro experiment was studied in two-compartment plates containing transformed carrot roots (Daucuscarota L.) mycorrhized by

Rhizophagusirregularis fungus. Hyphal compartment treated with Pb concentrations of 0, 0.01, 0.1 and 1 mM as Pb(NO3)2. After glomalin extraction, glomalin concentration in extracted samples was determined by indirect-ELISA using monoclonal antibody 32B11. After precipitation and digestion of glomalin, Pb-sequestrated by glomalin was determined by atomic absorption spectroscopy. **Results**: Immuno-reactive glomalin with monoclonal antibody 32B11 and Pb-sequestrated by glomalin increased as a linear function with rising Pb concentrations. The increased trend of glomalin produced by extra-radical hyphae and Pb-sequestrated by glomalin can be suggested that glomalin has high capacity for sequestration of toxic metals. **Conclusion:** Glomalin as a putative homolog of heat shock protein 60, and critical and effective component of extra-radical hyphal walls was most important in sequestration of Pb and reduction of its toxicity in the environment.

9816P

Vitamin D status has reduced in sulfur mustard exposed individuals.

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Introduction: Sulfur mustard (SM) is a strong alkylating agent with cytotoxic, carcinogenic and mutagenic features. Sulfur mustard complications occur in the form of acute and delayed complications. Vitamin D as an immunomodulatory agent is an effective tool to suppress inflammatory responses. It is shown that serum level of vitamin D has reduced in people with chronic obstructive pulmonary disease (COPD). Along with, some studies showed that vitamin D modulates with blood cells (WBC) and lymphocyte cells particularly in inflammatory disease. Moreover, WBC and platelets have increased in people with periodontitis as an inflammatory disease. In the present study serum level of vitamin D and its correlation with lymphocyte, platelet, neutrophil cells and WBC count were investigated in SM exposed individuals compared to healthy control group. **Materials and Methods**: Participants were 100 SM exposed and 87 unexposed as control group. Serum levels of vitamin D were measured by ELISA and lymphocyte, platelet, neutrophil cells and white blood cells by cell counter method. Serum levels of calcium were measured by spectrophotometry. **Results:** The results showed that the serum level of vitamin D is decreased in SM exposed individuals ($P<0.001$). White blood cells and neutrophil have increased significantly in SM exposed group compared to control group ($P<0.001$). Lymphocytes have reduced in SM exposed group ($P<0.001$). But platelet cells percent showed no difference between two groups ($P= 0.083$).**Conclusion:** This study revealed that SM exposed individuals have been male-adjusted in relation and correlation between vitamin D and immune system components.

10799P

The Effect of Diesel Engine Particles (DEP) on Disease Severity and Peritoneal Macrophages Activity in Experimental Autoimmune Encephalomyelitis (EAE) mice

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Introduction: Air pollution affects the immune system in ways yet partially understood. One of the important pollutants is diesel engine particle. In this study, the effects of these particles on disease severity and peritoneal macrophages activity in experimental autoimmune encephalomyelitis (EAE) mice were investigated. **Materials and Methods:** Female C57BL/6 mice, aged 8 – 10 weeks, were divided into two groups. EAE occurred via injection of

MOG35-55 suspended in complete Freund's adjuvant (CFA) and pertussis toxin. The DEP treated group received 5µg/mice of DEP suspended in PBS for 21 days. PBS was injected to control group. Then weight, disease severity symptoms, nitric oxide (Griess test for nitrite) and MTT of peritoneal macrophages activity (MTT) were evaluated. **Results:** Disease severity symptoms were significantly diminished in DEP-injected EAE mice from 3.75 to 1.18 ($P<0.002$) compared to EAE control. Besides, there was significant decrease in levels of nitric oxide ($P<0.02$) and MTT of peritoneal macrophages in DEP-injected EAE mice ($P<0.01$**Conclusion:** These data indicated that injection of DEP may cause a decrease in disease symptoms and peritoneal macrophage activity. These observations may be due to the effect of DEP in augmenting TH2 responses. Although, it needs further investigation to clarify the exact mechanisms. **Keywords:** Experimental Autoimmune Encephalomyelitis, Diesel Engine Particles (DEP), nitric oxide, MTT

11100P

Evaluation of serum levels of Calcium and Phosphor in chemical victims 20 years after Sulfur Mustard exposure and their relationship with lung function

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Introduction: Sulfur Mustard (SM) as a chemical weapon was used in the World War I and also during the Iran-Iraq war. This mixture is absorbed through inhalation, skin, eye and digestive tract (with the consumption of contaminated food). The aim of this study was to evaluate the serum levels of Calcium and Phosphor in veterans 20 years after sulfur mustard exposure and to find their relationship with lung function. **Materials and methods:** This study was conducted on 370 SM exposed participants from sardasht city and 127 unexposed volunteers from rabat city. The serum levels of Calcium and Phosphor were assessed by photometric assay and lun g function were assessed by spirometery and lung function were measured (FEV1%, FVC% and FEV1/FVC %).**Results:** The serum levels of Calcium in SM exposed group were significantly lower than control group (9.830±0.560 vs. 10.010±0.750, $P_{value}=0.004$) and also the serum levels of Phosphor in the SM exposed group were significantly lower than control group (3.430±0.590 vs 3.660±0.840, $P_{value}=0.001$). FVC% in control group and FEV1% in exposed group showed weak positive correlation with calcium but no correlation was found with Phosphor. **Conclusion:** The serum levels of calcium and phosphor were declined in SM exposed individuals but more investigation is required to clarify the matter. **Keywords**: Calcium, Phosphor, lung, chemical victims, Sulfur Mustard, Long-term complications

Immunology of Infectious Diseases

Oral Presentations:

7637O

Evaluation of hepatitis B e antigen and related antibodies to find an appropriate surrogate marker for the viral load determination in diagnosis of HBV infection

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Introduction: Different hepatitis B serologic markers are used to identify different phases of HBV infection and to determine whether a patient has acute or chronic HBV infection. The aim was to determine the best substitute for viral load determination in subjects infected by hepatitis B virus. **Materials and Methods**: One-hundred one hepatitis B infected patients were examined for the HBeAg, HBeAb and HBcAb by ELISA. Viral load was also conducted by real-time PCR method. **Results**: The mean age of cases was 38.5±11.74 years and 57.4% were males. There was a significant correlation between viral load and HBeAg optical density (OD) 450 nm in ELISA test (r=0.31) (p=0.005), however, HBeAb and HBcAb ODs had no significant correlation with viral load (r= -0.035, p=0.823 and r=0.244, p=0.221, respectively). According to the ROC curve, an HBeAg OD level of 0.021 could be considered equal to at least 105 copies/ml viral load with a sensitivity of 46% and specificity of 87%. Moreover, an Anti HBeAb OD level of 0.027 was equal to same value of the viral load with 43% sensitivity and 72% specificity. **Conclusion**: Based on results, the increase of viral load was accompanied by HBeAg positivity. However, the sensitivity of serological method was much lower than the PCR method. Therefore, they could not be considered as acceptable substitute for viral load determination in detecting the infection. **Keywords**: Hepatitis B; HBeAg; HBV antibodies; viral load

7641O

The role of PI3K/AKT pathway, apoptosis and Regulatory T cells in development of Adult T cell leukaemia lymphoma (ATLL)

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Introduction: The human lymphotropic virus type 1 (HTLV-1) infects approximately 10–20 million people around the world. About 2% to 5% of infected people may develop two HTLV-I associated diseases; Adult T-cell leukemia /lymphoma (ATLL) or HTLV-associated myelopathy-tropical spastic paraparesis (HAM/TSP). In this study, the host–virus interactions in the manifestation of ATLL were investigated by assessing the HTLV-1 pro-viral load (PVL) and AKT1, BAD and Foxp3 as one of the factors of PI3K pathway, apoptosis and Treg cells in host, respectively. **Materials and Methods:** Eighteen patients with ATLL, 10 HAM/TSP and 18 HTLV-1 asymptomatic carriers (ACs) were assessed for PVL, HBZ, AKT1, BAD and Foxp3 expression using real time PCR, Taq-Man method. The data was analyzed by SPSS software. **Results:** The HTLV-1 PVLs were higher in ATLL than ACs (p=0.003) and HAM/TSP (p=0.041). The expression of AKT1 in ATLL was higher than that of ACs (p=0.059) and HAM/TSP (p=0.008). The expression of Foxp3 in ATLL was higher than that of ACs (p= 0.003) and HAM/TSP (p=0.014). In the BAD expression no significant differences were found among ATLL, ACs, or HAM/TSP. **Conclusion:** In infected PBMCs, PVL may be involved in inducing cell proliferation through the high expression of AKT1 in the PI3K pathway, which is the main player in ATLL manifestation. Moreover, AKT1 in our study affects ATLL progression more, as it suppresses the pro-apoptotic BAD molecule. Furthermore, the findings showed that T regulatory cells were the major affected cells in ATLL as Foxp3 increased radically. **Keywords**: ATLL, HAM/TSP, HTLV-1-proviral load, AKT1, BAD, Foxp3

9732O

Diagnostic approach in Tuberculosis considering Quantiferon, Tuberculin Skin Testing and clinical and paraclinical findings

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Introduction: Tuberculosis **(**TB) still remains as a major threat to global health. Although different diagnostic modalities regarding TB have been introduced, yet there is controversy about accuracy of each one. The aim of this study was to evaluate the predictive value of Quanti-FERON test (QFT) and Tuberculin skin test (TST) and their agreement with clinical manifestations and para-clinical findings. **Materials & Methods:** The data of all patients referred to QFT test were reviewed in 3 consecutive years. Sensitivity, specificity, negative & positive predictive value (NPV & PPV) of QFT and TST were calculated. Cohen's kappa analysis was performed to assess the concordance between the two tests and clinical findings. Classification and Regression tree (CART) model were

conducted to evaluate the value of each variable in approaching to TB suspected patients. **Results:** Of total 478 patients, 357 fulfilled the inclusion criteria. TB diagnosis was confirmed in 39 patients. There was a fair agreement between QFT and TST (k=0.316). The sensitivity and specificity for predicting TB in this study were 90.6% and 93.3% for QFT and 84.0% and 85.5% for TST. The PPV and NPV for these two tests were 90.6% and 93.3% for QFT, and 72.4% and 92.2% for TST. According to CART model, QFT was the most discriminative variable in diagnosing TB; and clinical findings showed significant role in distinguishing TB patients. **Conclusion:** QFT might be a suitable alternative/complementary test for TB. The results also highlighted the importance of clinical findings in approaching to a TB-suspected patient.

10907O

Measurement of serum levels of interleukin-17 A in patient with acute and chronic Brucellosis

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Introduction: Brucellosis is an infectious disease with worldwide distribution. The bacteria establishing this infections, involved some members of mammalians including human. Due to brucellosis, it seems that Th1/Th2 cytokines balance may be involved in the resistance or susceptibility to Brucella infection. In this respect, Th1 cytokines confer resistance, while Th2 cytokines predispose brucellosis. It is also clarified that IL-17 is required for the induction of IFN-γ and IL-12 in macrophages and dendritic cells. Then, it seems that IL-17 can affect the induction of Th1 immunity which is necessary for controlling Brucella as an intercellular pathogen. **Materials and Methods**: In the present study, the challenge was to investigate probable relationship between IL-17A serum level and susceptibility to the human brucellosis. 87 patients with brucellosis and 95 healthy person were included in this study. Serum levels of IL-17A in patients with Brucellosis were measured before and after of treatment and control group by ELISA kit. Statistical analyses have been conducted using ANOVA and T-test. **Results**: The obtained results indicated that there was a significant difference in serum levels of IL-17A in the patients with brucellosis prior to administration of drug compared to control group. Average amount of this cytokine in the case of the patients with brucellosis equals to 21.71±4.36Pg/ml which was significantly different from that of control group, 8.07±3.8Pg/ml, ($P<0.01$).Compared to control group, in chronic cases the level of serum IL-17A remains low in both cases, that is, before and after treatment. **Conclusion**: Results showed that IL-17A can have important role in cases with Brucellosis. Probably, low level of IL-17A leads to recurrent infectious in some individuals.

11093O

Decreased serum levels of anti-tetanus toxin antibodies in patients with type 2 diabetes mellitus

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Introduction: Many immunological disorders have been reported in patients with diabetes mellitus. The aim of this study was to evaluate the serum levels of anti-tetanus toxin antibodies (anti-TTA) in patients with type 2 diabetes mellitus (DM) and in a control group. **Materials and Methods:** Totally, 100 patients with type 2 DM and 100 age- and sex-matched healthy individuals were enrolled in the study. The presence of type 2 DM was confirmed according to the clinical and para-clinical criteria such as fasting plasma glucose above 126 mg/dl. A peripheral blood samples were collected from all subjects. The serum samples of participants were tested for the levels of anti-TTA by ELISA method. The serum antitoxin concentration 0.1 IU/mL was considered as a protective level of antibody. **Results:** The sero-protective rate in healthy group was significantly higher than diabetic group (99% vs. 92%; $p<0.02$). The mean titer of anti-TTA in healthy group (5.32 ± 0.26 IU/mL) was also significantly higher than diabetic patients (3.46 ± 0.26 IU/mL; $p<0.001$). In diabetic men, the mean titer of anti-TTA was significantly higher compared to diabetic women (3.94 ± 0.34 IU/mL vs 2.59 ± 0.36 IU/mL; $p<0.01$). In diabetic patients the sero-protective rate and the mean titer of anti-TTA in subjects with age >40 years were also lower compared to those with age <40 years (89.23% vs 97.14%; $p<0.05$ and 4.57 ± 0.38 IU/mL vs 2.86 ± 0.32 IU/mL; $P<0.002$, respectively). The mean titer of anti-TTA was significantly higher in patients with diabetes duration <5 years compare to patients with disease duration >5 years (3.91 ± 0.35 IU/mL vs 2.85 ± 0.38 IU/mL; $p<0.04$). **Conclusion:** These results showed lower levels of anti-TTA in patients with type 2 DM, in diabetic women, in patients aged >40 years and in diabetic patients with disease duration >5 years. **Key Words**: anti-TTA, diabetes mellitus, ELISA

11130O

The first report of Nocardia species of thigh abscess in a man suffering from Behçet's disease from Iran and literature review

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Introduction: The genus *Nocardia* is opportunistic pathogen that belongs to Gram-positive and partially acid-fast aerobic actinomycetes. In recent decade, the most instances of nocardiosis occur in immune disorder individuals such as Behçet's disease with immunosuppressive and corticosteroid therapy. In this paper, the first report of *Nocardia farcinica* in a patient with Behçet's disease was described. **Case report:** A 39-year-old white man with Behçet's disease admitted to emergency department that was suffering from pain in the left flank and left thigh. Physical and clinical examinations were diagnosed abscess. Discharge was cultured on blood agar and colony like pale yellow observed after three days. Conventional methods showed that the isolate was *Nocardia asteroides* complex. For accurate identification of the genus *Nocardia* in the level species, various molecular methods such as

PCR-RFLP (*hsp65* and 16S rRNA genes) and PCR-sequencing for 16S rRNA gene were used. The strain identified as *Nocardia farcinica*. Antibiogram was conducted with disk diffusion method and the isolate was sensitive to imipenem, amikacin, cefotaxime, ampicillin,and cotrimoxazole and resistant to erythromycin, gentamicin and clindamycin. Patient was initially prescribed oral antibiotic with cotrimoxazole for six months and improved of nocardial infection. **Conclusion:** Isolation and accurate identification of *Nocardia* spp. is important in immune deficiency diseases. **Keywords:** thigh Abscess,*hsp*65 gene, 16S rRNA gene sequencing, Behçet's disease , PCR-RFLP

11213O

Lower numbers and impaired functions of NK cells in individuals infected with HTLV-1

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Introduction: HTLV-I is the etiologic agent of a progressive neurological disease called HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T cell leukemia (ATL). Natural killer (NK) cells are innate effector lymphocytes, necessary for defense against virally-infected and stressed cells. **Materials and methods**: A total of 42 HTLV-1 infected subjects (21 Asymptomatic Carriers (ACs) and 21 HAM/TSP patients) and 8 uninfected Healthy Controls (HCs) participated in this study. NK cell numbers, phenotype, function and expression of cell-surface and intracellular cytotoxic effector molecules were analyzed by flow cytometry. In addition, CD107 degranulation assays were performed to assess the cytotoxic activity of NK cells. **Results**: The total NK cell count was decreased in HAM/TSP patients, but the relative frequencies of total CD3-CD56bright NK cells and CD3-CD56dim NK cells were unaffected. NK cells from HAM/TSP patients and ACs are hypo-responsive. The mechanism of this defect is unknown. NK cells from HAM/TSP patients and ACs had lower cytotoxic related molecules such as Fas, FasL and TRAIL but a normal levels of perforin, granzyme B, granulysin and higher level of granzyme A, compared to NK cells from healthy controls. **Conclusions**: It was concluded that lower numbers of circulating NK cells and specific defect in NK cells capacity to degranulation may play a role in HTLV-1 persistence. Restoration of this NK cell capacity, as achieved by viral load reduction, could therefore contribute to definite antiviral control and disease HAM/TSP.

12343O

Evaluation of the regulatory T cells in the brucellosis infection

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Introduction: Brucellosis is one of the most chronic diseases with widespread distribution. In spite of cell-mediated immunity (CMI) via mainly activated T-helper type 1 (Th1) cells, brucellosis infection goes on to establish chronic form in about 10% to 30% of brucellosis cases via different strategies. In this way, regulatory T-cells (Treg cells) are

the most important related-pathways that are not specifically illustrated. **Materials and Methods:** Hence, in this study, Treg cells were evaluated in brucellosis infection by flowcytometery method. For this purpose, the percentages of CD4, CD25, and FoxP3 were analyzed in peripheral blood mononuclear cells (PBMCs) of acute brucellosis (AB) and chronic brucellosis (CB) compared to healthy individuals. **Results:** The results revealed reduced levels of the CD4 MFI in CB compared to AB and control groups (p value <0.05). Decrease in the percentage of Treg cells with CD4/CD25 expression in CB compared to control group (p value <0.05) was also found. **Conclusion:** Based on the findings of this study, it could be speculated that reduced numbers of CD4+ T lymphocytes and Treg cells with CD4/CD25 expression, leading to T-cell anergy and would be enough in moving into chronic form of the brucellosis infection.

12386O

The transcription level of IFN-α induced SOCS-1 gene, as a predictive factor for response to therapy in HCV infected patient

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Introduction: Hepatitis C virus therapy is a challenge. Response to standard therapy in genotype 1 is more frustrating than the other genotypes. Due to the side effects of standard therapy (peg interferon-alpha plus ribavirin) exploiting factors that could predict therapeutic response, especially in peripheral blood cells, can be very helpful. This study aimed at understanding the value of suppressor of cytokine signaling 1 gene transcription in predicting response to HCV treatment. **Materials and methods**: Totally, 12 non-responders (9 male and 3 female) and 10 responders (8 male and 2 female) all infected with HCV genotype 1, enrolled randomly and their PBMCs were isolated and distributed into 2 positions as 1 million cells/well. For all cases, one well left intact as control and the second treated by 500 IU/mL recombinant peg interferon-alpha. After 6 hours, cells were collected and then RNA was extracted. Evaluation of SOCS-1 gene transcription evaluation was performed by qRT-PCR. **Results**: SOCS1 gene transcription was also significantly increased (P <0.001) in responder groups after IFN treatment, which indicated the integrity of interferon signaling pathway. But at the same condition there wasn't any significant increase in SOCS1 gene transcription in non-responders. **Conclusion**: According to the results of this study, special genes like SOCS-1 following IFN treatment on PBMC exhibit different pattern of induction among responder and non-responder groups were completely congruous to in vivo behavior. So it can predict an individual's response to treatment with interferon before starting the therapy with an easy and available way to avoid the cost and side effects. **Key words**: HCV, SOCS1, IFN treatment

Poster Presentations:

7552P

New Insight to IL-23/IL-17 Axis in Iranian infected adult patients with gastritis: Effects of Genes Polymorphisms on Expression of Cytokines

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Introduction: Chronic inflammation is the hallmark of the pathogenesis of *H. pylori*-induced gastric cancer. IL-17A and IL-17F are inflammatory cytokines expressed by a novel subset of CD4+Th cells and play critical function in inflammation. The relationship between IL-17A G197A, IL-17F A7488G and IL23R+2199 A/C polymorphisms with IL-6, IL-17, IL-21, IL-23 and TGF-β1 mRNAs expression with regard to *H. pylori* infection with chronic gastritis were evaluated.**Materials and Methods:** Total RNA and genomic DNA were extracted from gastric biopsies of 58 *H. pylori*-infected patient with gastritis. Afterward, mucosal IL-6, IL-17, IL-21, IL-23 and TGF-β1mRNAs expression and polymorphisms in IL-17A G197A, IL-17FA7488G and IL-23R +2199A/C in gastric biopsies were determined by real-time PCR and PCR-RFLP. **Results:** The results showed that IL-17A G197A, IL-17F A7488G and IL23R +2199A/C polymorphisms have no effect on mucosal expression of IL-6, IL-17, IL-21 and TGF-β1 mRNAs expression in *H. pylori*-infected patients with chronic gastritis. **Conclusion:** These results suggested that IL-17A G197A, IL-17F A7488G and IL23R +2199A/C polymorphisms doesn't alter mucosal cytokine pattern in Iranian patients with *H. pylori* associated gastritis diseases.

7627P

Evaluation of autoimmune markers in HTLV-1 carriers compared to uninfected people

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Introduction: HTLV-1 infection with high prevalence in the North East of Iran, particularly in Mashhad, can lead to the T-cell leukemia and HTLV-1 associated with myelopathy/tropical spastic paraparesis (HAM/TSP) with a variety of autoimmune diseases. **Materials and Methods**: Serum samples were characterized from blood donors in Mashhad, Northeastern Iran. One hundred five HTLV-1 positive (cases) and 104 age- and sex-matched HTLV-1 negative donors (controls) were assessed for the presence of serum autoimmune markers by ELISA. **Results**: The mean ages of cases and controls were 40.8±9.4 and 41.5±9.3 years, respectively (p=0.5). In case group, 81.9% and in control group 83.7% were males (p=0.74). The frequency of positive ANA and anti-CCP in the serum of two

groups were not significantly different (p =0.68 and p =0.62 respectively). Only one ANCA-positive (1%) was observed in the group and no anti-phospholipid IgG positive was observed. The frequency of RF was high in case group than in control group, although the difference was not significant (p =0.08). The amount of RF in all 12 RF positive sera, was higher than normal levels (33 to 37 IU / ml). **Conclusion**: Due to the findings, to achieve no meaningful relation between serum autoimmune markers and HTLV-1 infection, and because of the relatively low prevalence of autoimmune diseases, it could be concluded that healthy HTLV-1 carriers do not produce rheumatologic related auto-antibodies more than healthy population. **Key word**: HTLV-1 infection; autoimmune markers; case-control study.

7653P

Homologous prime-boost injection with HCV core virus-like particles induces a Th1 response in BALB/c mice model

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Introduction: Development of an effective vaccine against hepatitis C virus (HCV) needs immunogens to induce both humoral and cellular immune responses. Virus-like particles (VLP) could be improved into successful immunogens, but, however, the recent expression systems for VLP production have some limitations. Herein, a novel strategy was developed to produce HCV VLPs using stably transfected *Leishmania tarentolae* promastigotes. **Materials and Methods:** HCV core gene was cloned into the inducible pLEXSY expression vector. The expression induced by tetracycline in *Leishmania tarentolae* was detected by SDS-PAGE and western blot analysis. The protein purification was performed by affinity chromatography under native conditions. VLP assembly was determined by TEM microscopy and its immunogenicity was assessed in BALB/c mice model. **Results:** TEM microscopy revealed HCV core VLP assembly with average size of 30-40 nm after purification using affinity chromatography. BALB/c mice were injected by three types of strategies including core DNA-based immunizations, core VLP-based immunizations, and core DNA prime/ VLP boost immunizations. The data showed that HCV core VLP-based immunizations significantly induced anti-core antibody responses, as well as secretion of IFN-γ cytokine compared to other groups. In addition, DNA-prime/VLP-boost regimens elicited importantly higher levels of IFN-γ and antibody responses compared to homologous DNA/DNA regimens. **Conclusion:** The data demonstrated that HCV VLPs generated by *leishmania* expression system could be developed into a vaccine component against HCV infections. **Keywords:** HCV, Viral like particle, Core, *Leishmania* expression system, Immunization

7693P

Evaluation of hepcidin polymorphism and serum iron in tuberculosis patients

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Introduction: Iron acquisition is essential for the growth of Mycobacterium tuberculosis. Hepcidin is known as an antimicrobial peptide and as a component of the innate immune response which has central role in iron metabolism.

Hepcidin inhibits M. tuberculosis growth in vitro. In this study, the aim was to identify −582A> G variants of the HAMP promoter and hepcidin concentration as well as iron in patients with tuberculosis (TB). **Materials and Methods**: The sample population consisted of 105 patients with TB and 104 healthy individuals. The −582A> G polymorphism was genotyped using a tetra-primers PCR set. Serum levels of hepcidin and iron were determined using an ELISA kit. Statistical analysis was performed using SPSS software. **Results**: The G allele is meaningfully associated with TB disease (95% confidence interval = 2–4.8, $p < 0.000$). Significant differences were observed in the levels of serum iron and hepcidin between the −582A>G polymorphism genotypes. There was significant reverse correlation between hepcidin and iron ($r = -0.849$, $p = 0.006$). **Conclusion**: A high association was found between serum hepcidin levels and the HAMP−582A> G variants in patients with TB.

9766P

Effect of tetanus-diphtheria (Td) vaccine on immune response to hepatitis B vaccine in healthy individuals with insufficient immune response

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Introduction: Hepatitis B virus (HBV) fails to produce appropriate immune responses in some healthy individuals; thus, different strategies have been adopted to promote immune responses. The current study aimed at evaluating the efficacy of HBV vaccine co-administered with tetanus-diphtheria (Td) vaccine compared to HBV vaccine in healthy individuals through measuring hepatitis B surface antibody (HBsAb) levels. **Materials and Methods**: This was a randomized controlled clinical trial, which was implemented in Isfahan, Isfahan Province (Iran) in 2013. One hundred and forty healthy individuals, whose HBsAb titers were less than 10 IU/L were recruited. The subjects were randomly assigned to either in intervention or control trials. The control group received 40 µg of recombinant HBV vaccines intramuscularly injected at 0, 1, and 6 months; however, the intervention group was simultaneously vaccinated by Td with the first dose of HBV vaccine. HBV antibody levels (titer) were measured before the vaccination and 6 months after the last vaccination. **Results:** Antibody titers of the subjects in the intervention and control groups increased from 5.07 ± 2.9 IU/L to 744.45 ± 353.07 IU/L and from 4.45 ± 3.4 IU/L to 589.94 ± 353 IU/L, respectively (both $P < 0.001$). Also, the mean difference of antibody titer was significantly different between the two groups ($P = 0.011$). **Conclusion:** Td vaccination can be applied as a feasible approach to promote efficient and persistent immunity in healthy individuals with insufficient HBsAb titers. **Key words:** Hepatitis B surface antibody (HBsAb) titer, hepatitis B vaccine, tetanus-diphtheria (Td) vaccine

10789P

H. pylori infection upregulate embryonic stem cell markers in gastric epithelial cells

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Introduction: *Helicobacter pylori*, a gram negative bacillus, naturally colonizes human stomach and has been recognized as class I Carcinogen. Many studies revealed that the expression of embryonic stem cell markers (ESC) increase in gastric cancer. However their molecular mechanisms has not been elucidated. **Materials and Methods:** In this study, the gastric epithelial cells (AGS) were infected with *H.pylori* and the expression of Sox2 and Nanog in this cells were evaluated. In addition, expression level of these genes were analyzed in biopsies of 59 *H.pylori* – infected and 37 *H.pylori* uninfected patients by qRT-PCR. **Results:** The data showed that *H.pylori* infection up-regulates both genes Sox-2 and Nanog in AGS cells and biopsy samples from patients with *H.pylori* infection showed a higher level of Sox-2 and Nanog. **Conclusion:** It was demonstrated, for the first time, that H.pylori infection enhances embryonic stem cell markers in gastric epithelial cells.

10790 P

Reticulated Platelet Rates Are Increased In Chronic Immune Thrombocytopenic Purpura (ITP) Patients

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Introduction: Immune thrombocytopenic purpura (ITP) is a common hematological disease and its pathophysiology is not fully understood. The purpose of this study was to determine the production rate of reticulated platelets (RT) in patients and compare the results with control group. **Materials and Methods:** A total of 32 newly diagnosed primary ITP patients (platelets $<100\times10^9$/L) and 32 non-thrombocytopenic controls were included in the study. Platelets were isolated from plasma samples of patients and reticulated platelet rates were analyzed by flow cytometry after incubation with anti-human fluorescein isothiocyanate (FITC, negative control), anti-CD41 IgG-FITC and staining with thiazole orange (TO) as a stain for RT platelets. **Results:** This study showed that RT platelets stained with TO from ITP patients were found to be significantly higher than control group and the number of RT platelets were inversely correlated with the number of platelets. **Conclusions:** Detection of RT platelets by flow cytometry is a suitable approach for enumerating of RT platelets in patients with ITP.

10793P

Infiltration of inflammatory cells in chronic gastritis associated with expression levels of embryonic stem cell factors

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Introduction: Recent studies showed that *Helicobacter pylori* infection leads to migration of inflammatory and mesenchymal stem cells (MSC) to gastric tissue and that MSC may transform to the malignant cells. It has been suggested that inflammatory cytokines and cells play a crucial role in this transformation. As the embryonic stem cells (ESC) factors have been shown to be up-regulated in many cancers, in the current study we sought to detect the level of two ESC factor (Sox-2 and Nanog) in gastric samples from patients with chronic gastritis and correlate their levels with the number of inflammatory cells. **Materials and Methods:** Expression of mRNA levels of Sox2 and Nanog were investigated by qRT-PCR from 95 human Gastric tissues with and without *H.pylori* infection and evaluated relationship between gene expression with inflammatory cells. **Results:**Our data showed that the number of both moncytes and polimorfonucleares (PMN) cells have been increased in *H.pylori* positive patients.the levels of Sox-2 were significantly higher in patients with severe than mild infiltration of monocytes and PMN(4.25 and 5 fold respectively) and similary the levels of Nanog were remarkably higher in patients with severe than mild infiltration of moncytes and PMN (3.6 and 5 fold 0 respectively).**Conclusion:** It was shown, for the first time, that the expression of Sox-2 and Nanog depends on chronic inflammation and the degree of infiltration of inflammatory cells.

10854P

Examining the effect of lymph nodes on dynamically immune response to Influenza A Virus Infection

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Adaptive immune response to the first sign of Influenza A Virus Infection in lungs, causes Dendritic cells to be placed in lungs and immune cells in lymph nodes for activation. In this research, for the first time the anatomy of the lymphatic network in adult human body was determined in such a way that the obtained information of this network resulted in the formation of a directed network. Each lymph node was considered as a node of the network and each lymphatic vessels was regarded as an edge in the network. By considering the total communication network of lymph nodes in an adult human body, the behavior of Influenza A Virus Infection at the first sign of its existence in lungs is modeled. The results of this research showed that some of the lymph nodes were stimulated by the Influenza A. Virus. Infection took actions to produce antibody and CD8 cells and delivering them to others lymph till reach them to lung. There are other lymph nodes which are not stimulated but could be considered as an interface for transferring antibody and CD8 Cells. The effect of network on the behavior of Influenza A Virus Infection, showed that compared to the models without considering this network, the value of such behavior has reduced in an optimal way.

10898P

Survey on the Association of IL-10 promoter polymorphism in patients with chronic hepatitis C

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Introduction: Host genetic factors play a major role in determining the outcome of hepatitis C infection. IL-10 promoter polymorphism will affect the production of IL-10 and susceptibility to inflammatory diseases.This study aimed at determining the association between promoter polymorphism of IL-10 and response to treatment in Iranian patients with HCV. **Materials and Methods:** In this study, 80 patients with HCV that 40 responders to treatment and 40 non- responders to treatment as well as 40 healthy individuals were randomly selected. Genomic DNA was extracted from buffy coat by saturated salt method. Genotypes IL10-819 (C / T) and IL10-592 (C / A) were determined by ARMS-PCR technique. PCR products were electrophoresed on agarose gel 1.2%. **Results:** In the group of patients who were responders to treatment, genotype IL10-592 (C / A) was seen more than other genotypes , whereas in non- responder patients to treatment, all three genotypes AA, CC, AC were observed . In both groups of responder and non- responder patients, genotype IL10-819TT was more than other genotypes.There was no significant difference between the two groups (P = 1.00) **Conclusion:** In the group of patients who were responders , the genotype IL10-592 (C / A)was more observed. There was no significant association between polymorphism-592C / A with, AST enzymes levels. **Keywords:** Interleukin-10, Single Nucleotide Polymorphisms, Hepatitis C, ARMS PCR

10926P

Effect of Pseudomonas aeruginosa lysate on nitric oxide production of Balb/c mice macrophages

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Introduction: *Pseudomonas aeruginosa* has become an important cause of gram-negative infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week, and it is a frequent cause of nosocomial infections. In this study the functional effects of cell wall and supernatant fractions of *P. aeruginosa* on peritoneal macrophages were evaluated. **Materials and Methods:** *P. aeruginosa* PAO1 standard strain was lysed by lysate solution then centrifuged. Peritoneal macrophages of female Balb/c mice were lavaged and cultured in 96 well plate and exposed to different concentrations of cell wall and lysate supernatant fractions. After 48h MTT assay were performed on macrophages and on culture supernatant nitric oxide measurement by Griess method. **Results:** MTT assay for supernatant fractions were not different ($P>0.05$) with control but for NO and TNF-α was decreased significantly in doses of 0.1 and 1 mg/ml ($P<0.05$). Cell wall had a cytotoxic effect in 1mg/ml dose resulted in MTT assay ($P<0.001$) but amount of NO production was not significant ($P>0.05$). **Conclusion:** In this study, it was shown that cell wall fraction of P. aeruginosa had a cytotoxic effect on macrophages as innate immune cells and cytoplasmic fraction of *P. aeruginosa* suppress macrophage functions. But method of fraction preparation and effect of other elements in these fractions would be considered and further evaluations should be conducted. **Keywords:** *P. aeruginosa* Infection, Macrophage, MTT assay.

11005P

Association of Hepatitis A exposure and allergic disorders in Birjand city, Iran

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Introduction: The prevalence of allergic diseases has increased during the recent decades around the world. Based on hygiene hypothesis, there is an inverse correlation between exposure to infectious agents and atopic disorders. Hepatitis A is very common in developing countries and mostly affects children. The aim of this study was to investigate the correlation between exposure to hepatitis A virus and allergic disorders in Birjand city, Iran. **Materials and Methods:** 105 allergic patients (mean age=28 y, M/F ratio=0.72) and 99 non allergic case ((mean age=30 y, M/F ratio=0.76) were enrolled in this study. Demographic data, history of hepatitis A and allergic disorders were collected by questionnaire. Presence of allergic disorders was confirmed by allergist and skin prick test. The level of anti –HAV antibodies (IgM+IgG) was measured by ELISA kit. **Results:** 86 (55.8%) of people with history of hepatitis A exposure, suffered from one type of allergic diseases while only 19 (38%) of people with negative HAV antibody reported allergic disorders and this difference was statistically significant (P=0.03, OR=2). **Conclusion:** The result of present study showed that there is a positive correlation between exposure to Hepatitis A virus and occurrence of atopic disorders. Further studies need to understand the mechanism of this effect.
Keywords: Allergy, Hepatitis A virus, Hygiene hypothesis

11007P

Prevalence of HbsAg+ Cases in Pregnant Women in kerman city (2014)

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Introduction: Hepatitis B virus infection is a very common cause of chronic liver disease worldwide. About 35 % of the population had contact with Virus and 2 to 3% of the population are healthy carriers of the virus Hepatitis B in Iran. Given the lack of comprehensive information on prevalence of the disease among pregnant women as well as the concern on virus transmission from infected mothers to fetus during the pregnancy, this research aimed to determine the HBsAg prevalence in pregnant mothers of Kerman city. **Materials and Methods:** In a cross sectional study all the 301 pregnant women referred to Obstetrics and Gynecology Clinic of Kerman were investigated in 2014. Experimental examinations of serum samples were performed using Elisa serology method and other information required were gathered by reviewing pregnant women health care records and analyzed using SPSS software. **Results:** Study group consisted of 301 pregnant woman, among them, (1.66%) were HbsAg+ with no positive results for HBe Ag. The highest percent of research units (89.3%) were in age group of 18-35.**Conclusion:** The highest percent of participants were in age group of 19-34 years. Kerman city is among the areas with intermediate prevalence of positive hepatitis B surface antigen and it seems increasing awareness of the couples could more effectively prevent the occurrence of disease in the fetus.

11046P

Relationship between IL28B rs12979860 & rs12980275 Polymorphisms on treatment outcomes in Iranian patients with chronic hepatitis C

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Introduction: Several host and viral factors have been associated with sustained virologic response (SVR). Genetic variations at the interleukin-28B (IL28B) locus are significant factors in predicting the therapeutic outcome for chronic hepatitis C virus (CHC) infection. The distribution of two IL28B SNPs (rs12979860 and rs12980275) and rapid virologic response (RVR), early virologic response (EVR) and SVR in HCV Iranian patients with CHC were investigated. **Materials and Methods:** A case-control study was designed on 190 patients with CHC and 120 healthy controls; IL28B genotyping was performed by amplification refractory mutation system (ARMS)-PCR. **Results:** In these patients, RVR, cEVR and SVR were achieved in 53.2%, 78.9% and 65.8% of the patients, respectively. Multivariate regression analysis indicated age<40 years (p=0.008), HCV genotypes (p=0.032), IL28B rs12979860 CC genotype (p<0.001), rs12980275 AA genotype (p<0.001), RVR (p<0.001) and cEVR (p=0.024) responses were significant predictor for SVR. Regarding RVR, rs12979860 CC genotype (p=0.033) and rs12980275 AA genotype (p<0.001) were two critical predictors. As for cEVR, only rs12980275 AA genotype (p=0.003) was the predictor. **Conclusion:** These results may help predict the outcome of CHC patients, and it was suggested the test for these biological markers before starting pegylated interferon and ribavirin (pegIFN-α/RVB) therapy.

11049P

Mutation frequencies at hepatitis B surface antigen (HBsAg) according to different immune epitopes of B, T helper (Th) and Cytotoxic T (CTL) cells during mono and combination antiviral therapies in genotype D chronic hepatitis B.

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Introduction: The mutation frequencies at hepatitis B surface antigen (HBsAg) according to different immune epitopes during mono and combination antiviral therapies is unclear. **Materials and Methods:** Retrospectively, 86 patients were selected who partially responded to different nucleoside analogues including: 8 lamivudine only (I), 30 adefovir only (II), 16 adefovir add on lamivudine (III), 32 adefovir + lamivudine (IV) and 100 controls (no therapy). The surface proteins were divided into three domains and the patterns of mutations in the surface proteins of HBV were investigated with LAM and/or ADF-resistant in partially-responded CHB patients. **Results:** lamivudine + adefovir combination therapy showed the lowest mutational profile than either lamivudine / adefovir monotherapy or adefovir add-on. Antiviral drug-associated potential vaccine-escape mutant (ADAPVEMs) analysis showed an

association between rtM204I/V and overlapped surface protein for compensatory mutations sI195M and sW196L/stop codon with prevalence of 6.6% to 56.2% between treated groups, respectively. The difference in surface protein compensatory mutations between drugs-treated and treatment naïve groups were significant. The highest degree of compensatory mutational patterns were found in overlapped surface CTL epitopes, especially for rtL217R (37.5%) which mirrored on surface as sI208T/S residues. The latter finding was, however, contradicting, as ADV monotherapy group showed the lowest number of compensatory mutations. **Conclusion:** Altogether, a variety of surface protein compensatory mutations were found following ADV therapy that have not been reported before, which might be related to the nature of pure genotype D circulating in this country.

11056P

How eotaxines are changed in pulmonary tuberculosis?

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Introduction: Chemokines are chemo-attractant which recruit immune cells to the injured regions with bellow subdivisions of C, CC, CX3C and CXC. Eotaxines (CCL11, CCL24 and CCL26) fit in CC Chmokines. Probable parts played by eotaxines in tuberculosis (TB) have not been identified. Therefore, this project was aimed to examine CCL11, CCL24 and CCL26 in plasma and broncho-alveolar Lavage fluid (BALF) of Pulmonary TB patients. **Materials and Methods:** Total of 300 patients were collected from Afghan immigrants and Iranian patients in Kerman and Khorasan provinces. The serum and BALF levels of eotaxines CCL11, CCL24 and CCL26 were examined in patients and controls using ELISA. Student's independent sample T-Test as well as Mann-Whitney test were employed to find the significance of the observed differences. **Results:** The results indicated marked elevated serum levels of all eotaxines CCL11, CCL24 and CCL26 in serum and BALF of pulmonary TB patients, as compared to controls. **Conclusion:** Regarding to the results of the present study, elevated eotaxines may probably contribute to the migration of eosinophiles to the TB pleural space. Increased eotaxines level in these patients may also be due to drug sensitivity as well.

11060P

Status of cc chemokines CCL1, CCL2, CCL4 and CCL5 in pulmonary tuberculosis

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Introduction: Chemokines, as a subfamily of cytokines, are chemo-attractive for immune cells. They are subdivided into four following subdivisions as, C, CC, CX3C and CXC. The role of CC Chmokines in tuberculosis

(TB) is yet to be cleared. Thus, the aim of the present study was to assess CCL1, CCL2, CCL4 and CCL5 as the inflammatory members of CC chemokine subfamily in plasma and broncho-alveolar Lavage (BAL) fluid of pulmonary TB patients. **Materials and Methods:** samples were collected from 300 patients from Afghan immigrants and native Iranian patients residing in Kerman and Khorasan provinces of Iran. The serum and BALF levels of chemokines CCL1, CCL2, CCL4 and CCL5 were measured in both patients and healthy controls by ELISA (R&D systems, UK). Two-tailed student's independent sample T-Test and Mann-Whitney test were performed to find the significance of the observed differences. **Results:** The results indicated marked elevated serum levels of CCL2 and CCL5 but not CCL1 and CCL4 in pulmonary TB patients, as compared to those of healthy controls. BALF and serum level of chemokines were following similar pattern. **Conclusion:** According to the findings, significantly higher levels of these chemokines may probably contribute to the migration of circulating inflammatory cells and macrophages to the TB pleural space. These markers may serve for the early diagnosis and disease severity.

11069P

Functional and Structural Characterization of Ebola Virus Glycoprotein (1976-2015) - An In-silico Study

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Introduction: Ebola virus (EBOV) is the causative agent of a severe hemorrhagic fever disease associated with high mortality rates in humans. In this paper it was attempted to characterize and predict physicochemical properties, B-cell epitopes, mutation sites, modification sites, and different protein structures of EBOV GP during a forty years course (1976-2015). **Materials and Methods:** GP sequences were obtained from NCBI gene bank from 1976 to 2015. Several programs were used to predict and analyze all sequences. **Results:** More variety of mutations were found in 2015 sequences. Mutations were related to huge changes in B-cell epitopes, phosphorylation and glycosylation sites. Prediction of secondary and tertiary structures have shown different disulfide bonds. **Conclusion:** Physicochemical properties of GP and the effect of mutations on GP properties were established. Here, it was suggested six positions for disulfide bonds and 4 phosphorylation sites for protein kinase C enzyme.4 conserve positions were found for N-link glycosylation because of numerous mutations. A mutation was determined that changed GP to allergen protein as allergenic properties of protein which has a significant role in vaccine designing. Also, 4 potent B-cell (380-387, 318-338, 405-438, and 434-475) epitopes in GP protein were found, as humoral response against EBOV infection is critical for recovery in human.

11087P

BCG stimulated Fibroblasts induce increase in TGF-β1/IFN-γ cytokine production of surrounded T lymphocyte

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Introduction: Fibroblasts as stromal cells in lymphoid organs contribute to the development of immune responses by providing chemokines and cytokines, being as scaffolds for cell trafficking, presenting antigen and producing inhibitory molecules. There is an intimate contact between fibroblasts and T lymphocytes in lymph nodes that can affect cytokine pattern of T lymphocytes in different inflammatory condition. **Materials and Methods:** To determine the effect of fibroblasts of BCG vaccinated mice on cytokine pattern of T lymphocyte, 10^6 cfu of BCG were injected subcutaneously to BALB/c mice. One month later, lymph node fibroblasts were isolated by Dispase and Collagenase digestion. CD45- adherent cells were purified by magnetic bead separation method, considered as fibroblasts. CD4+ T lymphocytes were isolated from non-stimulated spleen and co-cultured with fibroblasts. IFN-γ and TGF-β1 production were measured in the supernatant and compared with Fibroblast of non-stimulated lymph node. **Results:** Purity of isolated CD4+T cells and fibroblasts were confirmed by flowcytometry. IFN-γ and TGF-β1 cytokines were measured using ELISA. Results showed that both IFN-γ and TGF-β1 cytokine production were induced in fibroblast- T lymphocyte co-culture. However the ratio of TGF-β1/IFN-γ cytokine was significantly higher in BCG stimulated fibroblast group compared to non-stimulated fibroblast. **Conclusion:** Lymph node fibroblasts can affect T lymphocyte cytokine production depending on its inflammatory context. The results suggested a regulatory role for fibroblasts through TGF-β1 induction in BCG stimulated immune responses.

11195P

Evaluation of IL-32 gene expression in patients with HAM/TSP and HTLV-1 carriers

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Introduction: Human T cell Lymphotropic Virus type I (HTLV-I), infecting approximately 10-20 million people in the world, is endemic in some regions. This virus is the causative agent of two main types of diseases: ATL and HTLV-I Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP), which are neuro-inflamatory, disabling diseases that lead to destruction of central nerveous system. As cytokines have a major role in inflammation reaction occurred in HAM/TSP patients, this study aimed at measuring IL-32, which is a proinflamatory cytokine whose activity is associated with autoinflamatory disorders and other viral infections. IL-32 is produced by T lymphocytes, natural killer cells, and participates in the immunological responses in the viral infections such as HIV and influenza. **Materials and Methods:** In the present study, Peripheral Blood Monocyte Cells (PBMCs) of 21 patients with HAM/TSP, 21 asymptomatic carriers, and 21 healthy controls were separated by using ficoll method. RNA extraction and cDNA synthesis were also performed, their derived results were verified by PCR and IL-32 gene expression was finally quantified by real time Polymerase Chain Reaction (PCR). **Results:** Findings based on procedures mentioned above did not reveal a meaningful increase in terms of IL-32 gene expression during the process of disease. Results showed that, significant differences in IL-32 gene expression between groups were not

observed. **Conclusion:** Based on these findings, probably IL-32 might not have a significant correlation with pathogenesis and process of the disease. **Keywords***:* HTLV-I, HAM/TSP, IL-32

11255P

Diagnostic significance of Mycobacterium tuberculosis Recombinant Antigens for Detection of Recent Infection

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Introduction: Since an accurate test for detection of *Mycobacterium tuberculosis early* infection is urgently needed, this study was designed for development of an efficient screening test in diagnosis of *Tuberculosis* infection. **Materials and Methods:** In the present study, three recombinant proteins CFP-10, ESAT-6, Mpt64 were tested as antigens for the diagnosis of recent tuberculosis. The proteins were produced in *Escherichia coli*, purified and tested in indirect ELISAs with sera from 63 subjects with positive clinical results. Also, 56 sera from healthy persons were tested as controls. The results were compared with molecular and culture.**Results:** The levels of antibodies against M. tuberculosis antigens in patients with tuberculosis were significantly higher than those in healthy subjects. Among 63 patients, 58 were positive for ESAT-6, 54 for CFP-10 and 48 for MPT-64.**Conclusion:** Altogether, the role of M. tuberculosis recombinant proteins, as a suitable candidate for early diagnosis of tuberculosis infection was supported in this study. However, these strongly offer the potential of mixture or fusion of these recombinant proteins for better sensitivity and specificity. **Key Words:** *Mycobacterium Tuberculosis*, ESAT-6, CFP-10, MPT-64, ELISA

11260P

The Effect of IL28B Gene Polymorphism on Treatment Response in Iranian Patients with Hepatitis C Virus Infection

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Introduction: The aims of this study was to compare the allelic and genotypic frequencies of the IL-28B rs12979860 polymorphism in sustained virological response patients who didn't respond to the standard of care treatment and to verify whether there is a correlation between viral load and IL28B rs12979860 polymorphism. **Materials and Methods:** This cross-sectional study was carried out on 75 HCV infected patients, including of 45 responders (group 1) and 30 non-responders (group2) to treatment. Allele and genotype frequencies of the IL-28B rs12979860 between two groups were compared using PCR-RFLP method. **Results:** Genotypes frequencies of rs12979860 polymorphism in group 1 were CC (28.9%), CT (37.8%) and TT (33.3%) and in group 2, CC (6.7%), CT (43.3%) and TT (50%). There was a significant difference in genotype frequencies of IL28B polymorphism between the two groups (p=0.03). There was no significant association between the viral load and IL28B

rs12979860 genotypes in both groups 1 (P=0.3) and group 2 (p=0.2). **Conclusion:** The findings indicated that patients with the homozygous CC genotype in IL28B gene had a significantly higher rate of response to treatment than TT or CT genotypes. Also, the IL-28B rs12979860 polymorphism didn't effect on the viral load. **Keyword:** Hepatitis C virus, Interleukin 28B, genetic polymorphism, Viral genotypes, Viral load , sustained virological response

11261P

Relative Expression of Toll-Like Receptors 2 and 7 mRNA in Peripheral Blood of Patients with Hepatitis C

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Introduction: Hepatitis C virus (HCV) is an important human pathogen affecting an estimated 120 – 170 million individuals in the world. Toll-Like receptors (TLRs) are pattern-recognition receptors that recognize pathogen-associated molecular patterns, and stimulate immune responses. The aim of this study was to determine the mRNA expression level of TLR2 and TLR7 in HCV-infected patients compared to normal controls. **Materials and Methods:** Nineteen consecutive patients with HCV infection and nineteen sex and age-matched healthy controls were studied in a case-controlled research. **Results:** The results showed that the expressions of TLR7 in HCV infected samples were significantly increased in comparison those of the controls (P = 0.02), while the expression of TLR2 was similar between the case and the control group (P = 0.8). There were no associations between the expression levels of TLR2 and TLR7 with HCV viral load and HCV genotypes. Also, there was no association between viral load and genotypes of the virus. **Conclusions:** The findings showed that HCV infection could lead to increased expression level of TLR7 mRNA in peripheral blood cells of HCV infected samples. The viral load and genotypes of HCV did not affect the mRNA expression levels of TLR2 and TLR7.

11328P

The effect of Cyclophosphamide on the gene expression amount of TLR2 in Balb/c mice with Systemic Candidiasis

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Introduction: TLR2 plays an important role in the process of detection and launching the immune response against Candida albicans. Cyclophosphamide is one of the most widely used chemotherapy drugs, which causes severe

neutropenia and suppression of the immune system. In this study Balb/c mice were infected to disseminated candidiasis and neutropenia induced in Balb/c mice and expression of TLR2 gene was measured. **Materials and Methods:** Twenty-eight mice were divided into 4 groups. Cyclophosphamide and *C. albicans* were injected to mice. Blood samples were used for RNA extraction and cDNA synthesis, and expression of TLR2 gene was measured by Real-time PCR. Statistical analysis was performed Kruskal-Wallis and $2^{-\Delta\Delta CT}$ method. **Results:** Gene expression was increased in the group receiving Candida albicans and also in group receiving both Cyclophosphamide and Candida albicans but decreased in the group just receiving Cyclophosphamide. **Conclusion:** There was no significant difference between the control group and experimental groups for TLR2 gene expression (p-value= 0.478). However, the results of this study could be studied in selecting TLR2 or its receptor as a therapeutic target with monoclonal antibodies or gene therapy techniques. **Keywords:** Systemic Candidiasis- Toll like receptor-2(TLR2)- Cyclophosphamide- Neotropenia- Balb/c

12387P

Monitoring of Bacterial Infection (Septicemia) by Nitrobluetetrazolium (NBT) Test in Skin Burned Patients

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Introduction: Bacterial infection is an important causative agent of mortality of skin burned patients. NBT test is a rapid, easy and cost benefit which indicates the decrease or increase of bacteria in infected wounds and correlates with statues of wounds as healing or sepsis as early as possible. **Materials and Methods:** during 3/5 years (2013-2016) 35 skin burned patients with (≥50% or ≤ 50%) burning and 40 control cases were studied. Patients based on their surface of skin burned size (≥50% or ≤ 50%) included in tow groups followed and compared 4 weeks intervals. Parameters such as Hb, HTC, and WBC, serum albumin level and NBT test, bacterial colony count were assayed. **Results:** Out of 35 burned patients, 6 cases have died from2 groups (≥50% or ≤ 50%) of skin burned and 2 cases with (≤ 50%) and 4 (≥50%) skin burning. NBT or formazan positive neutrophil number in the first 24 hours increased, but in second and third weeks decreased gradually, then increased in fourth week. In patients with the (≥50%) burning, NBT or formazan cells number in first 24 hours, second and third, Fourth weeks were, 60%, 46%, 37%, 50%, and 55%, respectively, but for patients with the (≤ 50%) burning, NBT or formazan cells number in first 24 hours, second and third, fourth weeks were,48%,27%,23%,33%,39% Respectively.**Conclusion:** In conclusion we supported that the NBT test is a simple, inexpensive and rapid investigation in clinical practice to assess neutrophil function, and to predict infection. **Keywords:** skin burn –Neutrophil function- NBT test

12393P

Main causal agent of brucellosis in Kermanshah

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Introduction: Brucellosis is a zoonosis disease caused by the bacteria of the genus Brucella. The aim of this present study was to find the main species of Brucella responsible for brucellosis in Kermanshah. **Materials and Methods:** Anticoagulant containing blood samples from 162 suspected patients with brucellosis were collected and included in this study. By means of commercial kit (GeneAll), DNA was extracted from the blood samples. Using three primer pairs, one pair for the genus Brucella, another pair for melitensis species and the other pair for abortus species, PCR were performed on extracted DNA. **Results:** By conducting PCR on DNA extracted from blood samples, it was cleared that from 162 blood samples, 63 samples were positive for the genus Brucella. From these PCR positive specimens, 57 samples (%90) were positive for Brucella abortus and 6 samples (%10) were positive for Brucella melitensis. **Conclusion:** Taken together, results of this study showed that the most common causal agent of brucellosis in Kermanshah was Brucella abortus. These results indicated that substitution of small ruminants by large ruminant, cow, have changed common causative agent of brucellosis from Brucella melitensis to Brucella abortus.

12600P

Polymorphisms of Interferon gamma promoter andit's Receptor one in Iraq population are associated with HBV infection.

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Introduction: The interferon gamma gene with approximately 5.4 kb is located on chromosome 12p24 and composed of three introns and four exons. The SNPs of interferon gamma gene region can influence the it's production which may increased the risk of viral infections. Susceptiblity of interferon gamma gene receptor 1 variants to hepatitis B virus infection, suggested that the gene polymorphism of *INFGR1* may have some effects on initiation and development of hepatitis B infection. The aim of our study was detection of the polymorphisms in *INF-γ* promoter and INFGR1 genes in Iraq HBV patients and controls. **Materials and Methods:** Genomic DNA was extracted from 200µl of whole blood from 95 Iraqi subjects (45 HBV infected patients and 40 control) by using Relip™ blood DNA Miniprep system Kit. *INF-γ* detected in PCR by using primers: Forward 5'-CGAAGTGGGGAGGTACAAAA 3' and Reverse 5'-CCCAGGAAACTGCTCTCTG -3', optimizing TA 65.3ºc. Whereas the *INFGR1*was detected by using primers: Forwar5-TCCTCGAAATATACTGCATCA-3 and Reverse 5-ATTGTAACTCATGCTGATGAT-3 Optimizing TA 57ºc, The PCR products were purified by using MEGA quick-spin™ and Wizard SV, then sent to sequencing company. **Results:** The results exhibited that IFN-γ production decreased in 24 patients who have A/T SNP at locus -125 with allele frequency 0.27. HWE values for interferon gamma receptor one sequenced gene were is 25.69 for patients and 33.8 for controls in $(CA)_{12}$ and in $(CA)_{27}$ were 35.65 and 8.89 for each patients and healthy control, respectively, where as the PIC 0.36 refered to responsibility of this allele for infection with HBV because of PIC value (0.36) between 0.25 and 0.5. while the PIC of $(CA)_{27}$ is 0.68 which is highly informative to HBV infection. **Keywords:** Allele, CA repeats, Polymorphic information count, Hardy Weinberg Equilibrium test, HBV infection, Single nucleotide polymorphisms.

Rheumatic Diseases

Oral Presentations:

10782O

Immunomodulation of apoptotic responses in systemic lupus erythematosus

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Introduction: Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease with a variety of clinical manifestations. SLE is more prevalent in females and ethiology of the disease is unknown. Apoptosis is a highly regulated process and plays a crucial role in the pathogenesis of SLE. Any disturbance in the course of apoptosis could lead to the breakdown of tolerance and generation of autoimmunity. As environmental factors are implicated in the pathogenesis of SLE, the anti-apoptotic effects of Lactibacillus Delbocki as a probiotics and vitamin D was studied on apoptosis induction and some genes involved in the apoptosis process. **Material and Methods:** PBMCs from 20 SLE patients and 20 healthy controls were cultured in the presence of Lactobacillus DelbrCki or 50 nM of 1,25(OH)2D3; then by using Annexin V and PI cells, apoptosis was determined by Flow Cytometry. For gene expression assessment of FasL, Bcl-2 and Bax, RNA was extracted. cDNA was synthesized and gene expression was assessed using Real time PCR. Cell cycle progression was analyzed using PI treatment and Flow cytometer. **Results:** Number of early apoptotic cells in vitamin D and probiotic treated cells were decreased significantly compared to untreated cells. Cell cycle analysis showed a significant increase in G1 phase in treated cells compared to non-treated ones. Probiotic and vitamin D treatment up-regulated the expression levels of Bcl-2 and down-regulated expression of Bax and FasL. **Conclusion:** Lactobacillus Delbrocki and vitamin D showed regulatory effects on cell cycle progression, apoptosis and apoptosis related molecules in lupus patients. **Keywords:** systemic lupus erythematsus, probiotics, vitamin D, immunomodulation

10797O

Interleukin 31 (IL-31): A new cytokine in the pathogenesis of rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease. The inflammatory cytokines have been recognized as important factors in the pathogenesis of RA. Enhanced expression of interleukine-31 (IL-31) has been hown to be associated with a number of inflammatory diseases. Accordingly, the possible association of IL-31 with RA pathogenesis has studied in the present study. **Patients and Methods:** Levels of IL-31 in the sera and joint fluids of 44 patients suffering from RA and 44 patients with osteoarthritis (control group) were determined by ELISA method. Simultaneously, CRP levels of serum in rheumatoid arthritis patients were measured. **Results:** The mean levels of IL-31 in the sera of patients were not significantly different from the control group (35.58 pg/ml and 14.7 pg/ml, respectively; p=0.4) while the levels of this cytokine in the synovial fluid of patients were significantly higher compared to the controls (16.01 pg/ml and 0.67 pg/ml, respectively; p=0.0001). Interestingly, after classification of patients according to the duration of morning stiffness (above and under 60 minutes), it has been revealed that the levels of IL-31 were significantly higher in the synovial fluid of patients with morning stiffness of longer than 60 minutes (24.21±32.53 pg/ml and 11.36±22.05 pg/ml, respectively; p=0.01). **Conclusion:** According to the results of the present study, IL-31 could be considered as a inflammatory cytokine which was produced locally in the inflamed joints of patients who suffer from RA. Further proof of this finding in future studies might be a candidate IL-31 as a new therapeutic target in RA patients. **Key word:** Cytokine, Interleukine-31, Rheumatoid arthritis, Pathogenesis.

11012O

Evaluation of the protein tyrosine phosphatase non-receptor type 22 Gene Polymorphism in Iranian Patients with Rheumatoid Arthritis

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Introduction: Rheumatoid arthritis is a common chronic inflammatory autoimmune disease characterized by inflammation of the synovium and pathological infiltration of lymphocytes. These diseases occur as a result of the loss of physiological tolerance to self-antigens and are characterized by persistent activation of immune cells, leading to tissue damage. The +1858C/T single nucleotide polymorphism in the PTPN22 gene has been associated with susceptibility to multiple autoimmune diseases. The human PTPN22 gene encodes a LYP protein, which is important in negative control of T cell activation and T cell development. **Material and methods:** Patients were acquired from the Rheumatology Research Centre at Shariati Hospital. RF, anti-CCP and ESR laboratory tests were carried out. Genomic DNA was extracted from the whole blood. Genotyping of the +1858C/T SNP was performed by the PCR- RFLP method. **Results:** Higher frequencies of PTPN22 C/T (14.1% vs. 4.1%) were observed in patients versus controls. Significant differences were also observed between patients and controls in the genotype distribution of PTPN22 SNP (p = 0.007, OR = 2.321, 95%CI = 1.063–5.067). In addition, it was

found that the frequency of the PTPN22 T allele in the patients was significantly higher than that of the controls (p=0. 008, OR=3. 583, CI=1. 3-9.878). **Conclusion:** This study has shown that the +1858T allele in the PTPN22 gene is a genetic risk factor for susceptibility to RA and confirm that, this SNP influences the autoimmune processes towards a development of RA in Iranian population.

11014O

Association of interleukin 4 receptor Gene Polymorphism with rheumatoid arthritis in Iranian patients

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Introduction: Rheumatoid arthritis is a common chronic inflammatory autoimmune disease characterized by inflammation of the synovium and pathological infiltration of lymphocytes. The imbalance between pro-inflammatory and anti-inflammatory cytokines is a feature of rheumatoid arthritis. The role of interleukin-4 (IL-4) and its receptor in the pathogenesis of RA is conflicting. The aim was to investigate the role of polymorphisms in the IL-4Rα gene in susceptibility of RA. **Material and methods:** Patients were acquired from the Rheumatology Research Centre at Shariati Hospital. RF, anti-CCP and ESR laboratory tests were carried out. Genomic DNA was extracted from the whole blood. Genotyping of IL-4Rα I50 V (rs1805010) and IL-4Rα Q576R (rs1801275) were determined by restriction fragment length polymorphism-polymerase chain reaction (PCR-RFLP). **Results:** It was observed that IL-4Rα I50 V genotype was significantly more frequent in patients with RA than in controls (OR: 1.97, 95% CI: 1–3.7, P: 0.015). Subjects with IL-4Rα V50 V genotype were significantly more likely to have erosive arthropathy (OR: 2.6, 95% CI: 1.1–6.1, P: 0.031). **Conclusion:** This study has shown that the IL-4Rα polymorphisms were associated with susceptibility to RA and may be helpful in early detection of erosive RA and confirm that, this SNP influences the autoimmune processes towards a development of RA in Iranian population.

11204O

Evaluation of *DNMT1* Gene Expression Profile and Methylation of its Promoter Region in Patients with Ankylosing Spondylitis

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Introduction: Ankylosing spondylitis (AS) is an autoimmune disease with a chronic inflammatory arthritis. Over the course of past few decades, epigenetic concept is a fast-expanding area to shed a new light on the disease development circumstances. DNMT1 is an enzyme that establishes and regulates patterns of methylated cytosine residues. The aim of the current investigation was to unveil if methylation circumstances of CpG sites from *DNMT1* promoter could affect the expression level of the gene in AS patients. **Materials and Methods**: PBMCs were isolated from whole blood of 40 AS patients and 40 healthy individuals. Total RNA and DNA contents of

leukocytes were extracted. Afterward, Quantitative analysis was carried out through real-time RT-PCR using the SYBR Green PCR Master Mix. Finally, to determine the methylation level, PCR products of Bisulfite-treated DNA from patients and control were sequenced. **Results**: Compared to healthy controls, expression level of *DNMT1* in AS patients was significantly down-regulated. Methylation of *DNMT1* promoter was significantly higher in AS patients compared to controls. Albeit a negative trend between methylation and expression level in AS patients, however, no significant correlation was observed between both methylation and expression level of *DNMT1* with clinical manifestations. **Conclusion**: Given that decreased expression level of *DNMT1* was associated with highly methylation status of DNMT1 promoter in PBMCs from AS patients, this survey suggested that dysregulation of *DNMT1* expression through altered methylation level would impress the methylation level of other target genes and contribute to AS development. **Keywords**: Ankylosing spondylitis, CpG Island, Methylation, DNMT1

Poster Presentations:

7549P

The effect of active form of Vitamin D1, 25(OH) 2D3) D in Rheumatoid Arteritis Patients

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Background: C- reactive protein (CRP), as an acute phase reactant and a reliable marker of inflammation, increases due to inflammatory diseases such as Rheumatoid Arteritis and infectious conditions. New evidence shows that Vitamin D may have important effects on adjusting and reducing the immune responses. The aim of this study was to evaluate the association between serum vitamin D as an immuno-modulatory factor and CRP as an inflammatory factor in Arteritis Patients. **Material and Methods:** The CRP and Vitamin D were evaluated in Rheumatoid Arteritis patients confirmed by medical records (40 men and 40 women) and in 80 healthy adult people with normal CRP and Vitamin D and no history of arteritis (40 men and 40 women). Torbidometry was used to measure CRP and Eliza for Vitamin D. **Results:** In patient group, the mean of CRP and Vitamin D were 95.9±9.1 Mlg/lit and 9.17±2.9 Mlg/lit, respectively. There was a significant inverse correlation between C-reactive protein and vitamin D in Rheumatoid Arteritis patients (Pvalue= 0.03; Pearson correlation:-0.62) and that was the case for healthy people (p value: 0.04; Pearson correlation: -0.73). **Conclusion:** Based on the findings, inverse correlation was observed between serum vitamin D and CRP level.

9737P

Influence of vitamin D3 on the expression of TLR3 and TLR8 in patients with systemic lupus erythematosus

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Introduction: Systemic Lupus Erythematosus (SLE) is a multi-factorial autoimmune disorder associated with loss of B and T cells tolerance. The studies have shown that disturbed expression of TLRs play a crucial role in the development and pathogenesis of SLE. Vitamin D3 was considered for its immuno-modulatory effects in patients with autoimmune diseases. In this study, the influence of vitamin D3 on the expression of TLR3 and TLR8 was assessed. **Materials and Methods:** The current study included 20 patients with SLE and 20 (age and sex matched) healthy controls. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured in the presence and absence of vitamin D3 (50 nM).Then RNAs were extracted and cDNAs were synthesized and gene expression levels of TLR3 and TLR8 were evaluated by Real Time PCR, Taq Man method. **Results:** The expression level of TLR3 in the PMBCs of SLE patients exposed to vitamin D3 were significantly down-regulated (8.8±18.8 versus 140.8±357.6) (p=0.01), whereas following vitamin D3 treatment the expression rate of TLR8 was up-regulated in SLE patients compared to control group (164.2±466.5versus 2.1±3.1) (p=0.022) .**Conclusion:** The findings demonstrated that vitamin D3in SLE patients could exert some of its immuno-modulatory effects by affecting the expression levels of some of TLRs. **Key Words:** systemic lupus erythematosus, vitamin D3, TLR3, TLR8

10841P

Is there a relationship between vitamin D level and CRP, RF and Anti-CCP in patients with active rheumatoid arthritis?

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Introduction: Rheumatoid arthritis (RA) is a severe, progressive, systemic inflammatory disease, due to T-cell abnormal function. The morbidity and mortality are the consequences of local and systemic inflammatory processes that damage cartilage, bone and soft tissue. Anticyclic-citrullinated-peptide (anti-CCP) antibodies hold promise for earlier and more accurate diagnosis of disease, improved prognostic information, and have been implicated in RA pathogenesis. Vitamin D is an effective factor in natural function of immune system. The aim of this study was to assess influence of vitamin D treatment to decline inflammatory condition of active rheumatoid arthritis. **Methods and Materials:** Blood sample of 97 RA patients with low vitamin D were taken. Anti CCP and RF was performed by ELISA, but CRP by nephelometry. Parameters were measured before and after using vitamin D IM injection form. Statistical analysis was performed by prism graph. **Results:** It was found that there was significant relationship between vitamin D and CRP and RF level as increase in vitamin level showed decrease in RF and CRP

amount, and also study showed lower level of anti-CCP after vitamin D therapy. **Conclusion:** According to evidences, vitamin D could be effective parameters to reduce inflammatory condition of active RA. **Keywords:** RA, vitamin D, Anti-CCP

10956P

Investigation and Comparison of Autoantibody Repertoires of Rheumatoid Arthritis and Systemic Lupus Erythematosus Patients Using Phage-Displayed Random Peptide Library

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Introduction: Autoantibodies of Systemic lupus erythematosus (SLE) and Rheumatoid arthritis (RA) patients under treatment with DMARDs and Corticosteroids, reflect the variety of activated pathways involving in resistance to treatment and susceptible to different cancer. **Materials and Methods:** A random phage display peptide library was used to isolate the phages with the ability to detect IgG antibodies in patients with SLE and RA. After several rounds of enrichment and poly- and monoclonal-phage ELISA, 20 clones from each group of patients were selected. After investigation of seven peptide sequences obtained from the NCBI database and on Blastp, 476 proteins were found classified in the Enrich-R database based on the previous studies. **Results:** Finally among 476 obtained proteins, 17 and 18 proteins in SLE and RA were found, respectively. These proteins which play important roles in the signaling pathways in the resistance to treatment and cancers, are FGF, PDGFR and Wnt signaling pathways in RA patients and ABC transporters and semaphorin signaling pathways in SLE patients. **Conclusion:** This was the first study showing the importance of the autoantibodies against proteins with critical roles in the response to treatment and the possibility of developing cancers. **Key Words:** Autoantibody, Systemic Lupus Erythematosus, Rheumatoid arthritis, Phage display, panning

11127P

Anti- Inflammatory Effect of Naloxone in the Rat Air Pouch Model of Inflammation

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Introduction: A bidirectional interaction between the nervous and immune systems was becoming increasingly well documented. The aim of the present study was to evaluate the effect of naloxone on the inflammatory parameters in a rat model for rheumatoid arthritis, namely air pouch model of inflammation. **Materials and Methods:** To induce air pouches, Wistar rats (200-250 g) were anesthetized; sterile air (20 ml and 10 ml) was 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 and naloxone (100, 200,400 and 800 microgram) were administered intra pouch at the same time as the carrageenan and then for 2 consecutive days. After 72h, the rats were sacrificed and the pouches

were opened with a surgical scissor; pouch fluid was collected in order to determine exudates volume and cells were counted using cell counter. Pouches were dissected out and the weight determined. **Results:** Naloxone with doses of 100, 200 and 400 microgram decreased significantly leukocyte accumulation in the pouch fluid ($p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively) versus the control group. Volume of exudates and the granulation tissue weights were markedly decreased only by 400 microgram of naloxone. **Conclusion:** The current study highlighted evidences for the promising anti- inflammatory effects of naloxone that could be mediated through attenuation of leukocyte migration. **Key words:** Naloxone, Rat, Air Pouch, Inflammation.

Immunoparasitology

Oral Presentations:

6521O

Comparison of capability of GST and FABP for sero-diagnosis of Fasciola hepatica infections

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Introduction: Fasciolosis is caused by liver fluke, Fasciola hepatica. Differentiation of the acute and chronic phases of this zoonotic disease could help to better diagnosis and prophylaxis of this disease. Previously, glutathione S transferase (GST) and fatty acid binding protein (FABP) were detected as main immunogenic proteins of F. hepatica. In this study, the capability of the recombinant form of these proteins were compared for sero-diagnosis. **Material and methods:** Total RNA was purified from fresh adult liver flukes which were isolated from infected sheep. Following cDNA synthesis, the coding sequences of immunogenic proteins were cloned in top ET-28. The recombinant proteins were expressed in BL-21(DE3). Sera from healthy individuals as well as from fasciolosis patients and other parasitic diseases were used to evaluate their immune-reactivity using ELISA method. **Results:** The results showed that the recombinant forms of FABP and GST are both immunogenic and had a considerable reactivity with patients' sera. The ELISA results revealed that the recombinant FABP had more sensitivity, specificity and efficacy for sero-diagnosis of fasciolosis than the recombinant GST. Evaluation of the cross-reactivity of the produced immunogens with patients' sera indicated a significant cross-reactivity with Taenia spp. **Conclusion:** The recombinant form of FABP could be a better choice than GST for sero-diagnosis of F. hepatica infections with acceptable sensitivity and specificity in endemic areas. **Key words:** Fasciola hepatica, Recombinant protein, immunogenic proteins, serodiagnosis

7515O

The Effect of Artemisinin with Glucantim and Shark cartilage extract on Leishmania infantum in in vitro condition

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2-immunology

Introduction: Leishmaniasis is one of the neglected tropical diseases. visceral leishmaniasis is the acute form of the disease and, if diagnosis and treatment unleft, the rate of mortality will be high level. Recently the use of derivatives plant instead of chemical drugs are common into consideration. In this study we examined effects of

Artemisinin with Glucantim and shark cartilage extract as immunomedulator, on promastigotes and amastigotes of L. infantum in in vitro condition. **Material and Methods:** In this experimental study the effect of Artemisinin, Glucantim and shark cartilage extract was evaluated at concentration range of (400,200,100 ,50,25µg/ml) on the L. infantum promastigotes, non infected macrophages and macrophages infected with L. infantum after 72hrs by counting and MTT assay and the apoptosis was evaluated with flow cytometry assay. **Results:** IC50 of drugs Artemisinin, glucantim and both were determined after 72 hours respectively 50, 400 and 100µg/m. Cytotoxicity in control was 2.41% ,at concentration levels of 50 artemisinin, Glucantim, A+ G and shark cartilage were 52% , 52.8% , 61% and 32% respectively. Statistical analysis showed significant differences between treated and control groups (P<0.05). **Conclusion:** This study supports Artemisinin, Glucantim and shark cartilage showed significant effectiveness on promastigotes and amastigotes, and can be used as alternative drugs in vivo studies.

7578O

Development of an antigen detection dot blot method for diagnosis of Cryptosporidium infection in cattle

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Introduction: Diagnosis of cryptosporidiosis is based on the use of routine microscopic methods, but these methods possess poor sensitivity and also need an expert technician. The aim of the present study was to develop an antigen detection dot blot method for diagnosis of bovine cryptosporidiosis. **Material and Methods:** In this study 131 fecal samples were collected from suckling calves during summer 2014. The collected samples were concentrated by formol-ether concentration technique for microscopic goals, modified-Ziehl-Nielsen (mZN) and auramine-phenol (AP) staining methods. Dot blot method was developed and the results of the three methods were compared and evaluated for diagnosis of the infection. **Results:** Oocysts of Cryptosporidium spp. were found in 40 (30.5%) and 41 (31.3%) out of 131 fecal samples using mZN and AP staining methods, respectively. In addition, 49 (37.4%) of the samples were positive for Cryptosporidium antigen using newly developed dot blot method. No significant differences were observed among the results of three used methods and the sensitivity and specificity of dot blot calculated as 85-85.3% and 83.5-84.4%, respectively. **Conclusion:** Based on the results of the present study, dot blot method showed rather equal performance as modified-Ziehl-Nielsen and uramine-phenol staining methods. **Key words:** Cryptosporidium, modified-Ziehl-Nielsen, uramine-phenol, dot blot, antigen detection

9763O

Evaluation of expression of TLR2 gene in PBMCs of volunteers Immunized with the gentamicin-attenuated L. major

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Introduction: Toll-like receptors (TLRs) are important components and play a critical role in the outcome of the leishmaniasis infection. They basically rely on the skewed balance between Th1/Th2 immune response. TLR2 has been found to be responsible for increasing parasite load and reducing recruitment of inflammatory cells. TLR-2 involves in parasite survival in macrophages which leads to decreasing IL-12 and increasing IL-10 production. In

the present study, TRL2 expression was compared in the volunteers before and after vaccination with gentamicin-attenuated L. major and after challenging with wild-type parasites. **Material and Methods:** The attenuated line of Leishmania major (L. major) (MHOM/Su73/5ASKH) was established in the presence of gentamycin. Twenty seven healthy volunteer (14 women and 13 men) subjects were enrolled in this project. The volunteers were vaccinated subcutaneously with the attenuated line of L. major and their peripheral bloods were collected prior and 60 days after immunization. At 60 days of post-immunization, the vaccinated volunteers were challenged with L. major wild-type and blood collected after 60 post-challenged. The peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll and the expression of TLR2 was assessed by qRT-PCR. **Results:** The expression of TLR2 was found in PBMC of vaccinated volunteers prior to vaccination with 60 days after vaccination and also 60 days after challenging was decreased. **Conclusion:** Vaccination with the attenuated L. major, down-regulation of TLR2 in PBMCs may lead to cellular immunity in volunteers.

10803O

Comparison of optical reporter genes to track of Leishmaniatropica in different tissues of infectedBALB/c mice

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Introduction: L. tropica is one of the major parasite of human cutaneous leishmaniasis in Iran. Due to the lack of an accurate, sensitive and noninvasive detection system and unsuitable susceptible animal model, studying and combating against this parasite is a difficult task. Here for the first time, a recombinant L. tropica was generated being stably transfected with two reporter fused genes egfp-luc in order to be used as a specific tool for detection and measurement of infection in both life cycle of parasite. **Material and Methods:** Linearized cassette containing egfp-luc genes was integrated into wild-type L. tropica18s rRNA locus of genome by homologous recombination (HR). Presence of genes and HR occurrance were confirmed by PCR. Western blotting and luminometer were used to define both EGFP and LUC expression. Different groups of BALB/c mice were infected with 1x107wild-type or recombinant L. tropica. At different time periods after infection, parasite load was estimated by classic, real-time PCR methods as well as fluorescence/bioluminescence in vivo imaging. **Results:** Recombinant L. tropicaEGFP-LUC was confirmed genotypically and phenotypically through PCR, western blot and luminometer. Progression of infection in infected footpads or ears were monitored by in vivo imaging during 6 months follow-up and compared with real-time PCR and conventional limiting dilution methods. **Conclusion:** The results demonstrated that quantitative measurement of LUC could be used as sensitive tool to detect the development of parasitemia in L. tropica infected footpads of mice up to 6 months post infection. In contrast, it was only detectable in infected ears up to two months post infection. In both locations, EGFP signal was not detectable.

Poster Presentations:

6520P

Immunogenic proteins of Fasciola hepatica: purification and identification

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Introduction: Fasciolosis is a parasitic liver infection with global distribution caused by Fasciola hepatica. Characterization and purification of the immunogenic proteins of this parasite could be advantageous for sero-diagnosis, treatment and control of fasciolosis. **Material and methods:** The adult flukes were isolated from infected sheep livers. After washing with PBS they were homogenized in cell lysis buffer by mortar and pestle. The lysate was clarified via centrifugation at 14000g, 4°C, in 30 min. The supernatant was concentrated by ultrafiltration through 4 kDa membrane filters and the quality of the extract was analyzed by SDS-PAGE. The immuno-reactivity of the electrophoretically resolved bands were analyzed by western blotting. Finally, the immunogenic ones were purified by ammonium sulfate precipitation procedure and they were characterized by mass spectrometry methods. Furthermore, other purification methods such as anion exchange chromatography and electro-elution were applied for separation of desired proteins. **Results:** The results of western blotting showed that protein bands of 11-15, 25-27, 35-37 and 40 kDa were immunogenic. These immunogenic proteins were identified to be fatty acid binding protein, glutathione S transferae, cathepsin l and aldolase, respectively. **Conclusion:** The main immunogenic proteins of Fasciola hepatica could be enriched by the simple salting out method.These proteins could be used for detection of fasciolosis, vaccination and control of the infection. **Key words:** Immunogenic protein, Fasciola hepatica, Mass spectrometry

8707P

Immunization evaluation and efficacy of a DNA Vaccine candidate containing LeIF gene of cutaneous leishmaniasis due to Leishmania major in BALB/c mice

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Introduction: The aim of this study was to evaluate the efficacy of protective immune responses induced by DNA vaccine encoding of LeIF gene against leishmaniasis due to Leishmania major in BALB/c mice. **Material and Methods:** Recombinant plasmid expressing of LeIF was constructed. Then, Immunization experiments were carried out three times with a time-intervals of 3 weeks in 13 groups of susceptible BALB/c mice (15mice per group). After 3 weeks of the late immunization in each group, 5 mice were sacrificed for evaluation of cytokines before challenging and 10 mice were subcutaneously challenged with 2×106 Leishmania major (MRHO/IR/75/ER) promastigotes in stationary phase into tail base. After 4 weeks of challenging, 5 mice were used to evaluate cytokine levels. Lesion size and survival rate were weekly evaluated for remained animals. Also, antibody levels were measured just before and after challenge. **Results:** Immunized mice with one antigen individually or jointly indicated a partial protective immune response against leishmaniasis compared to control groups characterized by increased levels of IFN-γ and IgG2a and decreased levels of IL-4 and IgG1. Also, lesion diameter was decreased for

immunized animals than that in control groups. **Conclusion:** DNA vaccination with an antigen was not able to induce a strong protective immune response. So, use of antigenic combination or an adjuvant increases immune response against leishmaniasis and could be used as a method to improve a DNA vaccine efficacy. **Keywords:** Leishmania major, DNA vaccine, LeIF,

6521P

Expression of Pro-Inflammatory Genes in Lesions caused by Burning and Cutaneous Leishmaniasis

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Introduction: Leishmaniasis is a worldwide disease prevalent in tropical and sub-tropical countries in the world. Characterization of inflammatory responses produced in cutaneous Leishmaniasis has not yet been completed. **Material and Methods:** The specific primers were designed for ten pro-inflammatory genes including CCL4, CCL3, TNF-α, IL-1α, IL-12P35, IL-12P40, CCL5, CCR5, IL-1β and IFN- γ and their expression were assessed and compared using RT-PCR in the lesions caused by either Leishmania or burning in BALB/c mice. **Results:** None of the pro-inflammatory genes were expressed in the healthy tissue and in the lesions caused by Leishmania, except IFN-γ the other genes were down-regulated by the parasite in untreated mice. In mice treated with Glucantime, the expression of the pro-inflammatory genes restarted. In contrast, the figure of expression of pro-inflammatory genes in lesions caused by burning, was different where the pro-inflammatory genes were expressed in untreated lesions and down-regulated in cured lesions. **Conclusion:** The results indicated a role for Leishmania in supression of pro-inflammatory genes and a role of pro-inflammatory genes in healing of burning lesions. **Keywords:** Leishmania, proinflammatory genes, burning, lesion

9755P

Activities of nitroimidazolylmethylene-3(2H)-benzofuranone compounds on *leishmania major*

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Introduction: Cutaneous leshmaniasis, a neglected parasitic disease, is one of the sanitary problems in most countries around the world. Clinical manifestations of cutaneous leishmaniasis (CL) are wide, including the mild cutaneous form, non-ulcerative nodules and the disfiguring mucosal form. The treatment choice of cutaneous leishmaniasis is pentavalent antimony compounds as first-line drug. The other two second line drugs are pentamidine and amphotericin B. Despite their toxicity, high coast and difficult administration, incidence of drug-resistant is over-grown. This article is based on the evaluation of the antileishmanial activity of three derivatives of nitroimidazolyl-methylene- 3(2H)-benzofuranone, named A, B and C against leishmania major. The structure of nitroimidazolyl drugs are based on benzofuranones and nitroimidazoles. Benzofuranones effect on chorismate synthase, the last enzyme of shikimate pathway, are responsible for the formation of aromatic amino acids in bacteria, protozoa, fungi and plants, as an inhibitor. On the other hand, nitroimidazoles effect was on crucial structure like DNA, protein and cell membrane and result in pathogens' death. **Material and Methods:** Levels of

cytotoxicity of the compounds were determined by MTT assay on THP-1 cell line and inhibitory concentrations by Parasite-Rescue-Assay. **Results:** The compounds were evaluated against infected THP-1 and both of them presented the best activity, with values A (IC_{50}= 0.1337 µg per ml) and C (IC_{50} = 0.7602 µg per ml). Furthermore, A was more active than standard antileishmanial agent amphotericin B (IC_{50} = 0.4 µg per ml). **Conclusion:** This study could be considered as a new window to the field of drugs in treatment of leishmaniasis in future.

9783P

Recombinant Leishmania major lipophosphoglycan 3 stimulates purified human B-lymphocytes

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Introduction: Lipophosphoglycan (LPG) is the most abundant glycophospholipid on the surface of Leishmania promastigotes that plays key roles in the pathogenicity and survival of Leishmania. LPG3, as a member of HSP90 family, is responsible for the biosynthesis of LPG. In this study, the recombinant LPG3 effects on human B-cells activation and cytokine secretion were evaluated. **Material and Methods:** Peripheral blood mononuclear cells were obtained from heparinized whole blood of 10 adult healthy volunteers. Then B-cells were isolated by MACS technique. Purified B-cells were incubated with recombinant LPG3 (2, 10 and 20 µg/ml). B-cells activation was evaluated by detection of CD69 expression level by flow cytometry. Moreover mRNA expression and secretion of TNF-α and IL-10 cytokines were analyzed by Real time PCR and sandwich ELISA, respectively. **Results:** Treatment of B-cells with different concentrations (10 and 20 µg/ml) of recombinant LPG3 led to significant increase of TNF-α secretion ($p<0.05$). mRNA expression of TNF-α in 10 µg/ml concentration of LPG3 was statistically significant ($p<0.05$). However, LPG-3 had no stimulatory effect on IL-10 production by B-cells. Increased expression of CD69 in harvested B-cells with 10 µg/ml of recombinant LPG3 was statistically significant ($p<0.05$). **Conclusion:** This study indicated that LPG3 could activate and stimulate B lymphocytes as an important acquired immune response and could be potentially considered as an effective adjuvant in vaccine developments.

9824P

Development of Recombinant Leishmania Major Expressing Green Fluorescent Protein

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Introduction: Leishmaniasis is an endemic disease. Hitherto, no effective vaccine has been made against leishmaniasis. The use of recombinant Leishmania species expressing fluorescent proteins is one of the methods to evaluate the effectiveness of anti-Leishmania drugs through measuring the parasite burden. **Materials and Methods:** The plasmid vector (pLexsy egfp-sat2) containing the coding sequence of EGFP protein was digested with SwaI enzyme, purified, and then integrated to 18s rRNA gene locus of L. major (MRHO-IR-75-ER) by electroporation. Transfected parasite was selected after addition of NTC antibiotic to medium culture. Integration of digested plasmid to the Leishmania genome was performed by PCR using specific primers for inserted sequence and

18s rRNA locus. Additionally, EGFP expression was directly evaluated in promastigotes, using an Epi-fluorescence microscope. In order to investigate fluorescent emission by recombinant parasite in lesions and lymph nodes, the recombinant parasites were inoculated to the footpad of BALB/c mice. After 35 days, the fluorescence intensity was evaluated at intervals of one-week using In-vivo imaging method. **Results:** PCR results confirmed the genomic integration. Both in-vitro and in-vivo studies indicated that EGFP expression was stable and traceable in infected mice. **Conclusion:** The results showed that expression of EGFP gene that was integrated to the parasite genome was stable. Additionally, the GFP protein was produced continuously, without requirement to any inducible expression system. The parasite can be used for assessing parasite burden in alive mice by using in-vivo imaging method.

10946P

Route of infection affects pathogenicity and visceral growth of Leishmania major in BALB/c mice

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Introduction: Leishmaniasis is a group of diseases caused by Leishmania parasite. Experimental models can be used for development of new methods of prevention and treatment for these diseases. Route of infection is one of the variables that have been reported to influence the immune responses as well as the disease outcome in experimental models of leishmaniasis. Aim of this research is to study the effect of infection route on the virulence of Leishmania (L.) parasite. **Material and Methods:** Low (103 parasites / mouse) or high (106 parasites / mouse) doses of L. major was injected subcutaneously into foot pad or intradermally into ear dermis of BALB/c mice .**Results:** Results showed that subcutaneous infection route has substantial differences with intradermal infection route which results in higher pathogenicity of L. major in BALB/c mice as assessed by lesion diameter, parasite load in the draining lymph node, and dissemination of the parasite to spleen. **Conclusion:** The different pathogenicity of L. major in subcutaneous in comparison to intradermal may be due to presence of different immunoregulatory mechanisms such as IL-10 and CD4+CD25+ TREG cells in these two infection routes.

11083P

An evaluation of the therapeutic effect of *Aloe vera* leaf exudate in sensitive BALB/c mice infected with *Leishmania major* (*in vivo*)

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Introduction: Leishmaniasis is caused by the intracellular parasite *Leishmania major* which is transmitted through the bite of the sand fly. The aim of the present study was to evaluate the probable effect of *Aloe Vera* leaf exudate to investigate the therapeutic efficacy of the extract on *L. major*- induced *leishmaniasis* in inbred BALB/c mice. **Material and Methods:** Four groups of mice were used in this study. In order to contaminate the mice with the parasitic agent, 0.1 ml of a solution containing 2×10^6 promastigotes of *L. major* in their stationary phase was

injected subcutaneously into the tail base of the mice using an insulin syringe. In order to investigate the therapeutic efficacy of the extract, various doses of an ointment prepared from the extract was applied onto the site of the lesions daily for a period of 4 weeks. The size of the lesions were measured and recorded at the end of each week. **Results:** The topical ointment prepared from the extract was effective in reducing the size of lesions with the best effects exerted by the 4% ointment. **Conclusion:** The results of the present study showed that topical ointment of the *Aloe Vera* leaf exudate can reduce the size of lesions caused by the parasite, *in vivo*. ***Keywords*: *Leishmania major*; *Aloe Vera* leaf exudate; ointment; lesion size**

11103P

Leshmania LPG3 activates Th1 response and IFN-γ cytokine production

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Introduction: Leishmania major is the main causal agent of Cutaneous Leishmaniasis (CL) that remains a serious public health concern in many tropical and subtropical countries. The use of a long-lasting protective vaccine against leishmaniasis has been highly expected. Lipophosphoglycan (LPG) is one of the important immune stimulating antigens on the surface of Leishmania promastigotes which was synthesized by LPG3. The aim of this study was to investigate the ability of recombinant LPG3 (rLPG3) to induce Th1 response in purified T lymphocytes. **Material and Methods:** In the present study, human T cells were purified from peripheral blood of 10 adult healthy subjects using Magnetic-activated cell sorting (MACS) technique. Subsequently, purified T cells were treated with recombinant LPG3 in different concentrations (2, 10 and 20 µgr/ml). Following 48 hours incubation, the expression level of γ-IFN was determined using Real Time PCR assay. **Results:** The results revealed that the recombinant LPG-3 could significantly increase the production of IFN-γ in purified human T cells in moderate and high concentration compared to control group ($p<0.05$). **Conclusion:** This study indicated that recombinant LPG-3 could effectively stimulate IFN-γ secretion and further investigations are needed for its anticipated adjuvant application against leishmania infection.

11316P

Purification of polyclonal antibody from dogs infected with Leishmania infantum

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Introduction: Visceral leishmaniasis is endemic in some area of Eastern Azarbaijan and Fars Province. The aim of present study is preparation and purification canine antibody specific Leishmania infantum for designing a diagnostic kit. **Material and methods:** In this study 10 dogs were infected with Leishmania infantum. The sera were collected and the anti-Leishmania was measured using IFA. The antibody was determined using polyacrylamide gel electrophoresis. Polyclonal antibody was purified through precipitation with ammonium sulfate solution and ion exchange chromatography. Purity of the fraction was confirmed by polyacrylamide gel electrophoresis in reduced condition (SDS-PAGE). The purified canine IgG was conjugated with FITC then reacted

with promastigote of Leishmania infantum. **Result:** The conjugated antibody indicated that the purity of produced IgG class was 95% .The canine FITC- conjugated antibody suitable for diagnostic kit for detection visceral leishmaniasis in dogs .**Conclusion:** In the present study, we found that the canine FITC-conjugated antibody reacted with promastigote L.infantum as secondary antibody.

12336P

Desinging an immunochromatographic test for rapid indentification of dog infected with Leishmania infantum using a purified 21kDa protein

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Introduction: Leishmania is one of the most important zoonotic diseases. Dogs, especially domestic dogs are the main reservoirs of Leishmania. While medical signs weren't been observed in 60% of dogs, so designing an immuno-chromatographic test was conducted to rapid indentification of dogs infected with Leishmania infantum. **Material and methods:** Leishmania infantum was cultured in HOMEM medium and lysate was provided from obtained cells. For separating the immunogen protein, the lysate was approached to serum of infected dogs, and immune complex was made by affinity chromatography, and separated. The product of chromatography was separated by SDS_PAGE, and then 21kDa antigen was cut from gel and purified. To confirm the purified protein, western blot technique was applied. For desinging an immuno-chromatographic test on strip, a 21kDa protein was used in first line at test station and also anti-protein A in second line as control. Gold nanoparticle was conjugated to protein A coated on glass pad. **Results:** The serum of infected dogs was added to the sample pad by binding the antibody to protein A at the surface of gold particle and also by reaction with 21kDa antigen in test line, the purple color was observed and control line became apparent by binding protein A to anti-protein A. **Conclusion:** Being rapid and cheap, this method doesn't need the specific equipment and expert, so it could be an appropriate method for determining leishmania infantum in endemic areas.

12349P

Protective effect of plasmid encoding GRA4 gene of Toxoplasma gondii and genetic adjuvant in BALB/c Mice

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Introduction: Sever damages of toxoplasmosis in immune compressive patient and fetus indicate the need of effective vaccine against this problem .One method to enhance the potency of DNA vaccine is genetic adjuvant. IL12 is extremely potent in enhancing cell mediate immunity and involved in differentiation of Th0 cells into Th1. GRA4 is dense granule antigen secreted from bradyzoite and tachyziote .This study evaluated the protective effect of plasmid encoding GRA4 gene of Toxoplasma gondii and genetic adjuvant in BALB/c mice. **Material and Methods:** Female BALB/c mice were divided into five groups (n=10 in each group) including three control groups (PBS, pc DNA3, pcIL12), and two experimental groups (pcGRA4, pc GRA4+pcIL12). Mice were immunized intramuscularly in days 0, 21, 42. In days 21, 42, 63 the mice were bleeding for antibody assay .Three weeks after last immunization, 5 mice of each groups were challenged with fatal RH strain of T.gondii. The survival time was

recorded daily. Seven weeks after last immunization, 5 mice of each groups were scarified and spleen lymphocytes were cultured with TLA. Supernatants were collected for cytokine assay. For lymphocyte proliferation assay MTT was used .**Results:** Mice immunized with (pcGRA4) and (pcGRA4+pcIL12) indicated significant low level of IL4 and high levels of IgG and INF-γ ($p \leq 0.05$). Comparing the controls suggested that Th1 immune response was active .The mean survival time for experimental and control groups were 10.5 and 8 days, respectively. **Conclusion:** This study indicated that immunization with plasmid encoding GRA4 and genetic adjuvant elicited partial protective effect against T.gondii. The presented results, provided a basis for further researches towards the use of multi-component DNA vaccines combined with cytokine plasmid and adjuvant .**Key words:** Toxoplasma gondii ,GRA4 gene, DNA vaccine ,Genetic adjuvant.

12364P

Therapeutic effect of Propolis extract on BALB/c infected with Leishmania major

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Introduction: Propolis is a natural resinous mixture produced by honeybee from substances collected from part of plants, buds, and exudate. Leishmaniasis is caused by the intracellular parasite Leishmania major which is transmitted through the bite of sandfly. The aim of this study was to evaluate the anti-leishmanial activity of Zagrosian propolis extract in vitro and in vivo. **Material and Methods:** The ethanolic extracts of propolis were prepared by using a modified technique described by Miorin et al. Increasing concentrations of Propolis Ethanolic Extract (EEP) were applied to a standard number of promastigotes. The number of promastigotes was recorded at 24, 48 and 72 hours post treatment and the parasites were then tested by counting and MTT for viability. Four groups of mice were used in this study. In order to contaminate the mice with the parasitic agent, 0.1 ml of a solution containing 2×106 promastigotes of L. major in their stationary phase was injected subcutaneously into the tail base of the mice using an insulin syringe. In order to investigate the therapeutic efficacy of the extract, various doses of an ointment prepared from the extract was applied onto the site of the lesions on a daily basis for a period of 4 weeks. The size of the lesions were measured and recorded at the end of each week. **Results:** The data obtained from counting of viable parasites and the MTT test indicated desirable effects of treatment with the extract on the promastigotes with the strong effects observed at doses of 37.5, 75, 150 and 300 µg/ml. The topical ointment prepared from the extract was effective in reducing the size of lesions with the best effects exerted by the 4% ointment. **Keywords:** Leishmania major; Ethanolic Extract of Propolis; MTT; lesion size

12389P

Evaluation of relationship between Sero-prevallance of IgG & IgM Specific –Parvovirus B19 Antibodies and behavior and environmental factors among childbearing Female Population in East Azarbaijan Area.

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Introduction: The human parvovirus B19 replicates in rapidly proliferating cells, such as erythroblast precursors. Clinical manifestations include erythema infectiosum (ie, fifth Disease), transient aplastic crisis, chronic red cell aplasia, myocarditis, arthropathy, and non-immune hydrops fetalis. Typically, erythema infectiosum presents with

mild and nonspecific symptoms .**Material & Methods:**In this cross- sectional study, totally 625 pregnant women were evaluated for IgG and IgM parvovirus specific antibodies using ELISA technique. They attended for prenatal care in health service centers associated to Alzahra Gynecology center of Tabriz University of Medical Sciences from April 2012 to May 2015.They referred 362 (57.92%), 143 (22.88%), 120 (19.2%) cases in first, second, and third trimesters respectively. In groups with recent infection (G+M+& G-M+), factors such as the number of children and their ages and civilization –education –occupation of mothers have been surveyed. All data were analyzed by statistical software SPSS version 15.0 (SPSS Inc, Chicago, USA). P values $\leq$ 0.05 were considered significant. **Results:** Totally 625 pregnant women were studied in five age groups (ranged by 4yr intervals 19-38) yr. sero-epidemiologic findings were analyzed based on serologic status of subjects, such as 432 G+M- (69.12%), 164 G-M- (26.24%), 26 G+M+(4.16%), 3 G-M+ (0.48 %). There were direct relation between infectivity of mothers and some behavior and environmental factors. **Conclusion:** Studies of sero-epidemiology of parvovirus B19 in several countries and Iran have shown that the majority of our child-bearing and pregnant women population had contacted with parvovirus B19, particularly, quality and environmental factors imposed an important role in epidemiology of parvovirus infection in pregnant women. **Keywords:** parvovirus B19- infection- female population

12399P

Western blotting analysis of gentamicin -attenuated *Leishmania major* using sera from volunteers vaccinated with attenuated *Leishmania major*

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Introduction: The gentamicin-attenuated *Leishmania major* (*L. major* H-line) has been established in the pressure of gentamicin. It has been reported that *L. major* H-line was safe and induced a cellular immunity in BALB/c mice which protected mice against wild-type parasites. In the present study the immunogenicity of the attenuated line of L. major was investigated using Western blotting. **Material & Methods:** Promastigotes of *L. major* H-line (MRHO/IR/75/ER) were cultured in HOMEM supplemented with 10% FCS and gentamicin at 20 ug/mL. Twenty seven volunteers were vaccinated subcutaneously with a single injection of *L. major* H-line. Two months after vaccination, the specific anti-*Leishmania* IgG antibody in the sera of vaccinated volunteers were measured using IFA. The immunogenicity of attenuated L. major was compared with *L. major* wild-type using Western blotting. **Results:** The presence of specific anti- *Leishmania* IgG antibody was determined in the sera of volunteers. Subsequently by Western blotting analysis, it was shown that in these sera a 28 KDa band was recognized **Just** from *L. major* wild-type (WT) but not from *L. major* H-line. **Conclusion:** Probably, 28kDa antigen of *L. major* H-line, has been changed in the *L. major* W , from structurally or expression levels and may therefore lack of parasite virulence.

Immunopharmacology & Medicinal Plants

Oral Presentations:

3472O

The effect of theophyllin and silymarin on TNF-α secretion from human basophils

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Introduction Human basophils play a key role in allergic diseases such as asthma and in a variety of immunological disorders. Basophil is a major source of cytokine. The aim of the present study was to examine the effects of Silymarin, and theophyllin on the secreted TNF-α, IL-4 from human basophlis in vitro. **Material &Methods:** Basophils were prepared by ficol gradients (purity, 10 ±3%, n=5). The release of TNF-α, il-4 was assessed and activated with either LPS and then treated with Silymarin and theophyllin for 4 or 24h. To measure TNF-α and IL-4 in supernatants, ELISA kits were employed. Results were analyzed statistically, by using ANOVA test. **Results:** This study showed that there is significant difference between silymarin and theophyllin on TNF-α, IL-4 secretion from human basophils (p=0. 003), and silymarin was an effective inhibitor of TNF-α and IL-4 secretion .**Conclusion:** Our data revealed that Silymarin could be a good candidate for anti-allergy therapy with superior efficacy and lesser toxicity compared to other drugs.

7527O

Immunosuppressive properties of the hydroalcoholic extract of Xanthium strumarium in wistar rat after challenge with REV-1 vaccine

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Introduction: *Xanthium strumarium*has has been used as a medicinal herb to alleviate many inflammatory ailments. Current survey was carried out to investigate the effects of hydroalcoholic extract of *X.strumarium* on immune system of the Wistar rats challenged with REV-1(a Brucella live vaccine).**Materials and Methods:** Twenty male Wistar rats were randomly grouped in two equal groups and immunized with Rev1 (0.1 ml Rev1+0.9 mlPBS) . Rats in the treatment group, orally received hydroalcoholic extract of the hydroalcholic and extract of *X. strumarium* (200

mg/kg) every day from the beginning of the study and continued for 2 weeks. Animals were bled 5 days after the last injection. Moreover, 48 h before bleeding time, Rev1 (0.05 ml Rev1+0.45 ml PBS) were injected into the left hind foot pad of Rats. The acquired immune responses against REV-1 were checked by sero-agglutination test and footpad thickness, respectively. Moreover, body weight, thymus index, spleen index and splenocytes proliferation rate, nitric oxide production, respiratory burst and phagocytosis were assumed in splenocytes. **Results:** The findings indicated that the extract significantly suppressed the levels of anti-Rev1 antibody, footpad thickness and Lymphocyte proliferation index in splenocytes. Furthermore, the level of respiratory burst and nitric oxide production of phagocytic cells of splenocyte population concurrent with spleen weight index were significantly decreased in the treatment group compared to control group. However, the body weight and thymus index didn't show any significant difference between groups. **Conclusion:** It seems that the hydroalcoholic extract of *X. strumarium*may could be used as a natural immunosuppressive compound. **Keywords:** *Xanthium strumarium*, Humoral immunity, Cellular immunity, innate immunity.

7704O

Effects of the aqueous extract of Cynodon dactylon in ameliorating Ulcerative Colitis

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Introduction: *Cynodon dactylon* is traditionally used to treat painful and inflammatory conditions. The present study was done to evaluate the effects of aqueous extract of *C. dactylon* in ameliorating ulcerative colitis induced by acetic acid in rats. **Materials and Methods:** Thirty male wistar rat were randomly allocated in 4 equal group and Ulcerative colitis was induced by intra-rectal Infusion of 2 mL of 4% acetic acid. Different groups orally received 200, 300 or 400 mg kg^{-1} of aqueous extract of *C. dactylon* (treatment groups), 1 mg kg^{-1} dexamethasone (positive control group) or normal saline (negative control group) from two hours before the induction of colitis and continued for four days. The rats were sacrificed 24 hours after the last treatment and the colon tissues were studied for the levels of macroscopic lesions, myeloperoxidase, TNF-α and IL-6. **Results:** In addition to macroscopic lesions, the Myeloperoxidase activity and the levels TNF-α, without any significant difference, were markedly regressed in rat received C. *dactylon* and dexamethasone compared to rat received normal saline. The levels of IL-6 were significantly decreased in treatment group, however, the level of this mediator was higher in the *C. dactylon* received group than in the dexamethasone group ($P < 0.05$). The effects of *C. dactylon* were not in a dose dependent manner. **Conclusion:** The extract of *C. dactylon* may alleviate the induced ulcerative colitis in rats. Therefore, it may be a suitable natural source for further evaluations in clinical trials on patients with irritable bowel disease. **Keywords:** Ulcerative colitis, *Cynodon dactylon*, Inflammation, Acetic acid-induced colitis

9720O

Effects of Thyme components on Cell-mediated immunity in ovalbumin immunized mice

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Introduction: Thymol and carvacrol, two main components of thyme have revealed several valuable effects on immune system. The aim of the present study was to evaluate the effects of these components on T helper cell responses and their subsets in mice immunized with ovalbumin. **Materials and Methods:** The effect of components on *in vivo* specific immune response was evaluated by delayed type hypersensitivity (DTH) and splenocyte proliferative response by Brdu assay. The gene expression of cytokines as well as key transcription factors involved in T cell subsets differentiation in mice splenocytes were examined by real time PCR. The cytokines production in splenocyte culture supernatants and in mice sera were measured by enzyme-linked immunosorbent assay (ELISA). **Results:** Treatment of mice with thymol reduced DTH response to 34.1% of untreated mice. Both components diminished splenocyte proliferation to nearly 65-72% of control ($p<0.01$). These components decreased cytokine levels of Th1 (IL-2, IFN-γ), Th2 (IL-4) and Th17 (IL-17A) subsets in both splenocytes cultures and mice sera but increased IL-10 and TGF-β. Treatment of immunized mice with components significantly reduced the gene expression of specific transcription factors of T helper subsets including T bet, GATA-3 and ROR-γc compared to untreated ovalbumin-immunized mice. **Conclusion:** Carvacrol and thymol could suppress the antigen specific immune responses by reducing the T helper cells related cytokines and specific transcription factors suggesting their potential for modulating destructive immune responses arising from T cell over-activation. **Keywords** Thymol, carvacrol, Ovalbumin immunization, immunomodulation, T helper subset

9730O

The effect of Nigella Sativa essential oil on enhancement of the NK activity in mice with BCL-1 induced cancer

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Introduction: *Nigella sativa* L., commonly named black seed, has long been used in traditional medicine to treat different types of cancers. In this study, the potential immune-modulatory and anti-leukemic effects of orally administered *N. sativa* essential oil (NSEO) were investigated in mice with BCL-1- induced leukaemia. **Materials and Methods:** 15 female BALB/c mice were divided into three groups (n = 5). Two groups were received 5×10^6 BCL-1 cells and treated with either NSEO (treated group) or solvent (untreated group) for 3 weeks. The third group received only NSEO (black seed control group). The blood leukocytes were counted in blood smear. NK activity in splenocytes against YAC-1tumor cells was assessed by LDH release assay. **Results:** It was demonstrated that the volatile oil of *N. sativa* significantly enhanced NK activity in treated group at both 5:1and 10:1 E:T ratio ($P < 0.05$).The results showed that the percent of lymphocytes in untreated group significantly augmented compared to two other groups and treatment with NSEO decreased the number of cancer lymphocytes in peripheral blood. **Conclusion:** The results of the current study indicated that part of anticancer mechanism of NSEO in vivo could be attributed to its effect on NK activity and it could be considered as a promising agent for the treatment of cancers.

9809O

Satureja hortensis induces cell cycle arrest and cell death in leukemia cell lines

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Introduction: *Satureja hortensis* known as "Marzeh" in Persian is being used for various purposes in folk medicine. This study was designed to investigate the effect of different extracts of this plant on growth, cell death and cell cycle of leukemia cells. **Materials and Methods:** The growth inhibitory effect of different extracts from *S. hortensis* on K562 and Jurkat leukemia cell lines was determined by MTT assay. The most effective extracts were analyzed by flow cytometry for apoptosis induction and cell cycle changes. Caspase-3 activation level was measured by a colorimetric assay. **Results:** Various extracts of *S. hortensis* showed growth inhibitory effects on leukemia cells, among which two hexane and dichloromethane extracts with IC_{50} values of 47.8-32.1 µg/mL for K562 and 44.3-45.7 µg/mL on Jurkat cells were the most effective. According to annexin V/PI staining, these extracts at 50µg/mL were able to induce apoptosis in K562 (73-96%) and Jurkat cells (78-94%) 48h after treatment. Cell cycle analysis showed an increased accumulation of cells in sub-G1 and G0-G1 arrest in cells treated with 25-50µg/mL of the extracts. The caspase-3 activity levels were increased 24 h after treatment of K562 (3.5-2.5 fold) and Jurkat cells (3.1-2.7 fold of untreated cells) with 50µg/mL of the extracts. **Conclusion:** These data indicated that the two *S. hortensis* extracts had the capacity of inducing cell cycle changes and death in leukemia cells, therefore they might be good candidates for more studies with regard to their possible therapeutic usefulness in leukemia.

11181O

The Effect of Echinops Species Extracts on Peripheral Blood Mononuclear Cells Proliferation and Cytokine Secretion

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Introduction: The immuno-modulatory effects of some *Echinops* species on human peripheral blood mononuclear cells (PBMCs) proliferation and interferon-gamma (IFN-γ) and Interleukin (IL)-4 secretions were studied. **Materials and Methods:** Different parts of the *Echinops ilicifolius*, *Echinops jesdianus* and *Echinops lasiolepis* were collected and different concentrations of 0.1, 1, 10, 100 and 200 µg/ml were prepared. PBMCs were cultured into the 96-well micro-culture plate with 10 µl of extract and with different concentrations at a final volume of 100 µl. The proliferation of PBMCs was determined using BrdU cell proliferation kit. IFN-γ and IL-4 concentrations were measured using ELISA method. **Results:** The aerial extract of *Echinops ilicifolius* in 0.1, 1 and 200 µg/ml concentrations ($p<0.05$) showed significantly an inhibitory effect on PBMCs proliferation. Root extract of *Echinops ilicifolius* in all concentration ($p<0.05$) significantly showed an inhibitory effect on PBMCs proliferation. The effect of aerial extract of *Echinops jesdianus* on proliferation of PBMCs was significant in concentration of 100 µg/ml ($p=0.003$). The root extract of *Echinops jesdianus* at 100 µg/ml concentration significantly induced cell proliferation ($p=0.001$). *Echinops lasiolepsis* root extract in all concentrations significantly had an inducing effect on PBMCs proliferation ($p<0.05$). The secretion of IFN-γ compared to the control group in all extract concentrations was not significant but IL-4 level increased compared to the controls. **Conclusion:** The aerial and root extracts of different species of *Echinops* showed contradictory effect on human PBMCs proliferation and cytokine secretion. The extracts may have immuno-modulatory effects. **Keywords:** *Echinops*, IFN-gamma, IL-4, PBMCs

7682O

Stimulation of cholinergic anti-inflammatory system by nicotine alleviate autoimmune encephalomyelitis via change in T cell polarization

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Introduction: Recently, it has been demonstrated the signaling by the α7 nicotinic acetylcholine receptors producing the anti-inflammatory condition in both macrophages and T cells. Moreover, activation of macrophages and T cells have a very important role in multiple sclerosis (MS). The present study was conducted to evaluate the therapeutic effect of nicotine, on experimental autoimmune encephalomyelitis (EAE), an animal model of MS, and its effects on T-helper cells responses. **Materials and Methods:** EAE was induced by guinea pig spinal cord homogenate and complete Freund's adjuvant in Wistar rats. Animals were allocated in two therapeutic groups (n=7 per group). Treatment with nicotine (2.5 mg/kg-daily) was started in treatment group at day 12 when the treatment group developed a disability score. EAE control received vehicle alone with same schedule. Signs of disease were recorded daily until the day 36 when mice were sacrificed. Splenocytes were checked for proliferation by MTT test and cytokine production by ELISA. **Results:** Nicotine administration after the occurrence of clinical symptoms significantly regressed the clinical features of EAE. Nicotine significantly inhibited the production of pro-inflammatory IL-17 as well as IFN-γ. The levels of anti-inflammatory IL-10 were not altered significantly. However, IFN-γ to IL-10 and IL-17 to IL-10 ratios decreased significantly. Lymphocyte proliferation was significantly decreased in treatment group compared to control group. **Conclusion:** This pharmacological approach may be as a useful strategy to control MS. **Keywords** Multiple sclerosis; Experimental autoimmune encephalomyelitis; Nicotine; lymphocyte response.

7706O

Antitumor and Apoptosis-inducing Effects of the Hexane Extracts from Three Native Euphorbia Plants

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Introduction: Several Euphorbia plants (Euphorbiaceae) have shown antitumor activity.The present study aimed at investigating the growth inhibitory and apoptotic effects of three native Euphorbia species (*E. microciadia*, *E. heteradenia*and *E. osyridea*) on various cell lines. **Materials and Methods:** The hexane extract of the plants was prepared and examined for growth inhibitory effects on solid tumor and leukemia cell lines. Their apoptosis-inducing effect was determined by measuring changes in caspase3 activitybycolorimetric assay and by analysis of the expression of apoptosis-related genes by Real-time PCR. **Results:** All three plants had the capacity to inhibit the growth of cells. Determination of the inhibitory concentration 50% (IC_{50}) of the extract showed that *E. heteradenia* extract with IC_{50} value of 26.91µg/ml for K562 leukemia cells had a greater inhibitory impact compared to *E. microciadia* with IC_{50} value of39.81 µg/ml for HeLa cells and *E. osyridea* extract with IC_{50} of 83.17 µg/ml for Fen bladder carcinoma. We conducted caspase 3 colorimetric assay and Real- time PCR to determine if the observed

growth inhibitory effects of the extracts were due to apoptosis induction. The effectiveness of all Euphorbia species against tumor cell lines was found to be through caspase-dependent apoptosis. Also all of the extracts suppressed antiapoptotic Bcl-2 gene expression and increased Bax and Fas expressions**. Conclusion:** Euphorbia extracts could induce apoptosis in various tumor cell lines through both extrinsic and intrinsic pathways of apoptosis. Additional studies are necessary regarding their beneficial effects as potential anticancer and antileukemia agents. **Key words:** Euphorbia Species, Apoptosis, Cancer Cell lines

8713O

Evaluation of the anti-inflammatory effects of Echium amoenum extract on J774.1 macrophage cell line

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Introduction: *Echiumamoenum* is a plant belongs to Boraginaceae *family* that grows in most parts of Europe and in northern parts of Iran.This plant is an important Iranian traditional remedy. In this report, the hexane extract of *Echium amoenum* was tested for its possible anti-inflammatory activity. **Materials and Methods:** The extract was prepared from the flower heads of the plant. Various extract concentrations (1-100 µg/mL) were examined for their possible cytotoxicity on J774.1 macrophage cell line by MTT assay. Lipopolysaccharide-stimulated macrophages were treated with the extract and then nitric oxide (NO) production was measured using Griss solution.The gene expression of various pro-inflammatory cytokines and mediators including inducible nitric oxide synthase (iNOS), Cyclooxygenase 2 (COX2), tumor necrosis factor (TNF)-α, and interleukin (IL-1β)were examined by real time-PCR. **Results:** MTT assay showed no inhibitory effect in different concentrations of the extract on macrophages.The levels of NO production showed a decrease in the cells treated with 25-100µg/mL of the extract compared to untreated cells. Real time-PCR analysis indicated a reduced levels ofIL1-β, TNF-α and COX2 gene expressions in the presence of the extract. The gene expression levels of iNOS also showed a decrease in all concentrations. **Conclusion:** Echium amoenum extract was able to decrease the release of NO and expression of iNOS as important mediators of inflammatory processes. The diminished gene expression levels of TNF-α, IL-1β, and COX2 as the key mediators of inflammation suggested this extract for more studies because of its potential anti-inflammatory therapeutic effects.

9721O

Evaluation of anti-inflammatory properties of thyme components on adjutant-induced inflammation in rats

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Introduction: Thymol and carvacrol are two important components of thyme. This study has sought to investigate the in vivo anti-inflammatory effects of these components by adjuvant-induced inflammation in rats. **Materials and Methods:** Inflammation was induced in the hind paw of rats by 0.1 ml injection of Complete Freund's adjuvant (CFA). The rats received components (100mg/kg) by gavages two hour before CFA injection and daily after induction of inflammation. Rat paw edema was measured daily by a digital caliper. After 72 hour of inflammation

induction, sera were collected and inflamed tissue extracts prepared for cytokine assay by ELISA. **Results:** The components significantly decreased inflammatory score from 2.6±0.8 in untreated rats to 1.5±0.4 in thymol-treated (p=0.001) and to 1.8±0.3 in carvacrol-treated (p=0.01) rats. Paw edema also decreased from 7.8±1.7 in untreated rats to 6.4±0.4 mm (p=0.01, thymol) and 6.5±0.1mm (p=0.05, carvacrol). In serum and tissue evaluation of the inflammatory cytokines levels, the following results compared to the control group were obtained: a decrease in serum IL-1β levels by thymol and decrease of TNF-α serum and tissue levels by both components (p=0.05). Thymol reduced IL-17A inflammatory cytokine from 57.3±8.9 to 21.2±0.4 pg/ml in sera and from 148.4±13.4 to 90.1±18.9 pg/ml in inflamed tissues. Similarly, carvacrol decreased the level of this cytokine in mice sera and tissues. **Conclusion:** Carvacrol and thymol could becontributed to modulation of inflammatory cytokines and therefore they might be useful targets for reducing the severity of inflammatory reactions. **Keywords:** Thymol, carvacrol, in vivo anti-inflammatory effects

10871O

Establishment of PKA test to evaluate immunoglobulin products

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Introduction: Prekallikrein activator (PKA) is a contaminant in intravenous immunoglobulin (IVIG) prepared from fractionation of plasma. Adverse events in patients may occur where IVIG are contaminated with significant levels of PKA. Therefore, measurement of PKA in IVIG is one of the pharmacopeia (EP) requirements. Since this test has not been conducted and set up in Iran. Test launch is considered, and then PKA was assessed and compared with commercial IVIG in two different IVIG preparations. **Materials and Methods:** The fraction containing IgG in plasma fractionation in two different processes precipitated as fraction-I+II+III or fraction-I and then fraction-II+III and purification were performed by chromatography for both.In order to purify the substrate prekallikrein from human plasma, human blood was taken and ion exchanged chromatography was performed on dialysis plasma on the DEAE cellulose. Finally PKA test was conducted by an enzymatic assay. Prekallikreien converted to kallikreien by PKA and the latter has the ability to separate paranitroaniline (PNA) in substrate S-2302 and production of dye was read at 405 nm. **Results:** The standard curve prepared in different concentrations of WHO International Standard. Based on the standard curve PKA calculated in IVIG of two different production methods in comparison with commercial IVIG. These characteristics satisfied the requirements of the European Pharmacopeia. **Conclusion:** Determination of PKA is one of the important aspects of immunoglobulin products. It is a mandatory test according to the European Pharmacopoeia (EP, 2008) to release these products. Therefore the test was properly set up and PKA content in IVIG was evaluated. **Keywords**: Prekallikrein activator, intravenous immunoglobulin, Plasma

11066O

Effect of Dracocephalumkotschyi extract on cytokines and transcription factors regulating inflammationin macrophages

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Introduction: Deracocephalum kotschyiis traditionally are consumed as antispasmodic, analgesic and anti-inflammatory to treat rheumatoid diseases. The current study was aimed at determining the anti-inflammatory properties of ethyl acetate extract of this plant in macrophages. **Materials and Methods:** Lipopolysaccharide (LPS)-stimulated J774.1 mouse macrophages were examined by real time-PCR for interleukin (IL)-1 β, tumor necrosis factor (TNF- α), inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2*)* gene expression in the presence of the extract. The levels of phosphorylated stress-activated protein kinases (SAPKs)/c-Jun N-terminal kinase (SAPK/JNK), signal transducer and activator of transcription (STAT-3), p38, IκB-α, nuclear factor (NF)-κB p65 and dephosphorylated form of NF-κB p65, as well as total levels of IL-1 β and TNF-α were determined by means of ELISA. Nitric oxide (NO) production was measured by Griess reagent. **Results:** *Deracocephalum kotschyi* extract significantly reduced both IL-1β and TNF-α at the protein and mRNA levels. This extract also reduced iNOS and COX-2gene expressions. Treatment of macrophages with the extract caused significant decrease in the cytoplasmic levels of NFκB-p65 ($p<0.001$), phospho-SAPK/JNK ($p<0.01$) and phospho-STAT3 ($p<0.001$). No significant change in phospho-IκB level was observed, however cells treated with 10 µg/ml of extract showed decreased levels of phospho-p38 ($p<0.05$) and NF-κB phospho-p65 ($p<0.05$).**Conclusion:** The capacity of *Deracocephalum kotschyi* extract in reducing transcription factors signaling and various inflammatory cytokines and mediators levels suggested that this plant might have beneficial effects on inflammatory diseases process.**Keywords** *Deracocephalumkotschyi*, Inflammations, transcription factors

11273O

Cell cycle arrest and Induction of apoptosis by Scrophularia megalantha in human leukemia cell line

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Introduction: *Scrophularia megalantha* 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 megalantha* (*S. megalantha*) extract on K562 human leukemia cell line (CML). **Materials and 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 K562 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. megalantha* extract. The treatment with extract significantly showed significant cytotoxicity effect on tumor cell line. In addition, flow cytometry analysis indicated that *S. megalantha* extract induced cell cycle arrest in G2/M phase and apoptosis on tumor cell. **Conclusion:** The results of this study indicated that *S. megalantha* extract could inhibit leukemia cell growth through inducing G2/M phase arrest and cell apoptosis. **Keywords** *Scrophularia megalantha,* cell cycle, apoptosis, leukemia cell line

Poster Presentations:

6513P

Effect of Hydroalcoholic Extract of Capparis Spinosa Fruit on Blood Sugar and Lipid Profil of Diabetic and normal Rats

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Introduction: Diabetes mellitus is a common disorder of endocrine glands worldwide. Caper as a medicinal plant has anti-oxidant properties and has been used traditionally to cure diabetes. The aim of present research was to evaluate the effect of 200 and 800 mg/kg of caper fruit extract on blood sugar, glycated hemoglobin and lipid profile in diabetic and normal male rats. **Materials & Methods:** In this experimental study 60 rats were divided into 6 groups randomly, in which three diabetic groups received distilled water (control) and 200 and 800 mg/kg caper fruit extract respectively and three normal groups were treated as diabetic groups. **Results:** The blood sugar decreased in all groups receiving fruit extract compare to control groups and the decrease in blood sugar was dose dependent. Blood triglycerides decreased in all diabetic groups receiving extract compare to control but in normal rats the changes were not significant. **Conclusion:** The results of present study showed that consumption of Caper fruit extract lead to a significant decrease in blood sugar and also a considerable decrease in blood triglycerides in diabetic rats, therefore it seems that Caper fruit consumption may has beneficial effects on blood sugar and lipid profile. Keywords: Hydroalcoholic Extract of Caper fruit, Blood Sugar and Lipid Profiles, HbA1c

7547P

Immunomostimulant properties of the aqueous extract of Ziziphusjujuba(jujube)

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Introduction: *Ziziphusjujuba* (jujube) is used in traditional medicine to alleviate stress. Moreover, the anti-inflammatory effects of jujube has been previously reported. This study was conducted to check the immuno-modulatory properties of the aqueous extract of jujube in NMRI-mice challenged with sheep red blood cells (SRBCs). **Materials and Methods:** The study population consisted of 14 mice that randomly divided into two equal groups and immunized with SRBC (1×10^9). Mice in the treatment group orally received 400mg/kg of aqueous extract of Z.jujuba every day from the beginning of the study for 2 weeks. Specific humoral and cellular immunity, susceptibility of macrophages in respiratory burst and phagocytic population of blood leukocytes were measured. **Results:** Attained data indicated a significant increase in the level of anti-SRBC antibody and the level of cellular immunity in treatment group compared to control group. Moreover, the phagocytosis and the level of respiratory burst of the phagocytic population of blood leukocytes was significantly increased in the treatment groups compared to blood cells from control mice. **Conclusion:** This data suggested that the jujube may be used as a natural source for purposes of stimulating the immune system.**Keywords:** *Ziziphusjujuba*, Humoral immunity, Cellular immunity, innate immunity.

7548P

Immunomodulatory properties of the aqueous extract of Lawsonia inermis(Henna)

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Introduction: Henna (*Lawsonia inermis*) is a traditional cosmetic agent and is used worldwide. Also, it is applied in the body on lesions in the treatment of seborrheic dermatitis or fungal infections. This study was set out to investigate the immuno-modulatory effect of the aqueous extract of Henna in NMRI-mice challenged with sheep red blood cells (SRBCs). **Materials and Methods:** The study population consisted of 14 mice that randomly divided into two equal groups and immunized with SRBC (1×10^9). Mice in the treatment group orally received 100mg/kg of aqueous extract of henna every day from the beginning of the study for 2 weeks. Specific humoral and cellular immunity, susceptibility of macrophages in respiratory burst and phagocytic population of blood leukocytes were measured. **Results:** The findings indicated a significant increase in the level of anti-SRBC antibody without any change in the level of cellular immunity in treatment group compared to control group. Moreover, without any advantage in phagocytosis, the level of respiratory burst of the phagocytic population of blood leukocytes was significantly increased in the treatment groups compared to blood cells from control mice. **Conclusion:** This data suggested that the *Lawsonia inermis* may be used as a natural source for purposes of modulating the immune system. **Keywords** *Lawsonia inermis*, Humoral immunity, Cellular immunity, innate immunity.

7558P

Hydro-alcoholic extract of Carumcarvi can modulate immunity responses

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Introduction: *Carumcarvi* (Caraway) is a medicinal herb with anti-inflammatory and anti-oxidant properties. The aim of the current study was to evaluate the probable immunomodulatory effect of Hydro-alcoholic extract of

*C.carvi*in in wistar rats challenged with sheep red blood cell (sRBC).**Materials and Methods:** Ten wistar rats randomly placed in two equal groups. Mice were intra-peritoneally immunized twice with one weak interval by 1×10^9 SRBCs. Rats were bled 5 days after last immunization. Moreover, 48 h before bleeding time, 1×10^9 SRBCs were injected into the left hind foot pad of rat. Crude hydro-alcoholic extract of Caraway was orally administered to treatment group in a daily doses of 100 mg/kg from the beginning of the study and continued for 2 weeks. The levels of anti-SRBC antibody and the specific cellular immune responses were measured by micro-hemagglutination test and Footpad thickness, respectively. Moreover, the phagocytic population of blood leukocytes were checked for phagocytosis by neutral red uptake and respiratory burst by NBT reduction assay. **Results:** Rats in treatment group showed a significant increase in the levels of anti-sRBC antibody concurrent with a significant decrease in the level of cellular immunity (footpad thickness). **Conclusion:** It seems that the hydro-alcoholic extract of carumcarvi (caraway) may be used as a natural compound to modulate the immune responses. The phagocytosis and the level of respiratory burst of the phagocytic population of blood leukocytes were significantly increased in the treatment groups compared to blood cells from control Rat.P<0/05. **Keywords :** *carumcarvi*, *caraway*, Humoral immunity, Cellular immunity, innate immunity.

7563P

Evaluation of FoeniculumVulgareextracts (aqueous – ethanol - acetone) in preventing migration of tachyzoites of Toxoplasma gondii to lymphoidorgans in murine model

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Introduction: Toxoplasma gondii is one the most important apicomplexan parasite of humans and other warm-blooded animals. **Materials and Methods:** The effect of FoeniculumVulgare extracts (aqueous – ethanol - acetone) on tachyzoites of Toxoplasma gondii have been studied in male BALB/c mice. A total of 70 BALB/c mice (control &experiment) were included, and 10^4 organisms of the RH strain Toxoplasma gondii were given intraperitonealy to each mouse. Foeniculum Vulgare extracts (Aqueous– ethanol - acetone) were administered in 7 groups. All of the experimental mice were given extracts intraperitonealy with 100or 500 µl/kg/day single dose 3 hours after infection. **Results:** One hundred percent of mice survived with all of used doses of Foeniculum Vulgare extracts (Aqueous– ethanol – acetone) at 5 days after infection but one hundred percent of positive control mice died (P<0.05). In comparing groups, tachyzoites of Toxoplasma in the spleen disappeared in 500 µl/kg/day of acetone extract (40%) group (P<0.00I). **Conclusion:** The results showed that Foeniculum Vulgare extracts (Aqueous– ethanol - acetone) are effective on Toxoplasma tachyzoites in mice. In comparing the control group with all experimental groups, number of Toxoplasma tachyzoites in spleen (P<0.001) and liver (P<0.05) were significant and found to be effective in the treatment and prevent migration of Toxoplasma gondii tachyzoites to lymphoid organs in murine Toxoplasmosis.

7593P

Acquired immunity altered by hydro-alchoholic extract of Medicago sativa

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Introduction: Alfalfa (*Medicago sativa*) is a perennial flowering plant with potent antioxidant properties. Current survey was designed to evaluate the probable immuno-modulatory effect of hydro-alcoholic extract of Alfalfain in wistar rats after challenging with sheep red blood cells (sRBC). **Materials and Methods:** Ten wistar rats randomly placed in two equal groups (treatment and control). Rats were intra-peritoneally immunized twice with one weak interval by 1×10^9 SRBCs. They were bled 5 days after last immunization. 1×10^9 SRBCs were injected into their left hind foot pad, 48h before bleeding. Crude hydro-alcoholic extract of Alfalfa was administered to treatment group per os in daily doses of 100 mg/kg from the beginning of the study and continued for 2 weeks. The levels of anti-SRBC antibody and the specific cellular immune responses were measured by micro-hemagglutination test and Footpad thickness, respectively. Moreover, the phagocytic population of blood leukocytes were checked for phagocytosis and respiratory burst. **Results:** The findings indicated that the extract significantly increased the levels of anti-aRBC antibody. Conversely, the level of cellular immunity (footpad thickness) significantly was suppressed in treatment group. Nevertheless, the phagocytosis and respiratory burst of the phagocytic population of blood leukocytes didn't show any significant difference between groups. **Conclusion:** It seems that the hydro-alcoholic extract of Alfalfa could be used as a natural compound to modulate the acquired immunity. The phagocytosis and the level of respiratory burst of the phagocytic population of blood leukocytes were significantly increased in the treatment groups compared to blood cells from control Rat.P<0/05. **Keywords :**Humoral immunity, Cellular immunity, innate immunity.

7652P

Topical administration of Lawsonia inermis hydroethanolic extract improve wound healing process, via Macrophage infiltration

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Introduction: Lawsonia inermis (henna) is one of the most effective medicinal plants. On the other hand, the Macrophage has been shown to play a critical role in the wound healing process. In this study, the wound healing activities of hydroethanolic extract of ***Lawsonia*** on experimentally induced excision wounds were investigated in rats. **Materials and Methods:** For this goal sixty male Wistar rats were used. One circular surgical wound with 1/5 cm^2 were performed on the back of each animal. Then, the rats were divided into four groups (control, 1.5%, 3% and 5%) each with 15 rats. Wound healing activity was performed by histological studies specially Immuno-histochemical staining CD68 for macrophages on the end of 3^{th}, 7^{th} and 14^{th} days after surgery. **Results:** All therapeutic dose of *Lawsonia inermis* hydroethanolic extract, have been promoted significantly ($P < 0.05$) and increased the macrophages infiltration to the wound site, in treatment groups compared to control group. Reducing the number of PMN, increasing the Mononuclear Cells, fibroblast cell migration and re-epithelialization in all treatment groups were observed. **Conclusion:** Regarding these effects it was concluded that *Lawsonia inermis* hydroethanolic extract could be useful for healing the wounds and reducing inflammation. **Keywords:** *Lawsonia inermis*, Macrophages, wound healing, hydroethanolic extract

7654P

Cytotoxic and anti-angiogenic effect of Iranian snake venom (Macrovipera lebetina) on human endothelial cells

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Introduction: Today many researchers have been focused on development of novel therapeutics from natural sources. Snake venoms contain many proteins and peptides with anti-cancer activity. *Macrovipera lebetina* is one of the poisonous Iranian snakes. Analysis of the anti-cancer and angiogenesis effect of *Macrovipera lebetina* venom on human endothelial cells was the main aim of this study. **Materials and Methods:** Snake venom of *Macrovipera lebetina* was purified by fast protein liquid chromatography on sephacryl S-200 HR column. The fractions were collected and evaluated by SDS-PAGE analysis. Human endothelial cells were isolated from human umbilical cord vein. MTT assay and matrigel tube formation assay were performed to investigate the cytotoxic and anti-angiogenic effect of crude venom and fractions on human endothelial cells. **Results:** The results suggested five fractions in fast protein liquid chromatography. *Macrovipera lebetina* crude venoms and fractions showed cytotoxic effect on human endothelial cells in dose and time dependent manner. Higher cytotoxic effect was related to fraction 2 and 5. *Macrovipera lebetina* venom inhibited tube-like structures of human endothelial cells in matrigel assay as compared to control group. **Conclusion:** Because of the potent cytotoxic and anti-angiogenic effect of *Macrovipera lebetina* on human endothelial cells, it could be promising tools for further analysis as anti-cancer therapeutic development.
Keywords: snake venom, cytotoxicity, *Macrovipera lebetina*, cancer

7661P

In Vitro Cytotoxic Effects of Cuscuta epithymum Whole Extract on Human Acute Promyeloblastic Leukemia Cell Line

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Introduction: One of the major paths for drug development is the study of bioactivities of natural products. Therefore, the aim of this study was to compare the cytotoxic effects of aqueous extract of whole *Cuscuta epithymum*, which is a traditional medicinal herb commonly used in Iran and other oriental countries, on the human Acute Promyeloblastic leukemia (NB4) and another human lymphocyte Jurkat (JM) cell lines.**Materials and Methods:** *In vitro* cytotoxic screening with various concentrations (200-12.5 µg/ml) of the extract was performed using methyl tetrazolium bromide test (MTT) three times (24, 48, and 72 h).**Results:**The minimum effective concentration of the plant extract was 12.5 µg/ml, and increasing the dose to 200 µM induced increasingly stronger effects. The inhibitory concentration 50% (IC50) of the extract against NB4 was about 69, 57 µg/ml in 24 and 48 hours and 33 µg/ml in 72 hrs. In contrast, the extract did not have any cytotoxic effect on JM cells at these doses. **Conclusion:** The findings of the present study suggested that *C.epithymum* is toxic against NB4 tumor cells. Whether or not such effects can be employed for the treatment of such tumors, must await future studies.

7662P

In vitro effect of Elaeagnus angustifolia fruit on production of some inflammatory cytokines

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Introduction: *Elaeagnus angustifolia* in Iranian traditional medicine has been used for treatment of patients with peptic ulcer. A major application of *E. angustifolia* has been as anti-inflammatory. Inflammation is a protective nonspecific immune response that includes some inflammatory mediators release such as IL-1 TNF-β and PGE2. Due to the lack of scientific research about the anti-inflammatory properties of *E. angustifolia*. The effect of *E. angustifolia* aqueous and alcoholic extract on these inflammatory mediators was examined. **Materials and Methods:** Aqueous and alcoholic extracts of outer and inner layers of the fruit of the plant were prepared and then various concentrations (0, 1, 10, 25, 50, 100, 250, 500, 1000, µg/ml, 2, 5, 25, mg/ml) of the extracts were selected and added to the whole blood cell cultures. The cells were incubated in Co2 incubator at 37°c for 24 hours and centrifuged, separated supernatant were assessed for cytokine levels by Enzyme-linked immuno-sorbent assay (ELISA). Data were analyzed using analysis variance Test.**Results:** Different concentrations of aqueous and alcoholic endocarp and mesocarp of *E. angustifolia* did not affect production of IL-1 TNF-β. Only in concentrations of 5000 and 25000 micrograms an increase in the amount of PGE2 was found. **Conclusion:** Due to increased levels of PGE2 and protective effects of PGE2 in ulcer, it seems that at high concentration of aqueous and alcoholic extraction of *E. angustifolia* could have protective effect on ulcer.

7670P

In vitro Cytotoxicity of Iranian Cobra crude venom and fractions on human endothelial cells

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Introduction: Snake bite was referred as one of the most important public health concerns world wild. Respiratory and cardiac problems and destruction of endothelium system occurs as a result of snake bite. Snake venoms consist of various molecules with physiological and pharmacological properties. Evaluation the cytotoxic effect of Iranian Cobra venom on human endothelial cell was the main aim of current study. **Materials and Methods:** Cobra crude venom was fractionated by fast protein liquid chromatography using G-75 column. Purity of crude venom and each fraction was monitored by SDS-PAGE. Cytotoxicity of Cobra crude venom and fractions on human endothelial cells was evaluated by MTT assay (3, 4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide). **Results:** Fast protein liquid chromatography results revealed eight individual fractions. Cobra crude venom and fractions showed dose-dependent cytotoxic effect on human endothelial cell. Higher cytotoxicity was related to crude venom (IC50= 6µg/ml), fraction 6 (IC50=7µg/ml) and fraction 7 (IC50=6µg/ml). **Conclusion:** Achieved results indicated the cytotoxic effect of Iranian Cobra venom on human endothelial cell and represented as a potential for further cytotoxicity analysis studies to drug discovery. **Keywords:** cytotoxicity, snake venom, endothelial cell.

7691P

Hydroalcoholic extract of saffron (Crocus sativus) improves the histopathological lesions and biochemical profile of blood in experimental autoimmune diabetes

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Introduction:Multiple low doses of Streptozotocin (STZ) can induce autoimmune diabetes in C57bl/6 mice with a similar appearance in human type I diabetes.This study was conducted to investigate the effect of hydro-alcoholic extract of saffron (*Crocus sativus*) on the histo-pathological lesions and biochemical profile of blood in experimental autoimmune diabetes in C57bl/6 mice.**Materials and Methods:** After stabilization of diabetes, mice were orally treated with hydroalcoholic extract of saffron (500 mg/Kg) for 3 constitutive weeks. The levels of blood sugar, triglycerides and cholesterol of mice were recorded at the first and end of study. Finally, the animals were euthanized and the pancreases tissues were evaluated. **Results:** Treatment with saffron significantly decreased the hyperglycemia and the blood triglycerides without any changes in cholesterol. Histo-pathological observations indicated that the destruction of pancreases islets and leukocytes infiltration were ameliorated by saffron treatment. **Conclusion:** It seems that hydro-alcholic extract saffron may have a therapeutic effect against destruction of β-cells and insulitis in the animal model of type 1 diabetes. **Keywords:** Saffron, Multiple-low-dose-streptozotocin, Type 1 diabetes

7692P

Effect of hydroalcholic extract of saffron (Crocus sativus) in ameliorating the immunological aspects of experimental autoimmune diabetes in C57bl/6 mice

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Introduction: Streptozotocin (STZ) is a potent alkylating toxin and damages islet β cells. When STZ is given in multiple low doses, it elicit T-cell-dependent immune responses, and induce autoimmune diabetes in a manner similar to human type I diabetes.This study was done to investigate effect of hydroalcholic extract of saffron (*Crocus sativus*) on the clinical and immunological aspects of experimental autoimmune diabetes in C57bl/6 mice.**Materials and Methods:** After stabilization of diabetes, mice were orally treated with hydroalcholic extract of saffron (500 mg/Kg) for 3 constitutive weeks. The blood sugar was recorded weekly. At the end, the mice scarified and the lymphocytes proliferation, nitric oxide production and respiratory burst were evaluated in splenocytes. **Results:** Treatment with saffron significantly diminished hyperglycemia. Moreover, saffron treatment decreased the lymphocytes proliferation and nitric oxide production in splenocyte population of diabetes mice. Moreover, treatment restored the respiratory burst of splenocytes. **Conclusion:** It seems that hydroalcholic extract saffron may

be a promising strategy to control type 1diabetes mellitus. **Keywords:** Saffron, Multiple-low-dose-streptozotocin, Type 1 diabetes

7705P

Immunomodulatory potential of Anethum grareolens

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Introduction: Pharmacological effects of *Anethum grareolens* such antimicrobial or antibacterial, anti-hyperlipidaemic, antihypercholesterolaemic, antioxidative and hypoglycemic activities have been shown. This study was conducted to grasp the immunomodulatory properties of the *Anethum grareolens*in Rat challenged with Rev1.**Materials and Methods:** The study population consisted of 14 male rats that randomly allocated in two equal groups and immunized with Rev1 (0.1 ml Rev1+0.9 ml PBS). Rats in treatment group were intraperitoneally received 10 mg/ Kg *Anethum grareolens*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- 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 decreased in treatment groups, while the level of lymphocyte proliferation was significantly increased in treatment group compared to control group. **Conclusion:** Hydroalcoholic extract of *Anethum grareolens*may was used as a natural source in immune system disorders. **Keywords:** *Anethum grareolens*, Humoral immunity, Cellular immunity

8710P

Effect of co-culture mesenchymal stem cellstreated with estrogen on phagocytic activity, apoptosis and respiratory burst of peripheral blood neutrophilsin Rat

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Introduction: Mesenchymal stem cells (MSCs) are type of pluripotent cells from bone marrow that have therapeutic potentials due to their immunomodulatory properties. Therefore, these cells are extensively used in cell therapy. Estrogen, as a sex hormone, is also an immunomodulator for the immune system. The current study was about the effect of MSCs of male rat adjacent with estrogen on the function and survival of neutrophil. **Materials and Methods:** In this experimental study, mesenchymal stem cells were treated with Estrogen (10nm and 20nm concentrations) for 72 hours at 37 ˚C. Then, the cells were pulsed with neutrophils, and function of neutrophil were measured. **Results:** The percentage of phagocytosis and the rate of respiratory burst in neutrophils in contact with mesenchymal stem cells treated with estrogen, was increased compared to the control group. This increase was significant for both estrogen concentrations of 10nm and 20nm. On the other hand, the amount of apoptosis in

neutrophils treated by estrogen with both concentrations of 10nm and 20nm was reduced compared to control group. **Conclusion:** Considering the regulatory role of estrogen MSCs on immune system functions, neutrophil interactions with MSCs should be considered for treatment and interventionist hormonal therapy. Mesenchymal stem cells treated with estrogen had a significant impact on neutrophil function that this results could be taken into consideration in treatment of diseases associated with neutrophil functions and physiological and pathological responses of neutrophil. **Keywords:** Mesenchymal stem cells, Estrogen, phagocytosis, Apoptosis

9719P

Aloe Vera and Intervention Effect in Albumin glycation Reaction in vitro

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Introduction: Diabetes mellitus is the most common disease in the world. Hyperglycemia in diabetes causes glycosylation of the serum proteins that lead to a change in their conformation and function. In this study the influence of the Aloe Vera's extracts on the inhibition of the Albumin glycosylation reaction and also breaking the bond between Albumin and glucose was surveyed in vitro. **Materials and Methods:** The influence of Aloe Vera leaf extract in 0.1, 0.2, 0.5 and 1 g/dl concentrations in two states; the influence of the inhibition reaction of Albumin glycosylation and also the influence of the bond breaking between Albumin and glucose were examined. The rate of glycosylation was measured by thiobarbituric acid (TBA). Investigation by the spectrophotometry method was $P<0.05$ that was considered significant. **Results:** Aloe Vera's extract can inhibit significantly the Albumin glycosylation reaction in all concentrations except in 1g/dl concentration and it has maximum inhibitory effect in 0.1g/dl concentration ($P<0.05$). Again, the rate of this inhibition was time-dependent. Additionally, it could break the Albumin-glucose bond and the maximum effect was seen in 0.1g/dl concentration ($P<0.05$). Conclusion**:** The results demonstrated that Aloe Vera inhibits the glycosylation of the Albumin and also breaks down the Albumin-glucose bond, thus it has a hypoglycemic effect. This result can be generalized to other serum proteins and molecules. The results presented that decrease of hyperglycemia effects and therefore, damaging effects of free radicals of a glucose- proteins band in patients with diabetes is accessible. **Keywords:** Diabetes mellitus, Aloe Vera, Albumin glycosylation

9729P

In vitro study of the effects of Lactobacillus acidophilus and L.reuteri on IL-12 production by human dendritic cells

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Introduction: Immunostimulatory or Immunomodulation effects of probiotics attract many researchers to study about the effects of different genera and species of lactic acid bacteria (LAB) on immune system functions. Many researchers have focused on mechanisms of their effects on immune system. Dendritic cells have essential roles in direction of immune system toward responsiveness or unresponsiveness, therefore ,they are important candidates for immune system-lactobacilli interactions. Interleukin-12 (IL-12) as a product of dendritic cells and some other cells , is a multifunctional cytokine, the properties of which bridge innate and

adaptive immunity, acting as a key regulator of cell-mediated immune responses through the induction of T helper 1 differentiation .In this study , in vitro effects of Lactobacillus acidophilus and L. reuteri and on IL-12 production by dendritic cells evaluated. Materials and Methods: Human PBMCs were obtained from blood of healthy volunteers, then dendritic cells were derived. After exposure of immature DCs to inactivated bacteria : Lactobacillus acidophilus and L. reuteri for 48 h, culture supernatants were harvested and stored at a temperature of −30 °C until use. The production of IL12 was quantified using Bead-based Multiplex immunoassay with Flowcytometry. **Results:**Lactobacillus acidophilus and L. reuteri enhanced IL-12 production by dendritic cells. The effect of L. acidophilus is more than L. reuteri .**Conclusion:** The Lactobacilli are effective on immune system functions such as production of IL-12 by dendritic cells, but the exact mechanisms of their effects and the relationship to species or strains and also the potentially therapeutic applications need to be elucidated

9752P

The Impact of DMSO on the Expression of Chemokine Receptors in T Cells.

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Introduction: Dimethyl sulfoxide (DMSO) is usually used to solubilize poorly soluble drugs in permeation assays. Studies advocated that DMSO exposure not only affects the phenotypic characteristics but also induces significant alteration in gene expression, protein content and functionality of the differentiated hepatic cells. The aim of this study was to investigate the effect of DMSO on the Chemokine Receptors of Th1 and Th2 cells. **Materials and Methods:** Peripheral blood mononuclear cells from healthy individuals were activated with Conconavalin 'A' and treated with DMSO, 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 T cells. Peripheral blood lymphocyte subpopulations were identified and evaluated by two colors flow cytometric analysis. A nonparametric paired samples Wilcoxon test was applied to compare the grouped data. Results were expressed as the mean ± standard deviation (S.D.). *P*-values<0·05 were considered to indicate significant differences. **Results:** Results showed that the expression of CXCR3 on Th cells was increased (8.94±3.40 vs. 16.22±15.25, P= 0.018). Moreover, 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 DMSO on the expression of CXCR3, CCR3 and CCR4. Therefore, in future, DMSO could be used instead of other drugs such as immunosuppressive with fewer side effects in autoimmune diseases.

9759P

Evaluation effects of aqueous and ethanolic extracts of Matricaria chamomile on cytokines in balb/c mice.

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Introduction: Matricaria chamomilla L. (MC) is used as an effective plant for inflammation, ulcers, wounds, gastrointestinal disorders, stomach ache, pharyngitis, rheumatic pain and infertility in both traditional and modern medicine for years. However, there is few invivo studies to prove its extract effects on cytokines of macrophages and lymphocytes in Balb/c mice. The aim of this study was to determine the invivo effects of aqueous and ethanolic extract of Matricaria chamomilla on IL17, IL4, IFNγ and TNFα in Balb/c mice. **Materials and Methods:** The study was conducted on 110 mice divided into four groups. Four groups were injected intra-peritoneally (IP) with 25, 50, 75 and 100 (mg/kg/day) aqueous extract. Four groups were injected with 25, 50, 75 and 100 (mg/kg/day) ethanolic extract. Five groups were treated with 250, 500, 750, 1000 and 100 (mg/kg/day) aqueous extract orally and five groups were diet with 250, 500, 750, 1000 and 100 (mg/kg/day) ethanolic extract for 15 days. The control groups were treated with salin. On day 16, animals were scarified and macrophages and lymphocytes were separated from Peritoneum and Spleen respectively and supernatant were collected for ELISA test.**Results:**The results showed that IL17 decreased in 100, 75 mg/kg aqueous extract and IFN-γ decreased in 75 mg/kg aqueous extract and 100, 750 mg/kg ethanolic extract and IL4 increased in 50mg/kg aqueous extract and 750, 1000mg/kg ethanolic extract and TNFα increased in 50, 100 (mg/kg/day) in injected aqueous extract groups.**Conclusion:**Matricaria chamomile can reduce TH1 and TH17 cytokines and increase TH2 cytokine and reinforced innate immunity. Also more investigations were suggested to find its effects on other cytokines such as IL12.

9761P

Umbelliprenin induced anti-inflammatory and regulatory cytokines in C57/BL6 mice

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Introduction: Umbelliprenin is a prenyloxy-coumarin with pharmacologically polyvalent activity. Several studies have shown it's anti-inflammatory, anti-tumor, antioxidant anti-genotoxic activity, and other functions. However, the exact effect of this compound on the naïve immune response has not yet been shown. Umbelliprenin effects on the predominance of Th1 and Th2 responses was investigated in normal C57/BL6 mouse. **Materials and Methods:** Umbelliprenin (2.5 mg/200 µl IP) were subjected to six C57/BL6 mice every other day for 8 days. Paraffin and PBS injected mice were enrolled as solvent and control groups, respectively (n=6 mice/group). IL-10, IFN-γ and IL-4 levels were determined in sera and also in splenocytes culture supernatants at the presence of Con A (3 µg/ml) after 72h. H & E staining of paraffin embedded blocks were performed for lung and liver tissues of mice. **Results:** Umbelliprenin could significantly increase the secretion of IFN-γ and IL-4 in sera and IL-10 in splenocytes cultures. Comparison of IFN-γ /IL-4 in the sera and splenocytes culture supernatants showed lower ratios in umbelliprenin treated mice than in solvent and the untreated groups. **Conclusion:**Umbelliprenin could induce anti-inflammatory responses via the predominance of Th2 cells and some regulatory responses in C57/BL6 mice.**Keywords:** Umbelliprenin, Prenyloxy-coumarin, T helper (Th), regulatory responses, Anti-inflammatory cytokine, Interferon gamma (IFN-γ)

9781P

Anti-inflammatory Effects of Flavenoid, Rutin, on Activated Human Peripheral Blood Neutrophils

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Introduction: Neutrophils represent the first-line of human defense against infections. Immediately after the stimulation, neutrophilic enzymes are activated and produced toxic mediators such as Nitric oxide (NO) and Myeloperoxidase (MPO). These mediators can be toxic not only for infectious agents but also for host tissues. Since flavonoids exhibit antioxidant and anti-inflammatory effects, they are subjects of interest for pharmacological modulation of inflammation. In the present study the effect of flavonoid, Rutin, on the stimulus-induced MPO and NO production in human neutrophils was investigated. When the cells were pre-incubated with Rutin, the MPO and NO generation induced by phorbol 12-myristate 13-acetate (PMA) was significantly suppressed, showing anti-inflammatory effects of this flavonoid. **Materials and Methods:** Human peripheral blood neutrophils were isolated via density gradient centrifugation using Ficoll coupled with dextran sedimentation. The cell preparations containing >99.9% granulocytes were determined by morphological examination through Giemsa staining. Neutrophils were cultured in complete RPMI medium. Neutrophils were pre-incubated with or without Rutin for 30 min, and stimulated with 20 nM PMA or left unstimulated. After 2 hours, the supernatants were collected; NO and MPO production were analyzed using Griess Reagent and MPO assay Kit, respectively. Samples without PMA treatment were used as control. **Results:** The results revealed that Rutin, strongly and significantly inhibited neutrophil NO and MPO production.**Conclusion:** In this study, it was shown that Rutin significantly inhibits the release of NO and MPO by human neutrophils. Treatment with flavenoids may be considered as therapeutic strategies for neutrophil-mediatory and inflammatory/autoimmune diseases. **Keywords:** Human Neutrophils, Rutin, Nitric Oxide, Myeloperoxidise

9808P

Anti-leukemia and apoptosis-inducing effect of Satureja bachtiarica extracts

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Introduction: Apoptosis defection is a major causative factor in tumor progression and resistance of leukemia cells to therapeutic agents. In the present study the apoptosis-inducing effect of different extracts from *Satureja bachtiarica*, a native plants, *on* leukemia cells was investigated. **Materials and Methods:** K562 and Jurkat leukemia cells were treated with various concentrations of *S. bachtiarica* extracts for 24 and 48 h. MTT assay was used to find out the cell proliferation inhibitory effects of the extracts. Effective extracts were analyzed for apoptosis induction by annexin V/PI staining using flow cytometry and a colorimetric assay for caspase 3 activity. **Results:** Two hexane and dichloromethane extracts of *S. bachtiarica* at 1-100µg/ml concentrations significantly inhibited the proliferation of leukemia cells in a dose and time-dependent manner. The IC_{50} values of these extracts on both K562 and Jurkat cells were 28.18-27.2 and 22.9-33.1µg/mL, respectively. Flow cytometry analysis for annexin V/PI positive cells showed a significant increase in the percentage of cells undergoing apoptosis. Both the extracts at 25-50µg/mL induced apoptosis in approximately 85-90.8% and 87.35-53.9% of K562 cells and 97.1-89.7% and 94.05-97.3% of Jurkat cells 48h after treatment. There was an increase in the level of caspase-3 activation in K562 and

Jurkat cells treated with both the extract; 3.96-3.16 and 2.9-4.0 fold of untreated cells at 25µg/mL of the extracts after 24 h. **Conclusion:** These results showed that the growth inhibitory effect of *S. bachtiarica* on leukemia cells might be due to induction of apoptosis through a caspase-3-dependent mechanism.

9838P

Establishment of anticomplementary activity test to evaluate immunoglobulin product

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Introduction: The presence of aggregated IgG in intravenous immunoglobulin (IVIG) is associated with activation and consumption of complement without involvement of a specific antigen. Aggregated IgG in IVIG was always considered as one of the main reason of adverse reactions. Since this test has not been conducted and set up in Iran, test launch is considered, and then ACA in two different IVIG preparations were assessed. **Materials and Methods:** In order to obtain IVIG, Fraction-I+II+III or Fraction-II+III pastes prepared by the Cohn method and the chromatography process was performed as purification methods. For the ACA measurement, complement mediated lysis of antibody-coated sheep red blood cells (SRBC) was performed. So immunoglobulin was incubated with a guinea-pig complement and the remaining complement was titrated. ACA was expressed as the percentage consumption of complement relative to the complement control. **Results:** The test properly was set up and ACA was calculated in IVIG of two different production methods in comparison with commercial IVIG as a control. These characteristics satisfied the requirements of the European Pharmacopeia. **Conclusion:** This test was established for the first time to evaluate of IVIG products in Iran. The measurement of ACA in IVIG has remained an obligatory parameter in pharmaceutical quality control. Standardization of the ACA assay is difficult due to complex biological test system and various parameters influencing the test results. Variation between batches of complement, storage conditions of SRBC and the sample preparation were scrutinized as critical parameters of the ACA assay.
Keywords: Intravenous Immunoglobulin, Anti-complementary Activity**,** Plasma

10785P

Antitumor effect of Ferula assa foetida oleo gum resin against breast cancer in mice

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Introduction: Breast cancer is the most common type of cancer among women especially in industrialized countries. Herbs have been identified as an important source of novel bioactive compounds for medicine

development including cancer chemotherapeutic drugs. Ferula assa-foetida L. is one of these species that has been considered to act as anticarcinogenic in many traditional medicine systems. **Materials and Methods:** 8-week old female BALB/c mice that were bred in animal house of Shahid Sadoughi university of Medical sciences were selected. 5 mice were inoculated with 1 × 104 4T1 cells/mice subcutaneously, respectively. As a negative control for 35 days after the cells were treated with normal saline. In treatment group, 2 weeks after injection of the cancer cells, the animals were treated for 21 days with asafoetida. After 21 days of post-inoculation, mice were sacrificed and body weight, tumors size, spleen index and tumor volume incidence were measured. **Results:** The results revealed that asafoetida could decrease weight of tumor and tumor volume significantly ($P<0.05$). It was also determined that body weight significantly increased in treated group with asafoetida. In this study, no significant difference was observed in spleen index **Conclusion:** These data showed that asafoetida has a significant effect in treatment of breast cancer in animal models. These beneficial effects probably was related to its antioxidant potential or active components such as ferulic acid and umbeliferon. **Keywords:** Asafoetida, Breast Cancer, Tumor Size, Spleen Index

10814P

Anticancer activity of a natural effective compound from shallot in MCF-7 cell line through cell growth arrest and apoptosis induction

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Introduction: Natural compounds are promising potential drugs in the treatment of many diseases such as inflammation-related malignancies. In this study, the anticancer activity of an effective compound from shallot plant, was evaluated on human adenocarcinoma breast cancer cell MCF-7. **Materials and Methods:** The effective compound was isolated from the ethyl acetate fraction of shallot. Then, the apoptosis induction and cell progression inhibition by the compound was examined on MCF-7 cell line. Flow cytometry and gene expression analyses were also used to evaluate the mechanisms behind these anticancer activities of the compound. **Results:** The effective compound in a concentration-dependent manner exerted anti-growth activity on the cancer cells through cell cycle arrest at G0/G1 phase. This was well correlated with alteration in cell cycle-related genes at mRNA levels. The compound also induced the apoptosis in cell line which had accordance with the results of the viability assay and also concomitant with up-regulation of the apoptosis-related genes. **Conclusion:** The findings suggested that the anticancer activity of the effective compound in MCF-7 cancer cells might be related to the cell cycle arrest and induction of apoptosis partly, by modulating the gene expression of key regulators involved in. However, further experiments and preclinical investigations are needed to more clarify the mechanisms underlying the anti-cancer activity of this compound as the potential candidate for treatment of human adenocarcinoma breast cancers. **Keywords:** shallot, cell cycle, apoptosis, cancer

10824P

The Zatariamultiflora essential oil induces apoptosis and enhances cytotoxicity of A549 non-small cell lungadenocarcinoma

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Introduction: Lung cancer is one of the major obstacles of civilized societies resulted from chronic exposure to tobacco smoke or air pollution. Since conventional therapies of cancer are insufficient and impose many adverse effects to the patients, there is a great interest to identify natural medicinal compounds that exert an efficient response and at the same time leave fewer damages to the normal cells. In current study, the antitumor potency of an Iranian native plant, Zatariamultiflora, was investigated on A549 cells. **Materials and Methods:** First, cells were cultured and incubated in various concentrations of Z. multiflora essential oil for 24 hours. To investigate whether the treatment reduces the growth of tumor cells, MTT test was used. Furthermore, the occurrence of cell death was assessed by using DNA ladder assay. **Results:** Finding the proliferation assay showed that Z. multiflora essential oil considerably suppressed the growth of tumor cells in a concentration-based manner. On the other hand, gel electrophoresis of treated samples confirmed the presence of apoptotic bodies. **Conclusion:** In sum, essential oil of Z. multiflora exhibited desired antitumor effects in lung cancer cells. Therefore, it may use as an alternative for chemotherapy of lung cancer. **Keywords:** Apoptosis, Lung cancer, Proliferation, Zatariamultiflora

10861P

Immunological study of the effect of Chard (Beta vulgaris) aqueous extract on experimental autoimmune diabetes in C57 BL/6 mice

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Introduction: Diabetes type I is a chronic disease mediated by the immune system. The prevalence of type 1 diabetes in the past two decades has dramatically increased worldwide with an approximate prevalence of 1.2 to 14.5% in Iran. Inheritance plays the main role in the development of this type of diabetes as a result of which, changes in the normal function of the immune system is observed which finally leads to react against insulin producing pancreatic beta cells. The aim of the present study was to investigate the effect of oral administration of the aqueous extract of the leaf of sugar beet on the immunological aspect of STZ (Streptozotocin)-induced diabetes in mice. **Materials and Methods:** For this purpose, 20 male inbred C57 BL/6 mice, 6-8 weeks of age were divided into 4 groups of 5 including: control, diabetic, treatment and pre-treatment groups. In the second, third and the fourth groups, five consecutive daily injections of 40 mg/kg STZ were performed to induce diabetes. During the study period, the fasting blood sugar was recorded on a weekly basis. At the end of the study, the mice were euthanized and after the isolation of the spleens and the preparation of cell cultures, the cells were tested to determine the extent of lymphocyte proliferation and the severity of respiratory burst. **Results:** The results of this study showed that the administration of Chard extract can significantly reduce the blood glucose levels in diabetic mice ($P<0.05$). The lymphocytes proliferation in the treatment and pre-treatment groups showed a significant decrease compared to the diabetic group ($p<0.05$). Moreover, treatment with the extract significantly restored the reduced respiratory burst in the diabetic group to levels similar to the control group ($p<0.05$). **Conclusion:** It can be concluded that the leaf extract of Chard has a positive effect on the immunological changes and can reduce the complications of autoimmune diabetes in mouse models which necessitates further studies herein . **Keywords:** Chard; Streptozotocin; Autoimmune diabetes; Lymphocyte proliferation

10862P

Pancrease pathological study of the effect of the aqueous extract of Chard (Beta vulgaris) on experimental autoimmune diabetes in C57 BL/6 mice

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Introduction: Diabetes type I is a chronic autoimmune disease which is characterized by the infiltration of the immune cells into the Langerhans islets of the pancreas and destruction of insulin-producing beta cells. The prevalence of type 1 diabetes in the past two decades has increased dramatically worldwide. Reports indicated a prevalence of 1.2 to 14.5% in Iran. **Materials and Methods:** The animal models in the present study included inbred male C57BL / 6 mice with 6 to 8 weeks of age which were randomly divided into 4 groups of 5, after one week of accommodation. In this study, the induction of diabetes was confirmed after the intraperitoneal (IP) injection of 5 consecutive doses of STZ in 5 days. Finally, the pancreases of the mice were dissected and histological sections were prepared. **Results:** The results indicated significant differences in diameter, the size and number of pancreatic islets and fasting blood sugar levels among the diabetic and the control group ($p<0.05$). The pre-treatment group showed significant differences in diameter, size and number of Langerhans islets and also blood sugar levels during the first week compared to the control group ($p<0.05$). On the other hand, in the treatment group there was no significant changes in diameter, volume and number of Langerhans islets compared to the control group, while a significant difference was observed in fasting blood sugar from the third week. **Conclusion:** The results of this study showed that pre-treatment with the aqueous extract of sugar beet can ameliorate the histopathology of streptozotocin-induced diabetic mice. **Keywords:** Leaf of Chard; Streptozotocin; Autoimmune diabetes; Pancreas; Histopathology

10911P

The effects of cytoplasmic extract of Bifidobacteriumbifidumon cancer cell line K562

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Introduction: Probiotics are alive microorganisms which have useful effects on host health host by balancing its intestinal micro flora. Nowadays, probiotics are known as a factor for prevention of infectious diseases and cancer. The present study aimed at systematically reviewing the studies in checking positive effects of probiotics on health and their association with cancer. Studies showed that cytoplasmic extract and peptidoglycans derived from lactic acid bacteria inhibit cancer cell growth. Other studies have shown that *Bifidobacteriumlactis* induced apoptosis in response to genotoxic carcinogens after induction of carcinogens in descending colon of rat. Cancers of the hematopoietic system is the most common malignancies. In leukemia the normal production of white blood cells stopped and individual ability to deal with disease disappears. **Materials and Methods:** Bifidobacteriumbifidium bacterial cytoplasmic extracts (BCE) and bacterial non-cytoplasmic extract (BNCE) were prepared and after dialysis and autoclave, the protein content was measured by Bradford test and different concentrations were prepared. Number of 1×10^4 K562 cell/100 microliters, were pipetted into each well of 96-well tissue culture plate and were treated by various concentration of each treats in cell culture standard condition for 24, 48 and 72 hours. After incubation time, IC50 value (half maximal inhibitory concentration) in K562 line was measured by MTT assay for

all treats. PBMCs were obtained from heparinized peripheral blood of normal donor by Ficol method and 2×10^4 cell/ microliters were pipetted into 96-well plate. Cells were treated by the same concentrations of used treats for K562 cells. Cell vitality was measured by MTT assay. **Results:**data indicated that treating both cells by various concentrations of all treats in all time displayed a significant decrease in the percent of K562 and PBMC cells survival. It seems that, comparing with doxorubicin, the treats had weak selectivity function between normal and tumor cells. **Keywords:** Probiotic, Bifidobacteriumbifidium, BCE, BNCE, K562 cell line, Leukemia

10930P

Sesame seeds essential oil and Sesamolmodulate pro-inflammatory function of macrophage and Dendritic cells and promote Th2 response in mice

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Introduction: Commonly worldwide, herbal medicine is becoming progressively accepted for management of different diseases. Identification of the active components and the mechanisms of herbal medicines on the immune system and abnormalities are highly desirable. This experimental study aimed at investigating the effects of Sesame (*Sesamumindicum L.*), the essential oil and sesamol as sesame effective component on mouse splenocytes subsets, macrophages and dendritic cells (DCs). **Materials and Methods:** Obtained components affected on splenocytes and PHA (5µg/ml) and LPS (10µg/ml) activated splenocytes, macrophages and DCs in different concentration (0.01–100µg/ml). The cells proliferation/viability was assayed by MTT method. Macrophages nitrite levels were measured by using the diazotization method and TNF-α and IL-1β by ELISA. Treated DCs also assayed for maturation markers levels and cytokine production. **Results:** Analysis of the results indicated that both sesame components suppress PHA stimulated splenocytes with no effect on LPS stimulated subsets. Effects on lymphocyte cytokines release were diminution of IFN-γ release and induction of IL-4 secretion by sesame ingredients. Macrophages viability was not affected and production of NO, TNF-α and IL-1β were inhibited by sesame essential oil and sesamol. DCs phenotype skewed to immature and release of TNF-α and IL-1β were abrogated form DCs. **Conclusion:** These results indicated that sesame essential oil and its effective component as sesamol may be able to suppress the cellular but not humoral immune responses with domination of Th2 responses and also could modulate macrophages and the dendritic cells pro-inflammatory functions. **Keywords:** *Sesame, Sesamol, Splenocyte, Macrophage, Nitric oxide, Dendritic cells*

10941P

Determination of optimal effective dose of hydro-alcoholic extract of zingiber on PBMCs of patients with allergic asthma

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Introduction: Asthma is a chronic inflammatory disorder of the airways, characterized by excess production of Th2 cytokines and eosinophil accumulation in the lungs. Zingiber is a well-known herb used in oriental medicine for the treatment of asthma and bronchial inflammation. This study aimed at optimizing in vitro critical immuno-pharmacologic parameters, dose and dosage, of hydro-alcoholic extract of zingiber on human peripheral blood mononuclear cells (PBMCs) of patients with allergic asthma. **Materials and Methods:** Anticoagulant peripheral blood was derived from asthmatic patients. PBMCs were cultured in presence of different concentrations of hydro-alcoholic extract of zingiber(100, 200 and 300 µg/mL) for 24, and 48 hours. Then the anti-proliferative activity of extract was assayed by MTT assay and light microscopic examination revealed morphological alterations. In addition, induction of polarization in Th cells was determined using real time PCR. **Results:** The results revealed that anti-inflammatory properties of Hydro-alcoholic extract of zingiber were in a dose-dependent manner. On the other hand, 300 µg/mL was found the optimum concentration of zingiber which significantly altered gene expression levels of GATA3 and RORγtin PBMCs of patients with Allergic Asthma. **Conclusion:** Hydro-alcoholic extract of zingiber showed anti-inflammatory effect on PBMCs of patients with allergic asthma. This amelioration effect was dose-dependent. **Keywords:** Athma- zingiber- GATA3- RORɣt

10993P

1,3,7 Three MethylsXezanthine Alters Neutrophil's Functions by Affecting the Action of Pro-Inflammatory Mesenchymal Stem Cells

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Introduction: Former data showed that mesenchymal stem cells (MSCs) express some sub units of adenosine ligands. Moreover, it has been proved that LPS-primed mesenchymal stem cells (MSCs) secrete pro-inflammatory cytokines. Current study was conducted to evaluate the effect of the 1, 3 and 7 three methylsXezanthine (as an adenosine antagonist) on the interaction of lipopolysaccharide (LPS) activated MSCs on neutrophils. **Materials and Methods:** After isolation of mesenchymal stem cells from bone marrow of rats, these cells were stimulated with 10 ng/mL LPS for 1 h and then the condition medium threw out. After washing MSCs, they have been pulsed with different concentration of 1, 3 and 7 three methyls Xezanthine (0.1, 0.5 and 1mM) for 48 h. Then, MSCs were co-cultured with neutrophils and incubated for 4 h. Then, the functions of neutrophils were evaluated. **Results:**The lifespan and the respiratory burst of neutrophilsco-cultured with 1,3 and 7 three methyls Xezanthine treated LPS-stimulated MSCs significantly increased in used concentration, compared to unstimulated ones.Although, the neutral red uptake of these neutrophils significantly increased but didn't show any dose dependent effect.**Conclusion:**These findings may offer new insights into the potential mechanisms underlying the immuno-modolatory and anti-inflammatory effects of 1, 3 and 7 three methyls Xezanthine. **Keywords:** Mesenchymal Stem Cell, Lipopolysaccharide, Conditioned Medium, Neutrophil, 1, 3 and 7 three methyls Xezanthine

10994P

Anti-proliferating effects of Rose Bengal in cancer cells

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Introduction: Rose Bengal (RB) has been used as a safe photodynamic sensitizer for its anti-cancer activities. Its special toxicity through exposure with cancer cells was verified. In this study, the anti-cancer activities of Rose Bengal were determined. **Materials and Methods:** The toxic effects of RB against AGS cancer and NIH 3T3 fibroblast cell lines was studied using MTT assay. Cell death was studied using Annexin-V and PI staining. **Results:** AGS cancer cells exhibited significant decline in growth in response to Rose Bengal; these cells showed a greater reduced growth than non-malignant 3T3 cells. In AGS cancer cells exposed to Rose Bengal a significant increase in the apoptosis rate was determined. **Conclusion:** Findings of this study suggested that RB represses growth of gastric cancer cells through apoptosis. **Keywords:** Rose Bengal, anti-proliferation, cancer, apoptosis

11003P

Early effect of Urticadioica extract on mitochondrial stress oxidative in diabetic rats

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Introduction: It is indicated that increased oxidative stress plays pivotal role in the pathogenesis of diabetes mellitus in both experimental animals and human subjects. Glucose oxidation and protein glycosylation induced by hyperglycemia, result in enhanced formation of free radicals such as reactive oxygen species (ROS). Urticadioica, as a medicinal plant, has antioxidant activity. The objective of this study was to examine the effect of Urticadioica extract on mitochondrial oxidative stress in streptozotocin induced diabetic rats. **Materials and Methods:** Single dose of streptozotocin was injected intraperitoneally (i.p.) in adult male rats. On the 3rd day, fast blood glucose was measured. The diabetic rats were divided into 2 groups; diabetic rats with daily i.p. saline injection, diabetic rats with daily i.p. Urtica extract injection. On the 7th day, mitochondrial oxidative stress was measured in these two groups and healthy control group with daily i.p. saline injection by dihydrorhodamine (DHR) test.**Results:** DHR test's mean ±SD for control group, diabetic rats with saline injection, and diabetic rats with Urtica injection were 39.46±9.1, 39.49±9.12, 48.44±11.69, respectively. One-way ANOVA showed no significant difference between groups (p=0.184). **Conclusion:**It seems that diabetes only and diabetes with Urtica extract treatment has not early effect on mitochondrial oxidative stress.

11041P

The effect of crocin on decline of mast cells recruitment in testis of cyclophosphamide treated mice

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Introduction: Cyclophosphamide (CP), is extremely used as an antineoplastic agent for the treatment of various cancers, as well as an immuno-suppressive agent. Side effects in CP treated men were decreased the fertility and even sterility. This study was performed to evaluate the protective effects of Crocin in oxidative stress induced by CP on testis. **Materials and Methods:** In this study three groups (6 mice in each) of adult mice were used. Control group was treated with normal saline (0.2ml/day, IP), and CP group with CP (15 mg/kg/week, IP), and experimental group with CP along with crocin (200 mg/kg/day, IP). After 35days samples were taken and fixed in 10% formal saline and paraffin sections were prepared and stained by toluidine blue method. The mast cells were counted with latticed objective device in $1mm^2$field in three regions of each slide. All obtained data were analyzed by SPSS software in ANOVA and Duncan test. **Results:** Results showed that the amount of mast cells in crocin treated group were significantly lower than the groups that only received CP ($P<0.05$). **Conclusion:** This study showed that crocin decreased the mast cells recruitment and then ameliorated the oxidative stress effects of CP on male reproductive organ.

11042P

Protective effects of Crocin and Ethyl pyrovate on differentiationspermatogonia cells inCyclophosphamide treated mice

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Introduction: A high volume production of free radicals induced by cyclophosphamide (CP) with decreased vigor of immune system cause testicular tissue damage. This study aimed at evaluating the antioxidant effects of Crocin and Ethyl pyruvate in ameliorating the aforementioned damages. **Materials and Methods:** Four groups (6 mice in each) of adult mice were used. Control group was treated with normal saline.,ip, group 2 with CP 15 mg/kg/-week.,ip, group 3 with CP along with Crocin 200 mg/kg/day.,ip, and group 4 with CP along with Ethyl pyruvate 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 republication index (RI) was calculated based on the percentage. All obtained data were analyzed by SPSS software in ANOVA and Duncan test. **Results:** Observations showed that RI in Crocin and Ethyl pyrovate treated groups were significantly increased compared to CP group ($P<0.05$).**Conclusion:**This study showed the role of Crocin and Ethyl pyrovate in ameliorating immuno-deficiency system and increasing infertility in CP treated mice

11048P

Neuroinflammation in experimental autoimmune encephalomyelitis regressed by phenytoin

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Introduction: Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis. The hallmark of this disease is an autoimmune neuro-inflammation induced by Th17 and Th1 lymphocytes attack. Pervious documents indicated that phenytoin can protect axonal loss in EAE. Here, the immuno-modulatory effects of phenytoin in EAE were investigated. **Materials and Methods:** EAE was induced by guinea pig spinal cord homogenate and complete Freund's adjuvant in Wistar rats. Animals were divided into two therapeutic groups (n=7 per group). Phenytoin therapy (50 mg/kg-daily) was started in treatment group at day 12 when the treatment group developed a disability score. EAE control mice received vehicle alone with same schedule. Signs of disability were monitored daily until the day 36 when mice were sacrificed. At the end, Brain and spinal cord were removed for neuropathological analysis. Splenocytes were checked for proliferation by MTT test and cytokine production by ELISA. **Results:** Phenytoin therapy after the occurrence of clinical symptoms significantly regressed the clinical features and improved the histological schema of EAE. Phenytoin significantly inhibited the production of pro-inflammatory IL-17 as well as IFN-γ. The levels of anti-inflammatory IL-10 were not altered significantly. Lymphocyte proliferation was significantly decreased in treatment **Conclusion:** Phenytoin can improve EAE via direct neuro-protective effects and also immuno-modulatory properties. **Keywords:** Multiple sclerosis; Experimental autoimmune encephalomyelitis; Phenytoin

11078P

Up-regulation of gelatinase-β in human monocytes and mouse macrophages by phytohemagglutinin in vitro

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Introduction: Macrophages and monocytes can induce inflammation through different mechanisms including matrix metalloproteinases (MMPs) production. MMP-9 known as gelatinase-B degrades the extracellular matrix and plays an essential role in several inflammatory disorders including allergic asthma. Lectins are carbohydrate-binding proteins existing in many nutrients especially plants. Phytohemagglutinin (PHA), a mostly studied lectin, is a T-cell mitogen with allergenic and inflammatory properties. In this study, effect of PHA on gelatinase-B activity in human monocytes and mouse macrophages has been assessed in vitro. **Materials and Methods:** Human monocytic U937 cells and mouse peritoneal macrophages were cultured in complete RPMI medium. Then, the cells at logarhytmic growth phase were cultured in serum-free RPMI medium and subsequently were incubated with different concentrations of PHA (1-10 µg/ml) for 24 hours. The cell culture supernatants were collected. Next, the activity of gelatinase-B was evaluated by gelatin zymography. **Results:** PHA considerably increased gelatinase- B activity in human U937 cells and mouse peritoneal macrophages dose-dependently compared to non-stimulated control cells. **Conclusion:** The results showed that PHA could be a potential stimulator of gelatinase-B activity in monocytes and macrophages. Therefore, the inflammatory effects of PHA reported by others may be partly due to its enhancing effects on glatinase-B. Processing the PHA-rich nutrients such as kidney beans to remove, neutralize or decrease the PHA might be useful for prevention or alleviating of the inflammatory-based diseases such as asthma in which gelatinase-B is overexpressed. Besides, PHA could be useful in screening of MMPs modulators in immuno-competent cells. **Keywords:** Phytohemagglutinin, gelatinase-B, macrophages

11110P

Effects of Urtica dioica dichloromethane extract on cell apoptosis and related gene expression in human breast cancer cell line (MDA-MB-468)

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Introduction: Breast cancer is the most common cancer among women in worldwide, especially in developing countries. Therefore, a large number of anticancer agents with herbal origins have been reported against this deadly disease. This study is the first one in examining the cytotoxic and apoptotic effects of Urtica dioica in MDA-MB-468, human breast adenocarcinoma cells. **Materials and Methods:** The 3-(4,5-dimethylethiazol-2 yl)-2,5-diphenyltetrazolium (MTT) reduction and trypan-blue exclusion assay were performed in MDA-MB-468 cells as well as control cell line L929 to analyze the cytotoxic activity of the dichloromethane extract. In addition, apoptosis induction of Urtica dioica on the MDA-MB-468 cells was assessed using TUNEL (terminal deoxy transferase (TdT)-mediated dUTP nick- end labeling) assay and DNA fragmentation analysis and qPCR. **Results:** The results revealed that the extract significantly inhibited cell growth and viability without inducing damage in normal control cells. Nuclei Staining in TUNEL and DNA fragments in DNA fragmentation assay and increase in the mRNA expression levels of caspase-3, caspase-9, decrease in the bcl2 and no significant change in the caspase-8 mRNA expression level, showed that the induction of apoptosis was the main mechanism of cell death that ws induced by Urtica dioica extract. **Conclusion:** The results suggested that urtica dioica dichloromethane extract may contain potential bioactive compound(s) for the treatment of breast adenocarcinoma. **Keywords:** Urtica dioica, breast cancer, MDA-MB-468 cell line, cytotoxicity, apoptosis.

11180P

Protective effect of Tacrolimus in isoproterenol induced myocardial infarction

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Introduction: Myocardial infarction is associated with an inflammatory reaction, which is a prerequisite for healing and scar formation. Thus, understanding the molecular events associated with myocardial ischemia/reperfusion is needed to develop more site-specific interventions that could mitigate inflammatory injury during early reperfusion. **Materials and Methods:** Male Wistar rats were divided into six groups of control, isoproterenol, tacrolimus, and isopreteronol plus tacrolimus (0.5, 1 and 2 mg/kg). Isoproterenol (100 mg/kg) was injected subcutaneously to induce myocardial infarction, and tacrolimus was administered orally twice a day for two days along with isoproterenol. **Results:** Isoproterenol induced myocardial hypertrophy and necrosis as well as a marked reduction in hemodynamic and electro physiologic parameters. It was found that all doses of tacrolimus could amend the ECG pattern and ameliorate the isoproterenol-induced disturbances in cardiac function. Tacrolimus at dose 2 mg/kg twice a day for۲ days significantly increased (P<0.001) LVdP/dtmax from 2712.4±81.9 in myocardial infarcted rats to 4592 ±149.09 mmHg/sec. Similarly, tacrolimus lowered LVEDP from 17.6±0.68 in myocardial infarcted group to the value of 5.6±0.22 mmHg (P<0.001). Tacrolimus was found to reduce malondialdehyde concentration in serum and myocardium by 50-70% (P<0.001). Furthermore, tacrolimus (1mg/kg) significantly (p<0.001) decreased the

level of inflammatory cytokines (TNF-α andIL-6) in Isoproterenol induced myocardial infarcted rats. **Conclusion:** The results of this study showed that acute treatment with tacrolimus strongly protected the myocardium against the isopreteronol-induced myocardial infarction; this might be due to the antioxidant and anti-inflammatory properties of tacrolimus. **Keywords:** Tacrolimus, Myocardial infarction, Isoproterenol, Electrocardiography.

11279P

Antitumor effect of Glycyrizia glabra and Scloropholaria megalantha on transforming growth factorβ (TGFβ) production

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Introduction: Cancer is one of the major causes of mortality in the world. Cancers scape the immune system and could be one of the causes of failure of anti-cancer therapy. Tumors can help cancer development and immunosuppression in several ways, including TGFβ production. Traditionally, most people have used to treat diseases without any knowledge about the constituent elements of the plant. Therefore, anti-cancer properties of plants always have been considered. In this study, we decided to find out the effects of licorice and Scrophularia *Megalanta* extractions on TGF-beta production in colon cancer. **Materials and Methods:** HCT-116 cells were cultured under cell culture conditions in 75cm^2 cell culture flask. Cell growth curve was drawn**.** *MTT assay* in dose and time dependent manner was conducted to find *IC50 for each extraction. When Cells reached 50-60% confluence, they were exposed to different doses of plant extracts in triplicate for 48 hours.* Human fibroblast cells were considered as control. The levels of TGF-β were measured using ELISA kit. The effect of plant extracts on cell toxicity and survival was determined by MTT.**Results:** The results suggested that licorice and Scropholaria megalanta extractions could cause cell cytotoxicity on cancer cells in concentration of 5/43 and 62 mg/ml, respectively, and also 1000µg/ml of licorice decreased TGF-β level significantly. Although 10µg/ml of Scrophularia megalanta decreases TGFβ production, however the difference was not significant. A negative correlation between the concentrations of licorice extract and TGFβ level was observed. **Conclusion:** Based on the results of the present study, we can conclude that herbal extractions aforementioned, particularly licorice, could be a good option to reduce TGF-β and therefore could be used in colorectal cancer treatment. **Keywords:** TGF-β, *Glycyrrhiza glabra*, Scrophularia *Megalanta*, HCT-116

11274P

Anticancer effect of Scrophularia megalantha extract on the human stomach tumor cell line

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Introduction: Studies have demonstrated that plant extracts as nutritional and supplementary medicine possess various biological effects including anticancer activity with antioxidant mechanism. **Materials and Methods:** In the present study, the anticancer activity of *Scrophularia megalantha*, a native plant in Iran, was investigated. The total phenolic content as antioxidant agents of dry herb was determined by using the Folin-Ciocalteau assay. Free radical scavenging capacities of the extract and fractions, was measured by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay. The cytotoxic activity of the extract on tumor cell line using 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) colorimetric assay was determined. In addition, apoptosis of AGS (human stomach cancer cell line) was analyzed by flow-cytometry. **Results:** The IC50% DPPH radical scavenging activity and ascorbic acid equivalent of the antioxidant capacity of *S. megalantha* were 314.59 (mg/l) and 28.7(mg/g), respectively. The obtained results indicated that fifty-six percent inhibitions of AGS cancer cells due to exposure to *S. megalantha* were found at 200 µg/mL of the extract. In flow cyometry analysis, *S. megalantha* induced apoptosis in the AGS cancer cell. **Conclusion:** These results indicated that the extract as nutritional and supplementary medicine has antitumor effect through induction of apoptosis in the AGS cancer cell line. The antioxidant activity of the extract may be a mechanism of anti-cancer effect of this supplementary medicine, however, future studies are recommended. **Keywords:** Supplementary medicine, medicinal plant, *Scrophularia megalantha*, Antioxidant activity, Cancer.

11285P

Immunomodulatory Effects of some Allium species from Iran on mice Lymphocyte viability

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Introduction: Identification and application of immuno-modulators in natural ingredients can be effective in immune regulation. Lymphocytes are the cell components of adaptive immune system. In the present study, the aqueous extract effects of selective *Allium* species on viability of these cells were examined. **Materials and Methods:** Fresh bulbs of seven wild *Allium* species were collected from their natural habitats and the bulbs of cultivated *A. sativum* were supplied from a field in Hamadan. Aqueous extracts of the fresh bulbs were prepared. Lymphocytes population was prepared from mice spleen. The acquired cell pellet was cultured in RPMI/FBS. Different concentrations of aqueous extracts were added to the cell cultures, and cell viability was measured by MTT assay. **Results:** Based on the obtained results, significant differences ($P<0.05$) were observed among the aqueous extracts of *Allium* species in some concentrations on viability of lymphocytes. The results showed that bulb extracts of *A. sativum* at 0.001 and 0.0001, *A. jesdianum* at 0.05, 0.001 and 0.0001, *A. lenkoranicum* at 0.1, 0.05, 0.005, 0.001 and 0.0001, and *A. stipitatum* at 0.1 mg/mL had stimulatory effects and *A. Iranicum* at 1 and *A. elburzens* at 1 mg/mL had inhibitory effects on viability of lymphocytes.**Conclusion:**Our findings approved that the bulb extracts of all the examined *Allium* species had stimulatory or inhibitory effects on viability of lymphocytes in different concentrations, among them the best stimulatory results were obtained for *A. lenkoranicum*, followed by *A. jesdianum* and *A. sativum* in low concentrations.

11286P

Macrophage cell viability after treatment with some Allium species from Iran

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Introduction: Identification and application of immuno-modulators in natural ingredients can be effective in immune regulation. Macrophages are the cell components of innate immune system. In the present study, the aqueous extract effects of selective *Allium* species on the viability of these cells were examined. **Materials and Methods:** Fresh bulbs of seven wild *Allium* species were collected from their natural habitats and the bulbs of cultivated *A. sativum* were supplied from a field in Hamadan. Aqueous extracts of the fresh bulbs were prepared. Macrophages were isolated from mice peritoneum. The acquired cell pellet was cultured in RPMI/FBS. Different concentrations of aqueous extracts were added to the cell cultures and cell viability was measured by MTT assay. **Results:** Based on the obtained results, significant differences ($P<0.05$) were observed among the aqueous extracts of *Allium* species in some concentrations on viability of macrophages. The results showed that bulb extracts of *A. sativum* at 1, *A. jesdianum* at 0.1, *A. Iranicum* at 1 and 0.01, *A. lenkoranicum* at 0.1, *A. elburzens* at 0.01 and 0.001, *A. asarense* at 0.001 and 0.0001, *A. scabriscapum* at 0.0001 had stimulatory effects and *A. elburzens* at 1 and *A. stipitatum* at 0.1 mg/mL had inhibitory effects on viability of macrophages. **Conclusion:** Our findings approved that the bulb extracts of all the examined *Allium* species had stimulatory or inhibitory effects on viability of macrophages in different concentrations, among them the best stimulatory results were obtained for *A. elburzens*, followed by *A. asarense* and *A. Iranicum* in low concentrations.

11295P

Evaluation of the Cytotoxic Activity of Malva sylvestris Flower and Leaf against Murine Breast Cancer Cell Line (4T1)

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Introduction: Breast cancer is the leading cause of death in women worldwide. The majority of drug candidates, currently used in clinical cancer chemotherapy, have been originally derived from plants. *Malva sylvestris*, as a medicinal plant, is commonly used in Iran as a vegetable, namely Panirak. The cytotoxic activity of the aqueous extracts of this plant was evaluated against murine breast cancer cell line (4T1) by the MTT assay. **Materials and Methods:** Air-dried plant flowers and leaves were separately weighted (1g) and extracted by boiling and brewing in 50 mL PBS. 4T1 cell line was cultured in RPMI medium supplemented with 10% FBS. The diluted aqueous extracts were added, and after 24h and 48h incubation, the MTT test was performed. The extracts were also tested for peripheral blood mononuclear cells. **Results:** The results showed that *M.sylvestris* had significant cytotoxic effect with 30% viability on the 4T1 cell line. Flower extract showed better cytotoxicity than leaf extract. Furthermore, boiled extract of flower had higher cytotoxic effect than brewed extract. The viability of normal cells was under 50%. **Conclusion:** Although *M.sylvestris* had significant cytotoxic effect against 4T1 compared to the normal cells, the range of normal cell's viability was not acceptable according to the standards; therefore, did no clinical trial was performed. More research is needed about the extraction of the plant and alteration of its chemical structure.

Innate Immunity & Inflammation

Oral Presentations:

7526O

The effects of hydro-alcoholic extract of Hypericum Perforatum on acute peritonitis induced by Zymosan in NMRImice

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Introduction: Zymosan-induced peritonitis model can be used to study the recruitment of monocytes and neutrophils into the peritoneal cavity and to study the effects of existing and novel anti-inflammatory drugs. This study was conducted to evaluate the effects of hydro-alcoholic extract of Hypericum Perforatum on acute peritonitis induced by Zymosan in NMRI mice. **Material and Methods:** Sixty male NMRI mice were randomly allocated in 5 equal groups and treated with 100, 200 or 400 mg/kg of hydro-alcoholic extract of H.Perforatum (treatment groups), 25 mg/kg indomethacin (positive control group) or normal saline (negative control group) 1 hours before the induction of peritonitis. To induce peritonitis, each mice intraperitoneally received 10 µg of zymosan in 0.4 ml of saline. After 48 h, the peritoneal cavity was lavaged by 1 ml of cold PBS and the isolated cells used for differential count, nitric oxide production and respiratory burst potential. **Results:** The nitric oxide production and respiratory burst in exudate cells of the peritoneal lavage were significantly decreased in mice receiving H. Perforatumat 200 and 400 mg/kg orindomethacin, compared to mice received normal saline. Total cell number in peritoneal cavity showed significantly decreased in all treatment group. However, the proportion of different cells didn't show any

significant difference between treatment groups. **Conclusion:** The hydroalcholic extract of H. Perforatum may be a suitable natural source of control inflammation. **Keywords:** Peritonitis, Zymosan, Hypericum Perforatum.

10837O

The effects of LPS-primed mesenchymal stem cells after challenging with nicotine on the functions of neutrophils

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Introduction: Pervious documents suggested that LPS-primed mesenchymal stem cells (MSCs) secrete pro-inflammatory cytokines. It is also clear that these cells can express some subunits of nicotinic receptors. Moreover, functional interactions between MSCs and neutrophils upon bacterial endotoxin have been demonstrated. This study was conducted to investigate the effect of the nicotine on the crosstalk of lipopolysaccharide (LPS) activated MSCs on neutrophils. **Material and Methods:** After isolation of mesenchymal stem cells from bone marrow of rats, these cells stimulated with 10 ng/mL LPS for 1 h producing inflammatory condition) and then pulsed with different concentration of nicotine (5, 25 and 50 µl) for 48 h. Then, MSCs co-cultured and incubated with neutrophils for 4 h and the functions of neutrophils were evaluated**. Results:** The lifespan of neutrophils co-cultured with LPS-stimulated MSCs treated with nicotine at 25 µl significantly increased in dose independency compared to control groups. Furthermore the neutral red uptake and the respiratory burst of neutrophils co-cultured with LPS-stimulated MSCs treated with nicotine significantly increased compared to other groups. **Conclusion:** These findings revealed that nicotine can alter the crosstalk between MSCs and neutrophils after LPS challenge. **Keywords:** Mesenchymal Stem Cell, Lipopolysaccharide, Neutrophil, Nicotine.

11047O

Stimulation of innate immunity by Hibiscus tea

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Introduction: Hibiscus tea is an herbal tea made from sepals of Hibiscus sabdariffa. Pervious study has shown that drinking hibiscus tea may lower blood pressure in people with type 2 diabetes,or mild hypertension. The present study was conducted to evaluate the immumo-modulatory potentials of the aqueous extract of hibiscus tea. **Material and Methods:** The study population consisted of 20 male Wistar rats that randomly divided into two equal groups. Treatment group rats received orally aqueous extract of H. sabdariffa (500 mg/Kg) every day from the beginning of the study for 4 weeks. At the end of study, macrophages isolated from rat peritoneal cavity and the neutral red uptake, phagocytosis, respiratory burst and nitric oxide production were evaluated after challenge with opsonized yeast in these population. The Mann-Whitney test using SPSS 21 software was used to compare the results. **Results:** The results indicated that neutral red uptake,phagocytosis, respiratory burst and nitric oxide in treatment group were increased to 40%, 55%, 32% and 17%, respectively, compared to the control group. **Conclusion:** The

aqueous extract of hibiscus tea may be used as a natural source of innate immunity stimulation. **Keywords:** Hibiscus sabdariffa, innate immunity, Phagocytosis, Respiratory burst, Nitric oxide.

11094O

Increased serum levels of interleukin (IL)-27 in patients with ischemic heart disease

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Introduction: It has been reported that IL-27 has potent pro- and anti-inflammatory effects. The aim of this study was to evaluate the serum levels of IL-27 in a group of patients with ischemic heart disease (IHD) including unstable angina (UA), and also to clarify its association with traditional risk factors of disease. **Material and Methods:** A total of 60 patients with IHD as unstable angina (UA; n=60) and 60 sex- and age- matched healthy subjects as a control group were enrolled in this cross-sectional, case-controlled study. Serum samples were collected from all participants (for UA at admission time) and tested for the levels of IL-27 by use of ELISA method. **Results:** The mean serum levels of IL-27 in UA group (35.77± 18.93Pg/mL) was significantly higher than that observed in control group (24.91± 14.96Pg/mL; P<0.001, respectively). The mean serum levels of IL-27 in IHD patients with or without a certain traditional risk factor including hypertension, dyslipidemia, diabetes and smoking was significantly higher compared to control group. **Conclusion:** These results revealed that the higher serum levels of IL-27 were associated with unstable angina. The presence or absence of a certain traditional risk factors of IHD did not influence the serum levels of cytokine. **Key Words:** Ischemic heart disease, Unstable angina, Interleukin-27

11307O

The result of the gel extracted from the medical leech Hirudo Medicinalis in patients with OA of the knee

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Introduction: Knee osteoarthritis (OA) is a degenerative, progressive disease which destroys joint cartilage, thus, led to inflammation, joint pain, stiffness, limitation in range of motion and finally at long term, major disability. According to previous studies, compared to non-steroidal anti-inflammatory drugs (NSAIDs) as rution OA treatment approach, leech therapy seems to be safer. So in this study leech saliva extract (LSE) was used in the liposome base gel as supplementary treatment to relief the signs and symptoms of OA patients. **Material and Methods:** Saliva of medical leech (Hirudo medicinalis) was extracted. SDS-PAGE was conducted to confirm LSE protein complexes. Nano scale liposomes were used to formulate this supplement and to enhance skin absorption. Then, formation of the nano scaled complexes were assessed by Dynamic light scattering (DLS) and size of nanoparticles were measured by Zeta-Plus instrument. Clinical trial was designed to evaluate the therapeutic effects of LSE liposomal gel in 60 patients with confirmed OA in one month. Lenquesne and VAS questionnaires were applied as indexes of this supplement therapy efficacy. **Results:** LSE liposomal gel containing 15 mg protein with the size of nano-

liposomes about 200 nm was prepared. Results of questionnaire analysis demonstrated that after one month administration of LSE liposomal gel, patients' pain relived approximately up to 50 %. Also, due to the reduction in joint inflammation and stiffness, patient range of motion increased and finally, life quality was enhanced. **Conclusion:** LSE liposomal gel as an innovative supplement therapy in OA patients makes willingly therapeutic approach which seems to have a significant impact on patients' quality of life and self- care capability. **Keywords:** Knee osteoarthritis; leech therapy; leech saliva extract (LSE); liposome

Poster Presentations:

3470P

Effect of pentoxifylline on the serum level of interlukin-6 in patients with non-acoholic steato-hepatitis

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Introduction: Non-alcoholic steato-hepatitis (NASH) is a progressive form of non-alcoholic fatty liver disease (NAFLD). The pathogenesis of NASH is multifactorial, including inflammation. Pentoxifylline (PTX) is administered for treatment of NASH. It shows anti-inflammatory properties. We aimed to investigate the effect of PTX on the serum levels of interleukin 6 (IL-6) in patients with NASH and to compare its effect on placebo group. **Material and Methods:** Thirty patients with NASH enrolled in the study. Fifteen patients of the intervention group received PTX and 15 patients of the placebo group received placebo for 6 months. Patients were selected based on sonography and a 1.5-fold mean change from baseline of liver aminotransferases levels. Enzymatic photometry and enzyme-linked immune-sorbent assays were applied to detect the liver aminotransferases and serum levels of IL-6, respectively. **Results:** The serum levels of IL-6 and the liver aminotransferases were significantly decreased in the intervention and placebo groups at the end of six months ($P<0.0001$). However there was insignificant difference

between the placebo and the intervention groups. **Conclusion:** It seems that decrease in the serum levels of liver aminotransferases and IL-6 in both groups are related to low-calorie diets and exercise rather than PTX.

3471P

Interleukin 8 as a valuable biomarker for diagnosis of irritable bowel syndrome

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Introduction: Irritable bowel syndrome (IBS) is the most common gastrointestinal disorder and classified into diarrhea predominance (D-IBS), predominant constipation (C-IBS) and alternating forms (A-IBS). The gastrointestinal immune response is regulated by the balance between pro- and anti-inflammatory cytokines. Interleukin 8 is a potent inflammatory chemokine. The aim of this study was to investigate the relationship between the serum levels of IL-8 and IBS. **Material and Methods:** A total of 150 subjects, including 75 healthy controls from Kerman blood transfusion centre and the 74 patients with IBS, were enrolled in the study. Serum levels of IL-8 were measured by enzyme-linked immune-sorbent assay and compared between patients with IBS and healthy controls. **Results:** The serum levels of IL-8 were significantly higher in patients with IBS as compared to controls (P <0.001). **Conclusion:** Higher serum levels of IL-8 in patients with IBS than controls, suggested an important role for this cytokine as immune mediators in pathogenesis of IBS. Interleukin 8 might be a valuable biomarker for diagnosis of IBS.

4495P

Association of serum levels of PAI-1, MDA and hs-CRP in Non-Smoker and Diabetic patients withCoronary Artery Disease

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Introduction: PAI-1 (Plasminogen activator inhibitor), a novel biomarker in diagnosing CAD (Coronary artery disease), is a central component of the fibrinolytic system. It has reported that CAD is associated with atherogenic lipid profile, higher plasma levels of PAI-1, increased risk of intravascular thrombosis, stress oxidative (MDA) and inflammation (hs-CRP). The study was aimed to evaluate the association of fibrinolysis marker PAI-1 with stress oxidative and hs-CRP in CAD with Non-smoker and Diabetic. **Material and Methods:** 100 subjects (50 patients and 50 controls) were selected for testing. Risk factors PAI-1, MDA and hs-CRP were compared between 50 angiographically diagnosed CAD patients and 50age/sex matched healthy subjects. Furthermore, association between these risk factors with CAD was evaluated. **Results:** Association between Plasma PAI-1with serum levels of MDA and hs-CRP with CAD were significant. The main factors including PAI-1, MDA, hs-CRP and blood sugar were significantly different between two groups of patient and control ($p<0.05$). **Conclusion:** A significant association was found between serum level of PAI-1, MDA and hs-CRP with Coronary Artery Disease in Non-

Smoker and Diabetic patients. **Key Words:** Plasminogen Activator Inhibitor-1, MDA, hs-CRP, Coronary Artery Disease, Non-Smoker, Diabetic

7607P

Immunosuppressive effects of 4-nitroaniline in NMRI-mice

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Introduction: 4-nitroaniline is widely applied as an intermediate in the synthesis of dyes, antioxidants, pharmaceuticals, gasoline and poultry medicines, and as a corrosion inhibitor. This study was conducted to check the effects of 4-nitroaniline on immunity system of NMRI-mice challenged with sheep red blood cells (SRBCs). **Material and Methods:** The study population consisted of 14 male mice that randomly allocated in two equal groups and immunized with SRBC. Mice in the treatment group received trifluralin (75mg/kg-orally-0.01 LD50) every day from the beginning of the study and continued for 2 weeks. **Results:** The results of the present study indicated a significant decrease in the level of anti-SRBC antibody and simultaneously a significant decrease in the level of delayed type of Hypersensitivity (DTH) in the treatment group compared to control group. Furthermore, the level of respiratory burst phagocytic cell and also lymphocyte proliferation of splenocyte population concurrent with spleen weight index were significantly decreased in the treatment group compared to control group. **Conclusion:** This study indicated that 4-nitroaniline even in low dose led to a significant suppression in immunity system. **Key words:** 4-nitroaniline, Humeral immunity, Cellular immunity.

10908P

Molecular Cloning, Overexpression and Binding Analyze of Serum Amyloid A (SAA) to Bacteria

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Introduction: SAA (an acute phase protein), has two basic functions in immune system. The first is the induction of extracellular matrix-degrading enzymes which are important in tissue damage repair. The second function is the chemo-attraction of immune cells. In spite of SAA role in innate immunity, identity of potential SAA ligand(s) remains unclear. In order to understand its innate immune mechanisms in the antibacterial response, the present study investigated the molecular characteristics and binding activity of the SAA.**Material and Methods**: SAA cDNA was cloned and expressed. The recombinant SAA protein was purified by affinity chromatography with Ni-agarose. The resulting protein was analyzed by SDS-PAGE. SAA binding assays were performed for different bacteria. The microbes were incubated with the SAA protein, and the microbial pellets were assessed by western blot using anti-SAA serum. **Results:** After cloning cDNA of SAA, plasmid pE T21a (+)-SAA was transformed into *E. coli* BL21 for expression. Then, recombinant protein was purified and subjected to SDS-PAGE analysis and one

band at 20 kDa was visualized by stainig. Our results demonstrated that SAA is bound to a variety of Gram-negative bacteria in suspension including *E.coli*. **Conclusion:** It was shown that SAA binds strongly to a range of Gram-negative bacteria and that the ligand OmpA may well have a pathogen-associated molecular pattern, since it is conserved across the Gram-negative bacteria.

10983P

Comparison of inflammatory factors in patients with oral lichen planus and squamous-cell carcinoma (SCC) of the oral cavity

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Introduction: Lichen planus is a relatively common chronic skin-mucous membrane disease that affects the oral mucous. Some evidence suggested that the immune system may play a role in the lichen planus formation and progress. This survey was carried out with the aim of exploring serum levels of CRP, IL1α, IL6, and TNFα in oral lichen planus and SCC of the oral cavity. **Material and Methods:** In this cross-sectional study, 25 patients with lichen planus and 25 patients with oral SCC were chosen, as well as 25 healthy individuals, as the control group. IL1α, TNFα, IL6, and CRP were measured in serum and in order to compare the means one-way variance analysis and SPSS16 was applied (α=0.05).**Results:** The mean level of IL6, TNF, and CRP in a group of patients with lichen planus were 37.23ng/ml, 26.5pg/ml, and 41.16 ml/dl respectively, in addition to the group of patients with SCC of the oral cavity they were respectively 49.53ng/ml, 10.64pg/ml, and 48.35ml/dl and in the control group 39.37, 33.29 ng/ml and 9.7pg/ml. IL6 and CRP means were higher in the carcinoma group than the other two groups. On the other hand, CRP in oral lichen planus group was significantly higher in normal group, however; the value of IL1 α mean was not significantly different among various groups. **Conclusion:** According to the results, although IL6 and CRP serum levels were increased in patients with SCC of the oral cavity and IL6 and TNFα levels rise in patients with lichen planus, there was no meaningful difference in IL1α level of the two mentioned groups compared to the control group. **Key words:** oral lichen planus, SCC of the oral cavity, IL6, CRP, serum

11010P

Sex and Exercise Interaction to Alter the Salivary Immune Response

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Introduction: The incidence of immune deficiency is connected with the disorder of immunoglobulin levels secretion. So the aim of this study was the gender differenciation and salivary immune response to graded exercise test. **Material and Methods:** Fifty- six healthy male and female children (10-12 years old) were volunteered in this study. male and female separately according to gender differences randomly divided to the control groups (n=12 females, n= 12 males) and exercise groups (n=12 females, n= 12 males). Participants' salivary samples, body composition, VO2 max were collected in the two stages, before and immediately after a session of graded exercise test. ELISA method was used to measure the immunoglobulin A. Our data have been analyzed by independent t-test on SPSS software. **Results:** Immediately after exercise, IgA salivary levels in females exercise group compared to control females group were decreased significantly (p=0.012). But in males, IgA salivary levels in the exercise group compared to control group showed a significant increase (p=0.021). **Conclusion:** Intensive exercise has different effects on salivary immune response in gender differenciation. The results revealed that the females' immune system is weakened. However, graded exercise test led to improvement of the males' immune system.

11011P

Sex Differences in Serum Cortisol Levels in Response to Graded Exercise Test

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Introduction: Cortisol stimulates and activates inflammatory pathways under the conditions that result from over-activity of the B-cell-mediated antibody response such as inflammation and allergies. The aim of this study was to examine the efffect of sex differences in serum cortisol levels in response to graded exercise test. **Material and Methods:** Inactive healthy males (11 ± 1 yr) and females (11 ± 1 yr) participated in this study and randomly divided into the control and exercise groups (females: n=12 control, n= 12 exercise; males: n=12 control, n= 12 exercise). Exercise groups did a graded exercise test (grade: 5%, speed: 12 km/h, time: 20 minutes) on the treadmill. Participants' blood samples were collected in two stages; before and immediately after a session of graded exercise test (GXT). ELISA method and demediteckit was used for measurement of serum cortisol levels. The data have been analyzed by independent t-test in SPSS (v. 21) software. **Results:** The result revealed hat GXT can change the serum cortisol levels. In both exercise groups of males and females, serum cortisol levels after exercise compared to control groups and base conditions indicated a significant increase (p=0.001), but in the control groups cortisol levels didn't have a significant change before and after exercise(p=0.85). **Conclusion:** The result revealed that incremental exercise in non-active pediatrics led to moderate inflammation and allergy by changing the hormonal markers.

11030P

Inflammatory and Immune Markers Response to Combination Test LIST-Hockey Skills in National Team of Field Hockey Players

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Introduction: Immune system and inflammatory markers could be changed; since exercise is a complex stimuli involving mechanical loading, metabolic disturbances, neuronal activation and hormonal alterations. The aim of the present study was to examine the responses of inflammatory and immune markers to combination test LIST-Hockey Skills in national team of field hockey players. **Material and Methods:** 11 elite hockey players with 2 years background of national and international tournaments (BMI = 23.09, VO2Max = 50.80) after completing the health questionnaire and consent form participants in research. Before an hour (t1), two minutes (t2) and one hour (t3) after performing the combined test LIST-Hockey Skill that consists of two parts of the hip protocol and lafburu shuttle test skill, blood samples were collected from the brachial vein. Then, blood samples were analyzed for detection of MPO (Myeloperoxidase), lactate concentrations and hemoglobin mass. The research data were analyzed by statistical test of the repeated measure method. **Results:** The results showed that lactate concentrations in (T2) compared to (T1) and (T3) increased significantly ($P \leq 0.001$). But MPO levels in three modes (T1, T2, T3) had no significant difference ($P \leq 0.0$ 86). Also, the concentration of hemoglobin mass in each three times (T1, T2, T3) were not significantly different ($P \leq 0.0$ 22). **Conclusions:** The present study revealed that combination test of LIST-Hockey Skills on MPO levels and hemoglobin mass in elite field hockey players had no significant effect but, lactate levels had significant increase after exercise which can affect the immune- inflammatory response.

11082P

G2013 suppress inflammation via targeting IL-1 receptor associated kinase 1(IRAK1)

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Introduction: Toll like receptor 4 (TLR4) is one of the receptors involved in inflammation that can be activated by Lipopolysaccharide (LPS) of gram negative pathogens. One of the signaling molecules of activated TLR4 is IL-1 receptor associated kinase 1 which can induce inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) are broadly in use but unfortunately they are not completely effective besides high cytotoxicity. G2013 is a new designed NSAID with low molecular weight and cytotoxicity can be a new candidate for anti-inflammatory treatments. Here, the anti-inflammatory effect of G2013 on expression of IRAK1 in HEK 293-TLR4 cells was investigated. **Material and Methods:** The cytotoxicity of G2013 was assessed by MTT. Then, HEK293-TLR4 cells were treated with different doses of)5 and 25 µg/ml) G2013 with and without LPS (100ng/ml). Then the expression level of IRAK1 was evaluated in comparison with β actin by quantitative real time PCR. **Results:** Our results showed that IC50 of G2013 was 25µg/ml ($p<0.05$). The expression level of IRAK1 in HEK293-TLR4 cells after treatment by 5 and 25µg/ml of G2013 decreased to 7.14($p=0.0002$) and 7.69 ($p=0.0002$) fold, respectively. After pretreatment with LPS, the expression ratio of IRAK1 with the same concentrations of G2013 declined significantly to 4.34 ($p=0.0003$) and 6.25($p=0.0002$) fold. **Conclusion:** G2013 as a novel NSAID can suppress TLR4 signaling pathway which makes it a new substitution for prevalent toxic anti-inflammatory drugs. Indeed during LPS related inflammation, G2013 suppressed the TLR4 activation in competition with LPS.

11085P

M2000 as a new agent, inhibit inflammatory pathways by targeting suppressor of cytokine signaling (SOCS1)

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Introduction: M2000 as a novel non-steroidal anti-inflammatory drug (NSAID) with low molecular weight, high tolerability and efficacy, has shown its therapeutic effects in various experimental models. Toll like receptor 2 (TLR2) is one of the innate immunity receptors involved in inflammation that can be activated by various types of ligands such as, Lipopolysaccharide (LPS). Activation of TLR2 leads to producing inflammatory cytokines via inhibiting suppressor of cytokine signaling (SOCS1). Here, the anti-inflammatory effects of M2000 on expression level of TLR2 downstream adaptor molecule (SOCS1) in the HEK 293 TLR2 cell line were investigated. **Material and Methods**: The cytotoxicity of M2000 was evaluated by MTT assay. Afterward, HEK293-TLR2 cells were treated with different concentrations of M2000 and LPS (1µg/ml). Total RNA of treated cells and control group was extracted by trizol and c-DNA was synthesized. The expression level of SOCS1 was assessed by quantitative real time PCR. **Results:** The MTT results demonstrated that optimum dose of M2000 was up to 25µg/ml ($p<0.05$). The expression level of SOCS1 in HEK293-TLR2 cells after treatment by 5 µg/ml and 25µg/ml of M2000 increased 1.425(1.42±0.1207 p=0.0237) and 2.014 (2.014±0.04424 p=0.0003) fold induction respectively. Its expression level after treatment by LPS, decreased 2.12 fold induction (0.470±0.2635 p=0.0090). After co treatment with LPS and M2000 in low and high dose, expression level of SOCS1 increased 1.38 (1.389±0.1746, p=0.0317) and 1.70 fold induction (1.70±0.0206 p=0.0024) compared to control group. **Conclusion:** M2000 as nontoxic NSAID could decrease the production of inflammatory cytokines and can be utilized in inflammatory diseases. **Key word:** SOCS1, M2000

11088P

TLR4 signaling and Intestinal epithelial

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Introduction: Intestinal homeostasis depends on balanced innate immune responses and a healthy micro biota. We still do not know what constitutes a healthy micro biota, nor do we understand the interdependence of host signaling and cognate flora. If innate immune signaling is too weak, the host may be exposed to potential pathogens. If too vigorous, innate immune signaling may lead to detrimental inflammation in face of the harmless commensals. **Results:** In IBD, either as a cause or an effect of the disease process, epithelial expression of TLR4 is increased. In addition to IBD, TLR4 expression is increased in colitis-associated cancer as well as in some sporadic colon cancers. Several studies have demonstrated that the micro biota composition of the host is influenced by the status of TLRs and their adapter proteins. Although in vivo diseases demonstrated TLR over-expression, most studies have used global knock-outs or epithelial knock-outs of innate immune receptors (or adapters) to address the role of innate immune signaling on the epithelium. The studies demonstrated that the micro biota is altered in terms of quantity, composition and richness in response to increased TLR4 signaling. Also the micro biota differences between villin-

TLR4 and WT littermate mice were apparent at the mucosal lining and in the lumen, and were transmissible. Studies showed that epithelial TLR4 signaling has differential, site-specific effects on epithelial cell differentiation, barrier formation and AMP gene expression. TLR4 regulates the development of goblet cells in the small intestine. Also it was shown that constitutive epithelial TLR4 signaling affects crypt-villus structure of the intestine. The results of these studies implicated that TLR4 expression regulates defensins expression by affecting Paneth cell differentiation in the small bowel. Paneth cells play a role in limiting bacterial translocation. **Conclusion:** Based on these findings, it can be concluded that increased epithelial TLR4 signaling has clear effects on epithelial function and the micro biota. Also changes in epithelial function and the micro biota may contribute to the perpetuation of inflammation.

11111P

Association of Manganese superoxide dismutase) MnSOD rs4880 (gene polymorphism with the risk of periodontitis disease

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Introduction: Generalized aggressive periodontitis as a subtype of periodontal diseases is defined by the rapid destruction of periodontal supporting tissues. The inflammatory responses and oxidative stress play an important role in pathogenesis of periodontitis disease. So, polymorphism analyses of genes involved in inflammation and oxidative stress response can be effective in better understanding of the pathogenesis of the disease. Partial reduction of O2 in electron transport chain leads to the formation of highly reactive oxygen species (ROS), including the superoxide anion (O_2^_). To confront oxidative stress caused by ROS, Manganese superoxide dismutase (MnSOD) catalyzes the ROS. Moreover, Superoxide anions have pro-inflammatory roles. So elimination of superoxide anions by MnSOD and its iso-enzymes can be considered to be anti-inflammatory. The aim of this research was to evaluate the association of Manganese Superoxide Dismutase polymorphism (MnSOD rs4880) with periodontitis disease. **Material and method:** DNA was extracted from peripheral blood leukocytes in 50 patients and 100 healthy individuals. The MnSOD Val-9Ala polymorphism was analyzed using restriction fragment length polymorphism (PCR-RFLP). **Results:** The genotypes frequencies of Ala/Ala, Ala/Val and Val/Val in healthy individuals were 25, 66 and 9%, respectively. In periodontitis patients, genotypes frequencies were as Ala/Ala (12%), Ala/Val (50%) and Val/Val (38%) genotypes. There was a significant association between distribution of MnSOD rs4880 genotypes and the risk of periodontitis disease ($p<0.05$). **Conclusion:** The results indicated that MnSOD Val-9Ala gene polymorphism had a positive association with the risk of periodontitis disease. **Key words**: MnSOD rs4880, Gene polymorphism, Periodontitis disease

11137P

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

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Introduction: Chemokines, a subclass of cytokine superfamily, have both pro-inflammatory and migratory roles and serve as chemo-attractant 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. The inhibition of secondary injury through targeted chemokine therapy, accurate knowledge about the temporal profile of these cytokines following SCI are required. **Material and Methods:** The serum levels of CXCL-1, CXCL-9, CXCL-10 and CXCL-12 at 3-6h, 7 and 28 days and 3m after SCI in male and female SCI patients (n=78) were quantitated by using the commercially available ELISA kits and compare to age- and sex-matched patients with non-spinal cord injuries (n=70) and healthy volunteers (n=100). ANOVA with Tukey post hoc analysis were used to determine the differences between the groups. **Results:** The serum levels of CXCL-1, CXCL-9 and CXCL-10 peaked on day 7 post-SCI and then declined to the control level. 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 for CXCL-12 compared to control and SCI female. Any change was observed in chemokines' level of NSCI. Further, the age of the patients did not influence chemokines expression after SCI. **Conclusion:** These observations along with SCI-induced Cerebrospinal fluid-chemokine level should contribute to the identification of selective and temporal chemokine targeted therapy after SCI.

11205P

Investigation serum angiotensin 2 and vitamin D in steatohepatitis patient.

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Introduction: Angiotensin is a peptide hormone, causes vasoconstriction and a subsequent increase in blood pressure. It is renin-angiotensin system, which is a major target for drugs that lower blood pressure. Angiotensin stimulates the release of aldosterone, another hormone. NAFLD includes a spectrum of diseases from simple fatty liver disease, to non-alcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma (HCC). NASH is the only bridge between NAFLD and liver cirrhosis. Statistical data show that 15%-20% of NASH patients will develop liver cirrhosis in10 to 20 years, suggesting the risk of disease progression is much higher. **Methods & material:** Serum of 95 patients with fatty liver disease and 50 healthy controls was taken and three parameters including: vitamin D, Angiotensin, CRP was done. The method of assay was ELISA for vitamin D and Angiotensin 2 and nephelometry for CRP. Statistical analysis was done by Prism. **Results:** Our data showed that patient with fatty liver had significant lower vitamin D, higher level of angiotensin 2 as an inflammatory factor. there was no significance difference between CRP level in control and patient. **Conclusion:** there is relationship between low

level of vitamin D and fatty liver, particularly when angiotensin 2 is high in positive. But in this study did not show relationship CRP between control and patient. **Keywords:** vitamin D, CRP, steatohepatitis.

11236P

Boiling water and released proteins from human skin fibroblasts

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Introduction: In scald burning (hot water burns) skin, cells are damaged and in turn some inflammatory substances are released leading to systemic inflammation. This inflammation especially when taking place in vital organs can result in death. Since fibroblasts are the most important cells in skin. We encouraged to determine the electrophoresis pattern of released proteins from fibroblasts of human skin that have been treated by boiling water in-vitro. **Material and methods :**Human dermal fibroblasts were cultured in 25cm² cell culture flask. After reaching the cells to %80 confluence, 2ml of boiling water was added into flask. After centrifugation, the total protein concentration of supernatant was measured by Bradford method. Then, after concentrating with cold acetone, SDS- Polyacrylamide gel electrophoresis was performed and the developed protein bands were stained with Coomassie blue and molecular weight of proteins were obtained using Standard curves. **Results:** The total protein concentration before acetone precipitation was 20µg/ml. Then, 20 micrograms of these proteins was added to each well of electrophoresis. As the result of electrophoresis, there were 7 types of proteins with molecular weights of 88, 78, 71, 59, 47, 38 and 37 KDa, which the band related to the protein with 71KDa appeared as the densest band. **Conclusion:** The data originated from this study showed that skin fibroblasts treated with boiling water can release some proteins which are good candidate for systemic inflammation inducing agents in scald burning.

11277P

8 Weeks Resistance Training Reduces Inflammatory Cytokine Index

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Introduction: Inflammatory system showed different reactions in response to physical activity. So the aim of this study was to investigate the effects of 8 weeks resistance training on changes of the inflammatory cytokine index. **Material and Methods:** A total of 24 inactive healthy middle-aged men (age= 40-50 years) after completion of a health questionnaire participated in this research and randomly divided into control (n=12) and exercise (n=12) groups. Exercise group were performed eight weeks resistance exercise training (60 min/day, 3 days/week) by 60% of one repetition maximum (1RM) on the first week until 90% (1RM) in last week. Blood samples of volunteers were collected in 24 hours before and after the exercise protocol. ELISA method was used to measure the TGF-α serum levels. Independent t-test for analysis of data by using SPSS (v. 22) software was performed with a significant level of P <0.05. **Results:** The results revealed the TGF-α level of serum, 24 hours after the resistance exercise

protocol in comparison with the control group and basal level decrease significantly ($p<0.002$). **Conclusion:** The results showed that 8 weeks resistance training in inactive middle-aged men by reduction of the TGF-α serum levels as the most important inflammatory cytokine can prevent the incidence of inflammatory diseases.

12400P

Regulation of neutrophil

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Introduction: Neutrophils are the most abundant white blood cells in the human circulation.it play a major role in non-specific host defenseagainst pathogen invasion, and they also participate in the development of the inflammatory reaction. Excessive inflammatio cause morbidity and mortality in diverse human diseases.The aim of this study was to perform a systematic review and meta-analysis to determine endogenous and exogenousmechanisms regulate the innate immune responses and prevent excessive inflammation espicially in neutrophils. **Material and Methods:** A systematic search was performed to identify studies published in PubMed up to July 2013 and recently published abstracts were also reviewed.The studies utilized one of thefollowing methods:tested on animal models,cellculture,flowcytometry and serums that collected from patians and normal control **Result:** Some article demostrated The cholinergic anti-inflammatory pathway is a mechanism that regulates innate immune.they found that α7 nAChR+ alveolar macrophages and neutrophils were present in bronchoalveolar lavage and injured lungs of mice. acetylcholine released and effect on immune cell,so downregulated proinflammatory chemokine/cytokine generation.some studies showed that acute morphine treatment leads to inhibition of neutrophil cytokines involved in regulation of wound healing.other article said: β-Endorphin modulates the functional activity of lymphocytes and macrophage and neutrophil secretory functions.other researcher showed that increase concentrations of neuropeptides significantly afterchallenge with antigen so they have regulatory effects on neutrophils and other immune cells. **Conclusion:** there is little information on the effects of neuropeptides and opioid on neutrophil and intiflammatory processes, moreover studies are necessary to identify this mechanism.

12403P

How to Create Inflammation and Tissue Defensive Role and Restoration of Damaged Tissue?

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The process of inflammation is a physiological response to various stimuli such as infection and tissue damage. Though a helpful host immune response and tissue necrosis, they themselves can cause tissue damage. The aim of inflammation is to eliminate harmful factor and repair damaged tissue and then to reduce the reaction andrepairing damaged tissue. It is activated in two second phases vascular response cellular response. Although the phenomenon of inflammation is an important factor in protecting the body against damage, however, formation of tissue inflammation and its protective role has not been precisely identified. In this paper, we investigate how

inflammation is created and immune response of the tissues to repair damaged tissue. **Keywords:** vascular response, suburbia, adhesion, chemo taxis, phagocytosis, leukocyte function, restoration

12602P

Naturally Occurring Enzyme Inhibitors: A Smart Way to Fight against Micro-Inflammation in Human Gut

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Introduction: The gastrointestinal tract consists of a set of microbes that is known as gut flora. These microorganisms consist of over 1000 species. These microbes not only contribute to the food digestion but also play role in determining the susceptibility of host to gastrointestinal infection. At lower taxonomic levels, the microbiota of mammals is highly variable, however, four important phyla are: *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*.Sometimes there is a microbial imbalance inside the body, mainly in digestive tract, termed as dysbacteriosis or dysbiosis. This is linked to the bowel inflammation along with obesity and cancer. The changes that occur in the microbes have certain effects on the inflammatory and metabolic processes. **Material and Methods:** The structures of three enzymes nitroreductase, azoreductase and beta-glucuronidase were analyzed. Data regarding enzyme structures and their inhibitors was found from literature, Protein Data Bank (PDB) and Chemspider. Target sites of enzymes were then docked with the ligands using PatchDock. After reviewing Dr. Duke's Phytochemical and Ethnobotanical Databases and literature, different plants were found to have these enzymes inhibitors. The docking results were analyzed using the software PyMOL. **Results:** The active site blocker of nitroreductase (nicotinamide) ; azoreductase (riboflavin and allopurinol) and beta-glucuronidase (aspartic acid, glucaric acid and silymarin) were analyzed. It was found that the inhibitors blocked the active sites of their respective enzymes. **Conclusion:** The gut flora plays significant roles in digestion and metabolism of the host.Moreover, these microbes direct and maintain the immune system. Dysbiosis is spotted in various inflammatory diseases of human digestive tract. As a solution to this problem, numerous plants has been found to produce certain phytochemicals that help keeping the microbiota in human GI at a normal level, hence, preventing dysbiosis and other drastic inflammatory problems.

Innovation in Cancer Immunotherapy

Oral Presentations:

7588O

Construction and evaluation of a humanized single-chain antibody (huscFv)against EGFR-overexpressing tumors

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Introduction: Targeted cancer therapeutics are drugs that block the growth of cancer by interfering with specific molecules of cancer cells. Monoclonal antibodies are among the most specific agents recognized and bind to cancer cell surface antigens. Several recombinant formats of monoclonal antibodies have been developed such as full length antibody, Fab, scFv and etc.ScFvs are powerful tools and new reagents in biological research, diagnostic imaging and tumor therapy. Humanized scFv antibodies have been proposed to reduce the size and immunogenicity of murine and chimeric full length antibody. Here we describe the production of an anti-EGFR huscFv employing CDR-grafting method. Materials and methods: In the design of desired huscFv, the murine scFv sequence was humanized by CDR-grafting method. The huscFv was cloned and expressed in E. coli and affinity purified. The reactivity of huscFv was assessed by ELISA and the MTT assay was employed to assess the cell growth inhibition of huscFv on A431 cells. Result: The humanization of murine variable regions resulted in 13.7% increase inhumanness of hscFv. Expression analysis showed that the desired huscFv was produced with high concentration in E. coli. Investigation of hscFv reactivity on A431 cells revealed higher binding affinity and better growth inhibition capability comparing to murine counterpart. Conclusion: Results of reactivity and inhibition assay indicates that hscFv produced in this study can serve as a potent humanized single chain antibody to target EGFR- overexpressing tumor cells.Key word: Cancer targetedtherapy, huscFv, CDR-grafting

7667O

Identification of functional amino acids of antiEpCAMScFv:as potential traetment for cancer

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Introduction: Epithelial (EpCAM) is a trans membrane glycoprotein, which has a key role in cell– cell adhesion, proliferation, maintenance of undifferentiated states, as well as regulation of differentiation, migration, and invasion. So EpCAM can be a potential target in prevention and treatment of cancer. Anti EpCAMscfvis a single chain variable fragment of a monoclonal antibody which binds to EpCAM and kills Ep-CAM-positive tumors by apoptosis. This scfv has been investigated for treatment in bladder, head and neck cancer. Bioinformatics' tools enable researchers for engineering of binding proteins in order to improve some of their features. Binding proteins such as scfvs can be easily engineered and their catalytic and other functionally important residues can often be mutated to yield more stable proteins as well as high affinity antibody. Here we want to extend our knowledge on the oportuzumab antibody structure to provide protein-protein docking and identify functionally important residues in ligand binding site for development of antibody with betterfeatures. Materials and methods**:** Hereon, weprediced antiEpCAM scfv structure by using phyre2 server and then using Paratome web server at predicted the antigen-binding regions (ABRs) of an antibody, given its amino acid sequence or 3D structure. Finally, the functionally and structurally important residues identified in protein sequences using conseq and proABC.**Conclusion:** According to significant role of antibody in diagnosis and treatment, antibody variants with high affinity to the EpCAM can be selected by computer based methods, and after confirming experimental results they can be used for prevention of several cancers.

10922O

Construction of a trispecificantibody (tsAb) encoding IL-15 for cancer immunotherapy via retargeting NK Cells to Newcastle Disease Virus (NDV)-Infected tumor cells

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Introduction: Activation of Natural Killer (NK) cells against the target-tumor cells gained high importance for cancer immunotherapy., Bispecific Antibodies (bsAbs) are the artificially designed molecules that can bind simultaneously to tumor cells and immune cells. To this end ortsAb which additionally encode for interleukins (IL) was invented Herein, we describe construction of a tsAb encoding for IL-15, anti-CD16 (FcγRIII) and anti-HN-hem agglutinin neuraminidase (NDV-HN) for activation of NK cells against NDV infected tumor cells. **Material and Methods:** The pcDNA3.1 plasmid encoding for anti CD16-anti NDV-HN bsAb (gifted by Dr. Momburg-Germany) used for insertion of the synthetic human IL15 cDNA. The final construct was used to stably transfect the Human Embryonic Kidney (HEK) cells using lipofectamine under G418 pressure. Secreted Abs were purified from media by protein A-columns and analyzed by SDS-PAGE, western blotting and Flow cytometry. **Results:** Restriction analyses and sequencing results indicated the proper insertion of the IL-15 sequence (378 bp) and construction of the tsAb encoding plasmid. PCR analyses on DNA extraction of the HEK cells (using IL-15 specific primers) indicated the proper integration of the insert (378 bp) in stably transfected-cells. SDS-PAGE and Western Blot analyses (using anti-human IgG-HRP conjugate) on purified Abs indicated the expression of the 98 kD band corresponding to the trispecific antibody. Flow cytometry results indicated the proper binding specificities of the constructed tsAb. **Conclusions:** The constructed tsAb properly encoded IL-15 while maintaining the proper binding properties of its specific Abs for further *in vitro/in vivo* tumor targeting studies via activation of NK cells.

11144O

Production and Characterization of Specific Antibody to Recombinant Fibromodulin Protein: A Potential Tool for Chronic Lymphocytic Leukemia Diagnosis

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Introduction: Fibromodulin (FMOD) is a 42 kDa protein which belongs to small interstitial leucine-rich repeat proteoglycans family. *FMOD* transcript and protein are uniquely overexpressed in B-cells of chronic lymphocytic leukemia (CLL) with lack of expression in normal B-cells. Therefore, FMOD is considered as a tumor-associated antigen which can be used for diagnostic and therapeutic approaches in CLL. In this study, recombinant FMOD protein was applied for production of anti-FMOD polyclonal antibody that may be a potentially valuable tool for CLL diagnosis. **Materials and Methods**: FMOD gene was amplified from leukemic cells of CLL patients by RT-PCR and the purified templates were cloned in prokaryotic expression vectors. Recombinant protein expression in bacteria was optimized using different vectors, hosts, induction and cultivation protocols. FMOD protein was purified by Ni-NTA and verified by immunoblotting. The purified protein was mixed with Freund's adjuvant and then intramuscularly injected to New Zealand white rabbits. Anti-FMOD antibody response was measured by ELISA. Anti-FMOD polyclonal antibodies were affinity purified by FMOD-affinity chromatography column. Finally, the reactivity of the purified polyclonal antibody with FMOD protein and CLL was checked by ELISA and immunoblotting. **Results:** Successful expression of FMOD protein was obtained in FMOD-pET22b(+) transformed Rosettagami cultured at 37˚C overnight. Purified FMOD protein was verified by immunoblotting using commercial anti-His-tag and anti-FMOD antibodies. Serum titration of immunized rabbits showed successful immunization with FMOD protein. Native CLL FMOD protein was detected by biotinilated anti-FMOD polyclonal antibody in both sandwich ELISA as well as immunoblotting. **Conclusion:** Our data suggested that the produced polyclonal anti-FMOD antibody can detect native FMOD in CLL and may potentially be applied for the diagnosis of CLL cells.**Key words:** chronic lymphocytic leukemia, ELISA, Fibromodulin, immunoblotting, polyclonal antibody, recombinant protein

11156O

Vaccination with Recombinant HER2 Extracellular Subdomains ProteinsInduces Th1 Responses and inhibitsTumor Growth in HER2 Positive Breast Cancer Mouse Model

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Introduction: HER2, as an EGFR tyrosine kinase family member, is overexpressed in 25-30% of breast cancer patients. Successful therapeutic effects of commercial monoclonal antibodies high lights the importance of this molecule in cancer targeted therapy. In this study, we investigated the protectivity and immunological responses induced by active vaccination using recombinant proteins of extracellular HER2 subdomains**. Materials and methods:** Prokaryotic recombinant proteins of HER2 extracellular subdomains (DI, DII, DIII, and DIV) were purified using Ni-NTA. Balb/c mice were subcutaneously immunized with these emulsified proteins in Fraund's adjuvant. Anti-Her2 antibody responses were checked in immunized mice sera by ELISA. Furthermore, sera reactivity with eukaryotic HER2 protein was tested by ELISA, immunoblotting and flow cytometry. Subsequently, Immunized mice were challenged with HER2-transfected 4T1 cell line and tumor sizes were measured systematically. Besides, immunized mice splenocyteswere harvested and stimulated with corresponding HER2 recombinant proteins for 72 hr and then cytokine production was measured byELISA kits. **Results:** The immunized

mice sera strongly reacted with the corresponding subdomains indicating immunogenicity of all HER2 subdomains. Vaccinated mice sera could recognize eukaryotic HER2 protein by immunoblotting but not ELISA or flow cytometry. Growth rate of implanted HER2-transfected 4T1 tumor was delayed in HER2 vaccinated mice compared to control group during 48-day interval. In addition, tumor mass sizes were significantly smaller in DI (mean=92.5mm^3), DII (mean=131mm^3), DIII (mean=94mm^3) and DIV (mean=100mm^3) of HER2 vaccinated mice groups compared to control group (mean=217mm^3) at 18 days after tumor challenge. Finally, cytokine assay results indicated that HER2 subdomains could significantly induce Th1 and Th17, but not Th2 responses. **Conclusion:** Vaccination with prokaryotic recombinant HER2 subdomains could engage the cell-mediated immunity and inhibits the tumor growth in a mouse xenograft model. **Keywords:** breast cancer, vaccination, HER2, mouse model, recombinant protein, tumor challenge

11217O

Production and assessment of immunogenicity of recombinant HER2 extracellular subdomains: potential tools for tumor vaccination

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Introduction: HER2 is a member of the receptor tyrosine kinase family and an immunogenic target for breast cancerimmunotherapy. Structurally, the extracellular region of HER2 is composed of four subdomains which subdomains-II and -IV are targeted by the two therapeutic antibodies, trastuzumab and pertuzumab, respectively. In the present study, we produced and characterized all HER2 extracellular subdomains which are potentially valuable tools for active tumor vaccination. **Materials and Methods**: The extracellular subdomains of human *HER2* were individually amplified and subcloned in prokaryotic expression vectors and transformed into E.coli. Different vectors, hosts, induction and cultivation protocols were employed for the optimum expression. HER2 recombinant proteins were purified by Ni-NTA and verified by immunoblotting. The purified proteins were then injected together with Freund's adjuvant to Balb/C mice and anti-HER2-antibody levels were measured by ELISA. **Results:** Successful expression of subdomains-I and -III and subdomains-II and IV were obtained by pET-22b(+)-BL21-DE3 and pET32a(+)-Rosettagami, respectively. Optimization parameters showed that IPTG induction at OD_{600}=0.5 and incubation at 37˚C (5hr for I and III and overnight for II and IV) resulted in the highest expression levels as verified by immunoblotting using anti-His-tag-antibody. In addition, serum titration of hyper immunized mice demonstrated that all four HER2 subdomains induced anti-HER2-antibodies reactive with the corresponding subdomains. **Conclusion:** Our findings indicate that these subdomains are immunogenic and potentially valuable candidates for further studies such as protein vaccination and subdomain-specific monoclonal antibody production. **Key words:** breast cancer, HER2 extracellular domains, protein immunization, recombinant protein

Poster Presentations:

3493P

Evaluation of the cytotoxic effects of 1-3 bis(2-hydroxybenzilidene)thiourea on the K562 human tumor cell line.

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Introduction: Previous studies indicated that thiourea derivatives possess anticancer properties. This study was set out to evaluate the effects of 1-3 bis(2-hydroxybenziliden)thiourea, as an thiourea derivative, on K562 human erythroleukemia cell line and peripheral blood mononuclear cells (PBMCs) as normal control cells.Materials and Methods: The K562 cells or PBMCs (1×10^5 cells/100 µl/well) were incubated for different times (24, 48 and 72 h) with serial logarithmic dilution of analogue (0.25-250µg/ml) .At the end of incubation time , the survivalof treated cells was determined by MTT method. Results: Our data indicated that this compound had a profound cytotoxic effect on the k562 cell line in a dose dependent manner. Interestingly, the IC_{50} value of this compound for K562 was lower comparedto than IC_{50} value obtained from treatedPBMCs.Conclusion: As a result, this compound provide more favorable cytotoxicityin K562 cell line without any additive cytotoxicity inPBMCs.Key words: K562,PBMC, 1-3 bis(2-hydroxybenziliden)thiourea , MTT.

3494P

Evaluation of the cytotoxic effects of 4-bromo pyrimidine, on the K562 human tumor cell line

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Introduction: Previous studies indicated that prymidine derivatives possess anticancer properties. This study was set out to evaluate the effects of 4-bromo pyrimidine , as a pyrimidine derivative, on K562 human erythroleukemia cell line and peripheral blood mononuclear cells (PBMCs) as normal control cells.**Materials and Methods:** The K562 cells and PBMCs (1×10^5 cells/100 µl/well) were incubated for different times (24, 48 and 72 h) with serial logarithmic dilutions of analogue (0.10-100µg/ml) .At the end of incubation time, the survivalof treated cells was determined by MTT method. **Results:** Our data indicated that this compound had a profound cytotoxic effect on the k562 cell line in a dose dependent manner. Interestingly, the IC_{50} value of this compound inK562 cells was lower incomparison with IC_{50} value collected in PBMCs.**Conclusion:** As a result, this compound provide more favorable cytotoxicity against K562 cell line without any additive cytotoxicity against PBMCs.**Key words:** K562,PBMC, 4-bromo pyrimidine , MTT.

3495P

Evaluation of the cytotoxic effects of 2-methoxy pyrimidine, on the K562 human tumor cell line

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Introduction: Previous studies indicated that pyrimidine derivatives possess anticancer properties. This study was set out to evaluate the effects of 2-methoxy pyrimidine, as pyrimidine derivative, on K562 human erythroleukemia cell line and peripheral blood mononuclear cells (PBMCs) as normal control cells. **Materials and Methods:** The K562 cells or PBMCs (1×10^5 cells/100 µl/well) were incubated for different times (24, 48 and 72 h) with serial logarithmic dilutions of analogue (0.10-100µg/ml) .At the end of incubation time , the survival rate of treated cells was determined by MTT method. **Results**: Our data indicated that this compound had a profound cytotoxic effect on the k562 cell line in a dose dependent manner. Interestingly, the IC_{50} value of this compound inK562 was lower incontrast with the IC_{50} value obtained in PBMCs. **Conclusion:** As a result, this compound provide more favorable cytotoxicity against K562 cell line without any additive cytotoxicity against PBMCs.**Key words:** K562,PBMC, 2-methoxy pyrimidine, MTT.

3496P

Evaluation of the cytotoxic effects of 2-4-dichloropyrimidine , on the K562 human tumor cell line

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Introduction: Previous studies indicated that pyrimidine derivatives possess anticancer properties. This study was set out to evaluate the effects of 2-4-dichloropyrimidine, as pyrimidine derivative, on K562 human erythroleukemia cell line and peripheral blood mononuclear cells (PBMCs) as normal control cells. **Materials and Methods:** The K562 cells or PBMCs (1×10^5cells/100 µl/well) were incubated for different times (24, 48 and 72 h) with serial logarithmic dilutions of analogue (0.10-100µg/ml) .At the end of incubation time , the survival rateof treated cells was determined by MTT method. **Results:** Our data indicated that this compound had a profound cytotoxic effect on the k562 cell line in a dose dependent manner. Interestingly, the IC_{50} value of this compound in K562 was lower than IC_{50} in PBMCs.**Conclusion:** As a result, this compound provide more favorable cytotoxicity against K562 cell line without any additive cytotoxicity against PBMCs.**Key words:** K562, PBMC, 2-4-dichloropyrimidine, MTT.

4494P

Evaluation of the cytotoxic effects of (2(-2 hydroxy ethylamino) ethylamino) cyclohexanol, on the K562 human tumor cell line

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Introduction: Previous studies indicated that β-amino alcohol derivatives possess antibiotic properties. This study was set out to evaluate the effects of (2(-2 hydroxy ethylamino)ethylamino) cyclohexanol, as an β-amino alcohol derivative, on K562 human erythroleukemia cell line and peripheral blood mononuclear cells (PBMCs) as normal control cells.**Materials and Methods**: The K562 cells or PBMCs (1×10^6 cells/100 µl/well) were incubated for different times (24, 48 and 72 h) with serial logarithmic dilutions of analogue (0.25-250µg/ml) .At the of end incubation time , the survivalof cells was determined by MTT method. **Results:** Our data indicated that this compound had a profound cytotoxic effect on the k562 cell line in a dose dependent manner. Interestingly, the IC_{50} value of this compound in K562 cells was lower than IC_{50} value in PBMCs.**Conclusion:** As a result, this compound provides more favorable cytotoxicity in K562 cell line without any additive cytotoxicity in PBMCs.**Key words:** K562, PBMC,(2(-2 hydroxy ethylamino) ethylamino) cyclohexanol, MTT.

7528P

Evaluation of the immunotherapy with Low-density adherent splenocytes pulsed with the extracts of heated 4T1 and killed preparation of *Lactobacillus casei* in mouse model of breast cancer

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Introduction: The 4T1 mammary carcinoma is an easily transplantable, highly tumorigenic and invasive tumor cell line with low immunogenicity that can be used as an experimental model for human mammary cancer. Recently, the anti-cancer effects of probiotics have been noticed. This study was set out to investigate the efficacy of a new immunotherapy against breast cancer made by low-density adherent splenocyte population (LDS) pulsed with the extracts of heated 4T1 cells and *Lactobacillus casei*, as a probiotic. **Materials and Methods**: Mammary carcinoma was induced by injection of 4T1 cell line in the flank of female Balb/c mice. The first immunotherapy was initiated when all animals had developed a palpable tumor. Immunotherapy was done twice with one week interval. One week after the last immunotherapy, half of the mice were euthanized in order to determine the immune response profile. The remaining animals were kept until the time when death occurred spontaneously. **Results:** The findings indicated that mice with mammary tumor received LDS pulsed with combined heated 4T1 cells and *L. casei* showed a more favorable survival curve and slower rate of tumor development compared to the other groups. Moreover, LDS pulsed with combined agents significantly amplified the secretion of IFN-γ and conversely, diminished the secretion of IL-4, IL-10 and TGF-β and nitric oxide production in splenocyte population compared to splenocytes from other groups.**Conclusion:**LDC pulsed with heated 4T1 cells and *L. casei* promote beneficial outcomes in mouse model of breast cancer. Because of the low immunogenicity of 4T1 cells, these findings are beneficial. **Keywords:** 4T1 cells, Breast cancer, *Lactobacillus casei*, Low-density adherent splenocytes.

7539P

Evaluation of Different Levels of GreenTea Epigallocatechin-3-Gallate on T47D BreastCancer Cell Lineapoptosis

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Introduction: Breast cancer is the second cause of cancerdeathin women after lung cancer, and is the most common cancer in women after nonmelanoma skin cancer. According to the World Health Organization, every year more than 1.2 million cases of breast cancer were diagnosed and more than 500 thousand people die from the disease. Green tea has antioxidant, anti- tumor andanti-bacterial properties that may regulate endocrine glands. Epigalocatchi –gallate (EGCG) in green tea has been shown to induce apoptosis and cell death in cancer cells but not on normal cells. Due to problems in the treatment of this disease, this study is designed to investigate the anticancer effects of EGCG on gene expression involved in cell growth and apoptosis of breast cancer cell line T47D.**Material and method:** The breast cancer cell lines purchased from the cell bank of Iran Pasteur Institute were cultured in DMEM and then incubated with different concentrations of EGCG(50-80 µg/ml). Real time PCR was used for the study of apoptotic gene expression and apoptotis mechanism. The data collected were analyzed using spss software. **Results:** Results showed all concentrations of EGCG in terms of morphology caused increase the mortality and MTT assay in all experiments showed a significant decrease compared to the basic case ($P< 0.05$) and Cytotoxicity is observed during higher doses. EGCG significantly reduced BCL-2 anti- apoptotic gene expression ($P< 0.05$) **Conclusion:** In general, According to the changes in the geneexpressions evaluated in s study and in consistent with previous studies, wecan reported that the effect of EGCG on human breast cancer cells are undeniable. Although we recommended further studies on thiscompound to identify more precise mechanisms. **Key words:** Breast Cancer, green tea , epigallocatechin -3-gallate, gene expression

7561P

Application of Nanotechnology in Cancer Immunotherapy

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Introduction Cancer is one of the leading causes of death in the world. The aim of cancer immunotherapy is to stimulate the host immune system to detect and eliminate cancer cells. Current there are approaches to cancer immunotherapy such as the cancer vaccines, cytokine therapy, administration of immune activating antibodies and radio immunotherapy. But they have limitation for cancer suppression due to insufficient uptake by DCs(dendritic cells).In cancer vaccines to enhance antigen uptake by DCs, nanoparticle based antigen delivery systems have been explored. **Material and Method** This review summarizes the original and systematic review articles available in PubMed،Science Direct، Google Scholar. These databases were searched from 2000 to 2015 by the Nanotechnology and Cancer Immunotherapy keywords. **Results**Nanoparticles have several advantages for effective targeting of DCs and macrophages. Self-assembled polymeric micellar immunomodulator (SPI) based on cationic amphiphilic polymers, are used for cancer treatment. Antigen encapsulation into PLGA(polylactic-*co*-glycolic acid) nanoparticles is showed that enhanced cellular uptake of antigen and induced T cell responses. Smart pH-sensitive liposomes which correspond to the pH of endosome and taken up by murine DCs are used for targeting of cancerous cells. Photo thermal therapy(PTT) by gold nanoparticles destroys the near cancer cells by various mechanisms. **Conclusion** Nanoparticles due to their better accumulation within tissues and cells of the immune system are well

suitable for delivery of immune therapies such as vaccines or adjuvants. **Keyword**Nanotechnology , Cancer Immunotherapy

7589P

Recombinant immunotoxins as a new strategy for targeted cancer therapy

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Chemotherapy, radiotherapy and surgery are three common conventional treatments for cancer that have had a great successes in treatment, but the mortality rate due to cancer still remained high for several reason. Development of new cancer therapies is based on the design of drugs that targets specifically to cancer cells while have minimal adverse effects on normal tissues. Monoclonal antibodies are among the most specific agents that recognize and bind to cancer cell surface antigens. Despite high successes of monoclonal antibodies, they rarely are able to destroy completely cancer cells alone and it is thought they must be connected to a chemical agents or toxins to increase their efficiency. In immunotoxin based targeted therapy the targeting fragments such as antibodies, ligands and peptides conjugated to toxins, enzymes and other toxic agents. Immunotoxin (ITs) are very efficient so that one ITs molecule enough for killing a tumor cell while 10000- 100000 molecules of chemotherapy needed to kill a tumor cell. ITs according to the type of targeted moiety placed in three groups including: ITs based on the ligand, ITs based on the antibody and based ITs. Today, a variety of ITs have been developed which some of them are in the clinical trials. In 1999 the first IT, Denileukin Diftitox, has been approved by the Food and Drug Administration (FDA) for the treatment of cutaneous T cell lymphoma. In this review, various factors that are considered in construction of ITs as targeted moiety and as the toxic moiety have been discussed. **Key word**: Cancer therapy, Targeted therapy, Immunotoxin

7605P

Evaluation of the 1, 4 diaminobenzene cytotoxic effects on the K562 human tumor cell line

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Introduction: Previous documents indicated that diaminobenzene derivatives possess the ability of bind to DNA. This study was designed to investigate the effects of 1, 4 diaminobenzene, on K562 human erythroleukemia cell line and peripheral blood mononuclear cells (PBMCs) as normal control cells. **Materials and Methods:** The K562 cells (1×10^6 cells/100 µl/well) were incubated for different times (24, 48 and 72 h) with serial logarithmic dilutions of analogue (0.25-250µg/ml). Moreover, doxorubicin at concentration of 0.1 mM was used as standard control. At the end of incubation time, the cells survival was determined by MTT method. **Results:** Our data indicated that this compound had a profound cytotoxic effect on the k562cell line in a dose and time dependent manner. Interestingly, the IC_{50} value of this compound in K562 was comparable with IC50 value of doxorubicin in K562.**Conclusion**: As

a result, this compound provides more favorable cytotoxicity against K562 cell line.**Key words:** K562,1, 4 diaminobenzene, MTT.

7626P

Immunotherapy of lung cancer using anti-VEGF camel single domain antibody

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Introduction: Cancer recorded as one of the most leading cause of death around world. Angiogenesis plays an extremely important role in growth and progress of cancer cells. Among many factors involve in angiogenesis, vascular endothelial growth factor (VEGF) plays crucial role in angiogenesis promotion. Therefore suppression of VEGF is an effective approach to target cancer angiogenesis. Camel single domain antibody (also known as Nanobody) is novel class of antibodies with unique properties for drug development. According to the importance of cancer and angiogenesis, inhibition of angiogenesis and cancer cell growth was the main aim of current study. **Materials and Methods**: Selection of anti-VEGF Nanobody cross-reacting with human and mouse VEGF was performed on *camelus dromedarius* immunized library through phage display technology. Cross-reactivity of selected Nanoboy was analyzed by indirect and sandwich ELSA. *In vitro* Functional assays of selected anti-VEGF Nanobody on growth and angiogenesis of human endothelial cell was performed by MTT, Migration and tube formation assay. Mice bearing lung cancer tumor were modeled through injection of TC-1 cells on shaved right flank of C57Bl6. Anit-VEGF Nanobody injected near the tumor site five times a week. Tumor inhibition and growth was monitored and mice survival compared against control group. **Results**: Our results showed that anti-VEGF Nanobody significantly inhibited growth, migration and tube formation of human endothelial cells. In *in vivo* experiment anti-VEGF Nanobody significantly inhibited tumor growth and resulted in increasing of mice survival. **Conclusion**: Results indicated the potency of Anti-VEGF Nanobody for cancer immunotherapy through suppression and inhibition of VEGF. Key words; Angiogenesis, VEGF, Nanobody, Immunotherapy

9712P

Designing of new human recombinant PD-1as an inhibitory immune checkpoint

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Introduction: Programmed cell death protein-1/PD-1 ligand pathway is one of the possibile options which could be used as anti-tumor immunoregulatory strategy in tumor regression processing. This receptor pathway has negative-regulatory function on T lymphocytes by inhibition of immune responses., so blocking of PD-1/PD-L interactions with antibodies has been shown to be successful in preventing the inhibitory function of this pathway and improvedthe immune responses against advanced solid tumors, nevertheless these antibodies induce some adverse events in patients. Moreover, in animal models, Soluble PD-1 (consists of extracellular IgV domain of PD-1) has been used for blocking the PD-1/PD-L pathway. Blocking this molecule promoted proliferation and cytotoxic functions of T lymphocytes in murine models. So we attempt to design a new recombinant form of PD-1"human soluble PD-1"expressing gene for blocking this pathway in in-vitro model. **Methods and materials:**New recombinantPD-1 was designed based on protein and DNA sequences of Homo sapiens PD-1 extracellular IgV domains using multiple bioinformatics tools and software (NCBI data base)Inducible promoter of this recombinant protein was selected only according to sensitivity to tumor microenvironment. This new recombinant PD-1 was cloned into prokaryotic/eukaryotic vector (pcDNA3.1hygro+) and subsequently transformed into TOP10 competent cells. **Results and Conclusion:** The designed PD-1 was transformed in ToP10 cells successfully and was confirmed

by enzymatic digestion. Then human soluble PD-1 gene was ligated into pcDNA3.1hygro+ (vector) and then the productwas transformed into the cells. The processes were demonstrated by Clony-PCR analysis and DNA sequencing. This progression is an initial step for preparing the soluble PD1.

9725P

Apoptosis Effects of Chrysophanol onMCF-7 Cell Line

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introduction: Chrysophanol(1,8-dihydroxy-3-methyl-anthraquinone),a member of the anthraquinone family, is one of the components of Aloe Vera. breast cancer is the most common malignancy among women, , and it is the second leading cause of cancer death among women. The aim of this study was to investigate the cytotoxic effects of Chrysophanol on a cancer cell line, MCF-7. The rate of apoptosis induction and its mechanism were also evaluated. **Materials and methods:** MCF-7 cells were cultured in RPMI medium with 10% fetal bovine serum. The cytotoxic effect of different concentrations (5, 10, 25, 50, and 100 µM) of Chrysophanol on cultured cells were evaluated by MTT assay. Apoptosis and CD95 (Fas) expression were analyzed by flowcytometry using an Annex in V-FITC/PI kit and Fas(CD95) kit according to the manufacturer's protocol. **Results**: Chrysophanol decreases the viability of MCF-7 cell line in a time and dose dependent manner, so that the most effective concentration of this substance was 100 µM and 72 h after treatment. According to the data of Fas (CD95) expression and Annex in-PI, the highest apoptosis induction rate was seen in 100 µM and 72 h. **Conclusion:** Our findings indicated that Chrysophanol has some antitumor effects and can be used in the treatment of breast cancer. However, further investigation of its cytotoxic effects against tumor cells, both in vitro and in vivo, is recommended.

9750P

Anti-tumor effect of IP-10 using different strategies: comparison between DNA and live therapy in mice model

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Introduction: Breast cancer is the most common cancer among women worldwide. Immunotherapy improves the patient's own immune responses to attack tumors and disseminated metastasis with reduced side-effects. In our study,IP-10chemokine was applied as an immunotherapy agent in two different strategies. Herein,

Leishmaniatarentolae, a non-pathogenic parasite, was used as live delivery system. In another strategy, the pcDNAharboring IP-10 was applied as gene therapy. Therapeutic ability of these two approaches was compared by determining the arginase activity in 4T1-implanted mice as breast cancer model. **Materials and Methods:**pLEXSY plasmid containing(*IP-10-egfp*) fusion gene was constructed and transfected into*L.tarentolae*.Then, the therapeutic efficacy of pcDNA-(*IP-10-egfp*) and*L.tarentolae*-(IP-10-EGFP),was compared by measuring tumor-and spleen size, histologically examination of lungs and arginaseactivity determinationintumoral tissue and sera of 4T1 cells-inoculated BALB/c mice.**Results:**Reduction in tumor's growth and spleen and tumor's arginase activity also inhibition of metastasis to lung's tissue were seen in pcDNA-(*IP-10-egfp*)group. However, no significant difference was observed in arginase activity of sera and tumoral mass in the other therapeutic strategies. **Conclusions:** It was shown that IP-10 delivered by plasmid is a proper candidate for treatment of breast cancer inBALB/c mice model. However, *L.tarentolae*-(*IP-10-egfp*) as live delivery system with the applied regime needs dose modifications. Our results demonstrated that arginase assay canbe a good biomarker to differentiate tumoral tissues from normal ones.

9776P

Generation of *In Vitro Homogenous* Multicellular Spheroids to study therapeutic efficiency of anticancer drugs

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Introduction Multicellular spheroids are the 3D culture models that more closely mimic the physiologic environment of living organisms compared to conventional 2D culture and can be used to the development of anticancer drugs and this model provides close prediction of *in vivo* drug efficacy. **Materials and Methods**MDA-MB-231 cells were cultured as monolayer and then cells were harvested by trypsin zing. 1000 to 10000 cells per spheroid were placed in 96 well plates in liquid overaly method. In addition, the cells were distributed in to single drop and grown for 3 days in hanging- drop technique and then spheroids were placed in 96 well culture plates precoated with 50 µl 0.5% poly-HEM in 95% ethanol at 37° C. In this periodof time spheroidal growth was monitored daily and spheroid volume was calculated using the ImageJ software.**Results**In this study, 3D culture methods, liquid overaly and hanging- drop, were considered. In comparison of both methods, we showed the hanging-drop as a simple and rapid technique for homogenous spheroids generation. The spheroids are applicable for cellular assays to analyze the effect of cytotoxic drugs and recombinant proteins.**Conclution**This study present a rapid method to generate the homogenous single spheroids of MDA-MB-231cells in individual wells to study cytotoxic effects of drug prior of invivo study. This method is hanging drop which is suitable to consider therapeutic efficiency of anticancer drugs.

10888P

Cytotoxic effect of aqueous extract of *Trachyspermum copticum* (L.) Linkdried seedsonHuman Breast cancer cell line (MCF-7)

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IntrductionNowadays the use of medicinal plants to prevent or treat a variety of diseases is focus of many studies. Recognizing the positive and negative effects of these factors can be very important and will change the opinions concerning their consumption. Investigation of cancer cells growth inhibitory effects of some medicinal plants , is an important part of the studies on medicinal plants. Therefore, this study aimed to investigate the toxicity effects of aqueous extracts of the Ajwain (*Trachyspermum copticum* (L.) Link) dried seed on cancer cell line MCF-7.**Materials and Methods**: In this experimental study, to prepare the aqueous extract the stewing method was used. Anti-cancer activity was investigated using MTT method on 10 groups of cancer cell line MCF7 in different concentrations of the extract within 24 hours of exposure. Statistical analysis was performed by SPSS16 software, after data were collected. **Results**: The results of the study indicated a dose-dependent cell proliferation of the study extract at concentrations higher than 100 µg/ml. However, at concentrations lower than 100 µg/ml the relative inhibitory effect on the growth and cell toxicity was observed. **Conclusion**: This study showed that the aqueous extract of the Ajwain dried seeds had relative toxicity on cancer cell line MCF-7 only at lower concentrations and even at certain concentrations causes cancer cells proliferation. **Keywords**: Medicinal plants, Ajwain (*Trachyspermum copticum* (L.) Link), cell toxicity (cytotoxicity), MTT, cancer cell line MCF7.

10937P

The effect of Nano cisplatin cytotoxicity on cell line A-2780 in ovarian cancer in vitro

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Intrduction: Cancer is the most common cause of death after cardiovascular disease and a major challenge in cancer treatment is targeting and destroying cancer cells. In this field nanotechnology is the latest orientation, so this study tried a new formulation of the cisplatin drug by using polymeric nanoparticles on ovarian cancer cell line A-2780 and Cytotoxic effects of them was tested. **Materials and Methods:** Synthesis polymeric nanoparticles were synthesized by using emulsion polymerization. For determining the size and morphology of polymeric nanoparticles, scanning and transmission electron microscopy was used. The cytotoxic effects of cisplatin and nanoparticles with drug concentrations of cisplatin (2, 3 , 4 , 5 and 6 macromollar) were evaluated on cancer cell line A-2780.**Results:**The results of MTT assay showed that the IC50 of nanoparticles loaded with cisplatin in 24 hours is less toward free drugs. The amount of nano- drug toxicity is significantly more than free drugs also notable differences was observed between control and treated cell groups.**Conclusion:**This study describes that the efficacy of Nano- drug can be more than free drug. Thus, this finding may propose a new opinion about the effects of nanomaterials on cancer therapy by nanomedicine. **Keywords:**ovarian cancer,Cisplatin,Polymeric nanoparticles,Cytotoxicity

10944P

TDT inhibition caused anti turmeric effect on acute lymphoblastic leukemia

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Acute Lymphoblastic Leukemia (ALL) is the most common cancer in children, although it can occur in any ages. Despite the Considerable progress in ALL treatment with survival rate of 85% in children, yet ALL remains the important cause of cancer-related death in children and young adults. In nearly all of ALL, there is an increase in the expression of TDT (Terminal Deoxynucleotidyl Transferees). TDT is a unique DNA polymerase that adds deoxynucleotides to the V, D, and J exons during Immunoglobin and T-cell receptor gene recombination. In this study we evaluated the anti-carcinogenic activity of genistin (TDT inhibitor) against molt4 / nolm6 ALL cells .The MTT assay used to evaluate the growth inhibitory effect of genistin on leukemia cells. Flowcytometry was performed to analysis the effect of genistin on TDT expression using TDT antibody. In addition Proliferation and apoptosis measured by ki67 and caspase3 antibody. Genistin inhibited the expression of TDT in a dose and time dependent manner and also induced apoptosis by activation of caspase3 and proliferation reduction. These findings indicated that genistin exhibited anti-carcinogenic activities on acute lymphoblastic leukemia.Key words: TDT, Genestin, ALL, Apoptosis

10950P

Streptomyces levis ABRIINW111 has anti-cancer effect on human colon cancer

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Introduction: Previous studies showed evidences that natural products can be used as novel anticancer agents. *Streptomyces sp.* as a dominant genus in Actinomycetes have been interested recently because of their wide variety bioactive anticancer metabolites. Doxorubicin extracted from *Streptomyces peucetius* are as the best common anticancer and chemotherapeutic agent. Here we attempted to elucidate the anti-carcinogenic influence of the ether extracted organic metabolites derived from *Streptomyces*, bacteria isolated from Zagros Mountains, on SW480 colon cancer cells.**Materials and Methods:** The MTT assay used to evaluate the growth inhibitory effect of metabolites on SW480 cells. Flowcytometry was performed to observe the cancer cells undergoing apoptosis and cell cycle arrest using caspase3 and ki-67 monoclonal anti bodies and propidium iodide staining respectively. P53 measured by real-time PCR.**Result:** The metabolites exhibited robust inhibitory effects on SW480 in a dose/ time dependent manner. In addition, metabolites caused cell cycle arrest in the G_1 and G_2/M phase, which was accompanied by activation of caspase3 P53 and reducing in ki-67 expression .**Conclusion:** These findings indicated that the metabolites exhibited anti-carcinogenic activities in colon cancer cells.**Key words:** Streptomyces levis, Colon cancer, apoptosis, proliferation

10953P

Streptomyces levis ABRIINW111 increase cell death in Acute lymphoblastic leukemia cells

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Acute Lymphoblastic Leukemia (ALL) is the most common cancer in children, although it can occur in any ages. Despite the considerable progress in ALL treatment with a survival rate of 85% in children, it remains the important cause of mortality in children and young adults. Previous studies showed that natural products can be used as novel anticancer agents. *Streptomyces sp.* is a dominant genus in Actinomycetes which it has been interested recently because of their anti-cancer activity. Doxorubicin extracted from *Streptomyces peucetius*is the best common anticancer and chemotherapeutic agent. In this study we evaluate the anti-carcinogenic activity of the ether extracted organic metabolites derived from *Streptomyces* on nolm6 ALL cells. The MTT assay used to evaluate the cytotoxicity effect of metabolites on nolm6 cells. Flowcytometry was performed to evaluate apoptosis by caspase/3 and cell cycle arrest with propidium iodide staining.Proliferationalso measured by ki67 antibody. The metabolites exhibited robust inhibitory effects on nolm6 in a dose/ time dependent manner. In addition, metabolites caused cell cycle arrest in the G2/M phase, which was accompanied by apoptosis and reduction in proliferation. These findings indicate that the *Streptomyces sp* metabolites exhibited anti-carcinogenic activities on acute lymphoblastic leukemia cells.

10984P

The Optimum Gereration of Rat Dendritic Cells from Monocytes in Vitro

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Introduction: Dendritic cells (DC) are the most important cells in the initiation of immune responses. Because of their superior antigen-presenting capacities, DC may be useful for the initiation of antitumor responses. Current study was done to give rise to the method of differentiation rat monocyte derived DCs in vitro. **Materials and Methods:** Rats were anesthetized. Then, 6–10 ml heparinized blood was obtained by heart puncture. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll Hypaque density gradient centrifugation and were seeded in RPMI 1640 supplemented with FBS. After 2 hours of incubation at 37˚C, adherent cells were used for DC generation. These cells were cultured for 7 days in medium containing rat recombinant GM-CSF and IL-4 at a concentration of 50 ng/ml also 20 µg/ml lipopolysaccharides (LPS) was added at day 6 to induce maturation of the MoDC. **Results:** Morphological analysis of the MoDC showed most of the mature DC were appeared as single cells or loosely adherent aggregates viewed by light microscopy. **Conclusion:** Since DCs play an essential role in immune responses, a great deal of this research effort has been focused on the practical method for generation and maturation of these cells to obtain MoDC from PBMC.**Keywords:** Differentiation, Dendritic cell, Monocyte, Rat

10989P

Optimized Production of Tumor Cell Lysates from B92 Glial cells

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Introduction: Lysates from tumor cells are reported to induce maturation of dendritic cells (DCs) and are used in clinical settings for DC-based vaccination against tumors. Further studies showed that tumor lysates consist of HSP-peptide complexes (HSP-PC) that their expression increases in hyperthermia. This survey was designed to offer an efficient procedure for inducing the maximum amount of glial tumor cell lysates. **Materials and Methods:** Tumor cell line, B92 was cultured in RPMI supplemented with 10% FBS.To apply heat stress, culture flasks were sealed and immersed for 90 min in a water bath at 43°C, allowed to recover at 37°C for 8 h in a 5% CO 2 incubator. Then, cells were washed in condition medium and subjected to four freeze and thaw cycles to obtain a crude lysate. **Results:** Protein determination by the Bradford method showed a high level of the protein concentration in tumor lysate after exposure to heat. **Conclusion:** The present study proposes a valid method for generating of B92 rat tumor cell lysates consist of HSP-PCfrom cancer cells that form effective bases for immunotherapy approaches with unique properties. **Keywords:** optimized production, tumor cell lysate, Hsp-pc

10995P

Apoptotic effects of Artemisia khorassanica on melanoma cell line

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Introduction: Natural compounds derived from medicinal plants have been traditionally used to treat various diseases. Anti cancer effects of numerous plant products was investigated previously. In this study apoptotic effect of Artemisia khorassanica on melanoma cells was studied. **Material and Methods**: To explore the apoptotic effects of Artemisia khorassanica on cell death pattern, melanoma cells were stained with annexin-V/PI and apoptosis was determined by Flow Cytometry .**Results:** The results showed that SLAK caused a remarkable dose dependent increase in early apoptosis of melanoma cells. Early apoptosis percentages treated cells with 5, 25, 50, 75, or 100µg of SLAK/ml against untreated cells (3.3 [±0.5]% were 12.7 (±2)%, 8.4 (±3)%, 35.6 (±3)%, 44.4 (±5)%, and 44.6%(±4)%, respectively,.**Conclusion**: The presented results specify that *Artemisia khorasanica* may impact its anti-cancer properties via anti apoptotic effects. **Keyword:** Artemisia khorassanica - melanoma cell line – apoptosis

10996P

Artemisia khorassanica as an anti-cancer agent

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Introduction: Plant-derived natural products have been shown anti-cancer properties to control aberrant cell proliferation. *Artemisia khorassanica* has been used in folklore medicine for many years; according to its different biological properties. Lactone-bearing fraction isolated from *Artemisia khorassanica* (SLAK); was investigated in this study for its anti-cancer properties. **Materials And Methods**: Anti-cancer potential was evaluated by toxicity against human melanoma and fibroblast cell lines. Cell death properties were determined using Western blotting for evaluating Bax and cytochrome c protein expression .. **Results:** The results showed that SLAK caused inhibitory effects on proliferation of melanoma cells that was associated with remarkable increase in the over-expression of both Bax and cytochrome c. **Conclusion**: The current experiment indicates that *Artemisia khorasanica* may have anti-cancer activity. We anticipated that the ingredients may be employed as therapeutic candidates for melanoma. **Keyword:** Artemisia khorassanica - melanoma cell line - bax - cytochrome c

11105P

Immunological effects of Alloferon: asystematic review

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Introduction: Alloferon is a 13-amino acid peptide that was first isolatedfrom an insect immune system. It was reportedto show anti-tumor effects via upregulation of NK cell activity, and anti-viral effects, especially against herpes virus, through regulation of the viral life cycle. Itwas also recently reported that alloferon effectively downregulatesthe production of proinflammatory cytokines, such as IL-6, IL-8, and TNF-□, in UVB-induced skin inflammation. The results of studies shown that alloferon exerts its anti-asthmatic effect via downregulationof IL-5 production and eosinophil infiltration. **Materials and Methods:** In lack of statistical requirements, a systematic review of literature regarding effects of Alloferon was performed in order to assess a critical role in host immunity against cancer. Multiple databases (Cochrane, Embase, Pubmed and google Scholar) were systematically searched for studies published up to December 2015, and recently published abstracts were also reviewed. **Results:** Overall, 146 articles were screened and 5 retrieved for full-text evaluation. Studies showed that Alloferon enhances NK cell cytotoxicity against cancer cellsand virus-infected cells, induces expression of NK cell-activating receptors, and enhances the production of IFN-□ and TNF-□ and granule exocytosis from NK cells. **Conclusion:** Based on its immune-modulating activity, it seems that alloferon shows anti-tumor, anti-viral, and anti-inflammatory effects. The immunoregulatoryeffects of alloferon should be investigated intenselyin a cancer model as a research subject becausealloferon enhances NK cell activity, which play a criticalrole in host immunity against cancer.

11118P

Generation of a murine hybridoma clone producing monoclonal antibody directed against a newly established human acinar cell carcinoma of pancreas

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Introduction: Pancreatic carcinoma is the fourth-leading cause of cancer death and is characterized by early invasion and metastasis. Acinar cell carcinoma is a malignant epithelial neoplasm composed of cells with morphological and functional resemblance to pancreatic acinar cells and represent 2% of exocrine pancreatic neoplasms in adults and 15% in children. Faraz-ICR is a newly established cell line from a patient with acinar cell carcinoma which has been characterized in Shiraz Institute for Cancer research. **Material and methods**: After immunization of Balb/c female mice with Faraz cell line and fusion of splenocytes with SP2/0 myeloma cell line, high reactive hybridoma producing antibodies to Faraz-ICR were detected using immunocytochemical staining and flowcytometry. Western blot and 2D immunoblotting were used for further characterization of the target antibodies. **Result**: Among 10 high reactive clones, the reactivity of 7C11 clone was assessed with other epithelial tumors. The isotype of antibody was revealed to be IgM and the antibody reacted to a molecular weight membrane protein of 55 KDa in western blot analysis. To further characterize the target antigen, immunoproteome of Faraz cell line was prepared and has been sent for mass analysis. **Conclusion:** Pancreatic cancer is a fatal malignancy with no reliable biomarker for early screening and diagnosis. In this study by establishing a pancreatic cell line a panel of monoclonal antibodies was generated aiming to explore specific or associated cancer targets for possible diagnosis and therapeutic purposes. **Key words**: Acinar cell carcinoma, hybridism, monoclonal antibody

11132P

Designing of peptide ELISA kit for Evaluation of anti VEGF IgG in serum of mouse model

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Introduction: VEGF plays a pivotal role in neoangiogenesis of malignant tumors. In this study, VEGF peptide based vaccine was designed with immunoinformatics for blocking of VEGF molecule to inhibit its effects. **Material and methods**: Peptide sequences were adopted from Uniprot data base of different VEGF-A isoforms aligned with program mega4. Also we used IEDB site to study suitable epitopes for stimulating immune responses and BLAST program to avoid overlap with other human antigens. A conserved sequence of VEGF was conjugated with KLH used for immunization of mice. To measure the polyclonal anti-VEGF antibody titer in mice sera, an indirect peptide-ELISA was designed based on conjugation peptide with BSA.**RESULT:** The selected sequence has not overlapping regions with other proteins in body, provide enough antigenicity and ability to stimulate anti-tumor appropriate responses. SDS-PAGE analysis of the conjugated molecule showed efficient coupling of the peptide with BSA. Comparison of different ELISA procedures revealed that coating of plates with BSA conjugated antigens resulted more reproducible ODs than naked peptides. A substantial increase of the antibody titer was observed in vaccinated mice compared to controls. **Conclusion:** Our results reinforced the potential of KLH conjugated peptide for immunization and production of specific polyclonal antibodies against VEGF-A. Production of high titer antibodies against this auto antigen offers this peptide as a vaccine to stimulate humoral immune system which recommended to be evaluated in In vitro and In vivo models. **Key words:** Immunoinformatics, Peptide vaccine, VEGF, Peptide ELISA

11135P

Suppression of matrix metalloproteinases -2 and -9 in murine breast cancer cell line by β-d-mannuronic acid

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Introduction: Proteases, especially matrix metalloproteinase (MMP)-2 and -9play key roles in advanced-stage breast cancer invasion and metastasis. 4T1 is an animal model cell line for human breast cancer studies, which is able to produce highly metastatic tumors in BALB/c mice. B-d-mannuronic acid (M2000) is an anti-inflammatory agent derived from brown algae. In this study, the effect of β-d-mannuronic acid on the cell proliferation and activity of MMP2 and 9 in 4T1 cell line was investigated. **Materials and Methods:**4T1 cultured in the presence of various concentrations (0, 5, 25, 50, 100 and 200 µg/ml) of β-d-mannuronic acid. Analysis of tumor cells proliferation and MMPs activity in supernatants were carried out by LTT assay and gelatinase zymography respectively. **Results:**Our data demonstrated that β-d-mannuronic acid did not effectively reduced the cell viability and number of 4T1 in 48h. However, markedly reduced the activity of MMP2and 9in a dose-dependent manner. **Conclusion:** These findings suggest that the therapeutic potential of M2000 in treatment of tumor cell metastasis in breast cancer. **Key Words:**β-d-mannuronic acid, MMP-2 and MMP-9, 4T1 breast cancer cell

11136P

The effect of adipose derived stem cells (ASC) on the movement of MDA-MB-231 cell line *in vitro*: comparing normal ASC with cancer ASC

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Introduction: During the progression of carcinogenesis, mesenchyme stem cells as a subpopulation of cells in tumor microenvironment play critical role in tumor development and progression, including tumor cell proliferation and metastasis. The purpose of this study was to investigate the effect of adipose-derived stem cells (ASCs) conditioned media from healthy donors and breast cancer (BC) patients on the movement of MDA-MB-231 cell line *in vitro*.**Material and methods**: ASCs were cultured from breast adipose tissue of normal donors and breast cancer patients. After culture of MDA-MB-231 cell line uniformly, using a sterile pipet tip, a wound was scratched through the cells moving pipette top to bottom. Then cells were cultured in presence of conditioned media from ASCs. After 8 hrs cells were fixed and stained with crystal violet, pictures were taken after dryness and analyzed using Image J software.**Results:**Results showed that ASC caused higher movement and proliferation rates in MDA-MB-231 cells compared to standard culture media. Furthermore, the tumor derived ASCs especially those from higher stages of breast cancer have stronger effects on proliferation and movement of MDA-MB-231 cells than normal ASCs (P value <0.05).**Conclusion:** It can be concluded that cancer ASCs may be different in their chemokine and chemokine receptor expression profile from normal ASCs, resulting in cancer cells proliferation; invasion and metastasis. These findings have important implications in immunotherapeutic interventions for treatment of breast cancer. **Keywords:** Adipose-derived stem cell, MDA-MB-231 cell line, movement

11155P

Therapeutic Efficacy of Silibinin on colorectal cancer Cells: key roles of Akt and NF-κB Expressions in Silibinin-Induced Response

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Introduction: Colorectal cancer (CRC) as an elaborate heterogeneous tumor is associated with 10% of cancer related mortality. Chemoprevention treatment regime such as nontoxic synthetic or natural compounds are used to reduce cancer risk. Silibinin, a flavonolignan is a natural product which is used as the chemo preventive agent and may be effective in treatment of various malignancies. Indeed in proceed research, antioxidant activity and modulating of NF-κB /AKT signaling pathway were assessed. **Material and methods:** Direct antioxidant properties of Silibinin was investigated by 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) assay. MTT was used for evaluation of Silibinin cytotoxicity in CaCO2 cell line. Function of Silibinin in cell signaling was studied by real time PCR. **Results:** AAPH result showed that Silibinin has a direct antioxidant activity. In concentration of 100Nm, It is cytotoxic up to 80%. Silibinin reduced the expression of Akt which inturn induced apoptosis. Evaluation of other signaling pathways are in process. **Conclusion:** Silibinin as a natural product make a promising therapeutic approach in the field of supplementary cancer therapy. **Key word:** silibinin, antioxidant, AKT, signaling pathway

11175P

Effects of linum usitatissimum and scrophularia striataethanolic extracts on TGF-β production in MCF-7 cell line

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Introduction: Breast cancer development is dependent on the interactions between immune and cancerous cells. In this regard, cytokines play an important role in the tumor promotion or its regression and consequently in manifestation of the disease symptoms. Among them, the role of TGF-β as an immunosuppressive cytokine is prominent. It is well known that in many cancers including breast cancer, the level of this cytokine is increased. Thus the strategy to decrease the TGF-β production can be effective in breast cancer treatment. In this study, we investigated the effect of two herbal extracts, linum usitatissimum and scrophularia striata, on the TGF-β production in MCF-7 cell line. **Material and Methods:** *MTT assay was used to determine* IC50 *(inhibition concentration 50%) values for both extractions.* MCF-7 cells were cultured in RPMI-1640 supplemented with 10 % fetal bovine serum and 1% penicillin/ streptomycin in 75cm^2 cell culture flasks to reach 50-60% confluency. Then, the cells were treated with herbal extractions in 3 different doses (high, intermediate, low) based on IC50 value as triplicate for 48 hours. TGF-β levels were analyzed by ELISA. **Results:** The results suggest that both herbal extractions from flaxseed and linum usitatissimumcould increase TGF-β expression in MCF-7 cells as compared to control($P < 0.01$).**Conclusion:** Despite our expectation, both extractions increased the TGF-β production in MCF-7 cell line. However, these findings are useful for treatment of patients suffering from autoimmune diseases. **Keywords:** MCF-7,TGF-β, linum usitatissimum, scrophularia striata

11194P

An overview on Antibody-targeted drugs: a successful immunotherapy in cancer

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Introduction: Cancer is one of the main causes of human death in recent years. Nowadays, the most common treatment for cancer, is chemotherapy. It also affects the body's normal cells so researchers are studying on specific methods those just affect cancer cells. One of these methods is antibody-based targeted therapies. Antibody drug compounds help that Monoclonal antibodies deliver to cancer cells antigen especially. **Materials and Methods :** In this study we used several databases such as PubMed, Google Scholar and some journals such as Nature and Science to overview on papers as a systemic review. Eventually, by studying the mechanisms of effectiveness the methods systematically reviewed and a table was set. **Results:** In this study, the pharmacological and medicinal effects of FDA approved monoclonal antibodies drugs like as Herceptin® and Rituximab were studied and Monoclonal antibodies to surface receptors, such as Trastuzumab or Rituximab, have complex mechanisms of action leading to effective tumor regressions. At the end the results set in a unique table. **Conclusion:** Studies on mechanism of drugs was found that these drugscouldespecially affect tumors with several methods and decreased tumor size in patients. As well as their drug delivery mechanism systematically reviewed. These methods indicated that can be expanded in the future to find an alternative method for non-specific treatments.

11245P

In vitro analysis of T cell responses induced by Glial tumor cell lysate pulsed with autologous dendritic cells

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Introduction: Immunotherapy is a therapeutic strategy that manipulates immune responses against tumor cells. Previous studies have shown that multiple tumor antigens do exist which can be used to induce autologous tumor specific T cell responses in vitro; therefore an alternative strategy for effective vaccination may be the use of unfractionated tumor derived antigens such as tumor cell lysate. In this in vitro study, T cell responses, which are induced by monocyte-derived DCs pulsed with glial heated tumor cell lysate (hTCL) were analyzed in terms of specific cytotoxicity. **Materials and Methods:** Autologous T cells were isolated from the no adherent cells that were harvested during the procedure to the isolation procedure of the rat monocytes. These no adherent cells stimulated and incubated with hTCL pulsed MoDCs at a 1:10 ratio in 24 well microtiter plates for 7 days. For specific cytotoxic activity of tumor antigen-primed T assay, glial tumor cells and T cells were cultured in V bottom 96 well plates at final volume of 200 µl of CM for 3 h at an effector: target ratio of 100:1. **Results:** Flow cytometric analysis of specific lysis using Annexin V and PI showed that tumor cell lysate pulsed autologous DC could elicit specific cytotoxic T lymphocyte response against autologous tumor cells. **Conclusion:** Our result suggested that glial hTCL pulsed DCs could elicit effective specific antitumor T cell responses in vitro, therefore, hTCL pulsed DC vaccination may be considered as a novel strategy for immunotherapy of patients with brain cancer refractory to standard modalities. **Keywords:** Glial cancer, lysate, Dendritic Cells, T cell response, Tumor immunotherapiy

11246P

Cimetidine effects on human lymphocytes exposed to gamma ray

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Introduction: Radio protector agents reduce the effects of radiation on healthy normal tissues. Cimetidine, a selective histamine-2 receptor antagonist, has attracted interests because of its potential as an immune response-modifying drug. In this study two concentrations of Cimetidine were selected for treatment of radiation exposed blood cells. **Materials and Methods:** Blood lymphocytes of healthy man cultured and treated with 50 and 100 concentrations of Cimetidine. Samples exposed to one Gy gamma ray. Micro nuclei (MN) assay used for analyzing damaging effects of radiation to lymphocytes according IAEA protocol. **Results:** In lymphocytes exposed to one Gy gamma ray MN frequency was 0.2 % while in irradiated cells was 2.13%. When lymphocytes treated with 50 and 100 micromole per liter of Cimetidine, MN of samples was 1.32% and 1.72%, respectively. **Conclusion:** Cimetidine decrease radiation effect by improving immune system and variation on drug concentrations can reduce radiation adverse effects on lymphocytes .

11248P

Human blood lymphocytes protection by Cimetidine and IMOD against gamma radiation

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Introduction: Ionizing radiation has damage effect on the tissues or cells but some radio protective products such as aminothiols reduce these harms. Despite improve in radio protective products most of them poorly tolerate and using a combination of drugs especially with herbal medicines is suggested. In this Study Cimetidine, a selective histamine-2 receptor antagonist, with immune modulatory capacities – Setarud (IMOD) have been introduced as a new radio protectors. **Materials and Methods:** Lymphocytes of blood samples obtained from healthy man cultured treated with various doses of Cimetidine and IMOD and were exposed to gamma-ray. Micro nuclei (MN) assay used for analyzing damage effects of Lymphocytes according IAEA protocol. Drugs efficiency for radiation damage reduces was presented by DRF.**Results:** Irradiated lymphocytes showed high frequency of MN. Treatment of cells by Cimetidine and IMOD induced to a significant reduction on the MN frequency. DRF of Cimetidine and IMOD were about 1/7 and 2, respectively **Conclusion**: Combination of Combination with IMOD had acceptable reduction on the radiation damage on the lymphocytes within increasing of their tolerance due to herbal inherent of IMOD. This combined medicine in radiotherapy and hematopoietic syndrome by radiation accident patients are suggested.

11250P

Nano Immunology: a new era for resolving immunological issues

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Introduction: Nanomaterials are belonging to the new smart materials which dimensionally are in the range of 1-100 nm. In the last decades, these materials have attracted many attentions because of their new, improved and unique properties along with their dimension. Many kinds of biological entities and biological process are in the range of nanometers and one can manipulate, controls and monitors these process and entities with the nanomaterials. Engineered nanomaterial's and nanostructures give us these opportunities to deliver immunologically active agents to interested sites precisely and in a controlled manner. Improvement in designing vaccines, more precise and sensitive immunoassays systems are another workplace for nanomaterial's. **Materials and methods:** In this paper, we reviewed papers related to Nano immunology from 1990 to 2016.**Results:** Several studies have used varieties of nanomaterials such as nanoparticles, magnetic nanoparticles, quantum dots (QD), nanoemulsions, nanotubules, nanofibers and self-assembled peptides for targeted delivery of immunomodulatory agents, development of Virus-like particles (VLPs), vaccines, adjuvants and immunoassays systems. **Conclusion:** Nanoparticles and nanostructures hold great promise for resolving existing biological and medical especially immunological problems because of their Physico-chemical properties.

11292P

Comparing the Cytotoxic Activity of Taheebo Tea against Murine Breast Cancer Cell Line (4T1) in Different Boiling Times

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Introduction: Breast cancer is the leading cause of death in women worldwide. The majority of drug candidates, currently used in clinical cancer chemotherapy, have been originally derived from plants. Taheebo, extracted from the inner bark of the *Tabebuia avellanedae* tree (found in the Brazilian Amazon), exhibits selective anti-proliferative effects in carcinoma cell lines. Taheebo is under FDA approving process. **Materials and Methods:** Air-dried taheebo tea was weighed, and the extracts were prepared by boiling in PBS for 15, 30, 45 and 60 min. 4T1 cell line was cultured in RPMI medium supplemented with FBS. The diluted aqueous extracts were added, and after 24h and 48h incubation, the MTT test was performed. The extracts were also tested for peripheral blood mononuclear cells. **Results:** We observed a proliferative effect in the 15 min boiled extract on the 4T1 and normal cells while the 30, 45 and 60 min boiled extracts did not show any proliferative effect. There was a significant cytotoxic effect with 30% viability on the 4T1 cell line, especially in the 60 min boiled extract, whereas the normal cell's viability was up to 70%. **Conclusion:** The results showed that by increasing the boiling time, the proliferative effect of taheebo tea decreases, and its cytotoxic effect increases. It is concluded that boiled taheebo extract, as a chemotherapeutic agent, can be a proper candidate in clinical use in the context of cancer treatment.

12362P

Efficacy of the combined immunotherapy with the lysate of heated 4T1 and propranolol in mouse model of breast cancer

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Introduction: It has been shown that β-adrenergic receptor antagonists such as propranolol, promote immune responses toward Th1profile. The 4T1 mammary carcinoma is an easily transplantable, highly tumorigenic cell line with low immunogenicity that can be used as an experimental model for human mammary cancer. This study was done to evaluate the efficacy of a new immunotherapy against breast cancer, which was made by mixing the lysate

of heated 4T1 cells and propranolol, as an adjuvant. **Materials and Methods:** For tumor induction, female BALB/c mice of 6–8 weeks old were challenged subcutaneously in the right flanks with 4T1 cells. The first immunotherapy was initiated when all animals had developed a palpable tumor. Immunotherapy was done twice with one week interval. One week after the last immunotherapy, half of the mice were euthanized in order to determine the immune response profile. The remaining animals were kept until the time when death occurred spontaneously. **Results:** The combined immunotherapy in mice with mammary tumors caused a more favorable survival curve and slower rate of tumor development compared to the mice with tumors that received only heated 4T1 and/or negative control mice. Moreover, the combined immunotherapy significantly amplified the secretion of IFN-γ, and, conversely, diminished the secretion of IL-4, IL-10, and TGF-β in the splenocyte population compared to splenocytes from other groups. **Conclusion:** The combined propranolol and heated 4T1 cells promote beneficial outcomes in mouse model of breast cancer. Because of the low immunogenicity of 4T1 cells, these findings are beneficial. **Keywords:** 4T1 cells, Breast cancer, propranolol.

13577P

The Beberine effect on survivin mRNA expression in peripheral blood mononuclear cells of CLL patients

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Introduction: Chronic lymphocytic leukemia (CLL) is one of the most common hematologic malignancies.CLL is characterized by a typical defect in apoptosis and is still an incurable disease. Berberin, a naturally occurring isoquinoline alkaloid, has been shown to possess anti-inflammatory and antitumor properties in some in vitro systems. Survivin protein inhibit caspase activation, thereby leading to negative regulation of apoptosis or programmed cell death (apoptosis marker). The aim of this study was to investigate the effect of Berberine on survivin gene expression **Material and method:** 5 ml whole blood was collected from 16 CLL patients and 8 healthy donors from Kosar Hospital in Semnan and PBMC of these samples was separated with ficoll gradient. PBMC cells was treated by Berberine for 48h in RPMI 1640medium 37C^{o} – CO2 5%. RNA was extracted from PBMC cells and Real time PCR was run for evaluating the expression of survivin by using SYBR Green method (ABI 7900). **Results:** The surviving expression was compared in CLL patients and healthy subjects. Our data indicated that Berberin as an apoptotic agent significantly reduce the expression level of surviving mRNA in CLL patients ($P<0.05$) in compare to untreated CLL cells. However, there was no significant change on PBMC of healthy subjects. **Conclusion :** It seems that Berberine induces apoptosis in PBMC of CLL patients by decreasing survivin gene expression while it has no effect on survivin expression in PBMC of normal subjects. Down-regulation in survivin expression causes apoptosis and sensitization to anticancer drugs. These findings may help to consider Berberine as a safe anti-cancer drug for CLL patients. **Key words:** Berberin, Survivin Expression, CLL, Apoptosis

Medical Ethics in Immunological Researches

Poster Presentations:

11235P

The survey of moral sensitivity and depression in the fasting people working in teaching hospitals of Jahrom in 2014

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Introduction: moral Sensitivity enables a person's to detect ethical conflicts and Intellectual sense assumes from vulnerable situations and awareness to ethical results of making decision about others, This study aimed to investigate the ethical sensitivity and depression in the fasting people working in teaching hospitals was arranged in 2014.**Materials and Methods:** The present study was convenience that it performed in 2014 on the Nurses of therapy educational centers. After the presentation of an introduction letter, the list of nurses prepared and Proportional with number of nurses in each part, according to simple random sampling was done. Collecting instruments consisted of demographic and moral sensitivity and depression questionnaire was DASS. The data analyzed with using spss software version 21 and statistical t-test was performed. **Results:** the results of Chi-square test shows that there is a statistical significant between the level of moral sensitivity and depression in the fasting people (P-value>0.05).also there isn't statistical significant between the level of moral sensitivity and depression in the non-fasting people (P-value<0.05).**Conclusion:** The results of this study showed that there is a statistical significant between the level of moral sensitivity and depression in the fasting people.

Monoclonal Antibody (Diagnostic & Therapeutic)

Oral Presentations:

7533O

Isolation of recombinant single chain variable fragment (scFv) against human CD47 biomarker with desired affinity from phage display library

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Introduction: Overexpress of CD47 protein in bladder cancer stem cells (CSCs) cause to escape from immune system and act as a protective mechanism for survival of tumor cells. Studies showed the production of recombinant antibodies with desirable affinity and specificity replacing non-engineered monoclonal antibodies with significant function for more effective Targeting of biomarkers. The aim of this study was to isolate an specific anti CD47 ScFv through biopaning from our previously constructed library . **Material and Methods:** A scFv library was previously constructed from variable light and heavy region of immunized mouse genomic immunoglobulin repertoire. The library was infected with M13KO7 helper phage then phage particles displaying various scFvs fusion with surface protein III were panned against CD47 up to 4 rounds. Enrichment of the eluted phage was measured by polyclonal phage ELISA. To isolate individual CD47 scFv with desired affinity, various clones derived from eluted phages, were screened by monoclonal phage ELISA separately. **Results:** The results showed that the titer of phage bound to CD47 increased steadily from 10^6 pfu in the first round to 17×10^7 pfu in the fourth , implying that specific binding clones were selected and enriched during panning as confirmed by polyclonal phage ELISA. Finally, 7 phage clones was selected from the library by monoclonal phage ELISA that strongly interacts with human recombinant CD47 protein. **Conclusion:** These results suggested that by using an optimized protocol and sufficient repertoire diversity, a specific scFv, can be isolated for particular targets. **Keywords:** Bladder CSCs, CD47, Phage display, ScFv, Biopaning

7631O

Construction and characterization of HBV neutralizing chimeric antibody against Hepatitis B surface antigen with ability to recognize mutant HBsAg

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Introduction: Hepatitis B surface antigen (HBsAg) induces a protective antibody response which could neutralize hepatitis B virus (HBV). The "a" determinant is a hydrophilic immuno-dominant region presents in HBsAg of all subtypes and genotypes of HBV. We have recently produced a murine anti-HBsAg antibody (4G4) which recognizes an epitope within the "a" determinant and neutralizes HBV infection in HepaRG cells. Here, chimerization (human-murine) and characterization of this monoclonal antibody were described(c-4G4). **Material and Methods** Variable region gene of heavy and light chains of the 4G4 were cloned and fused to constant regions of human kappa and IgG1 by Splice overlap extension (SOE) PCR. The chimeric antibody was expressed in CHO cells and purified from culture supernatant. **Results:** The antigen-binding studies with ELISA and Western blot showed that this chimeric antibody retained the high affinity and specificity of the parent mouse antibody. The results of competition ELISA showed that both antibodies bind to the same epitope. Both mouse and chimeric mAbs showed a similar pattern of reactivity to 13 escape mutant forms of HBsAg and a potent HBV neutralization capacity in in vitro model even at low concentration. **Conclusion:** Due to the ability of c-4G4 to recognize a variety of escaping mutants of HBsAg and neutralize HBV, this antibody could be considered as a potential alternative for the commercial polyclonal hepatitis B immune globulin (HBIG), which is currently used for prophylaxis and passive immunotherapy against HBV infection.

7703O

Dimerization effect on efficacy of a novel bivalent single chain antibody directed to human CD123, as leukemic cancer stem cell marker

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Introduction: Current therapies for acute myeloid leukemia (AML), are associated with high relapse rates. Hence, development of new therapeutic strategies is crucial to circumvent this problem. Bivalent antibody technology has been used to engineer novel antibody fragments with increased avidity, by assembling two scFv in a single molecule. Here we describe the construction of a biscFv antibody targeting CD123, the most important biomarker of leukemic cancer stem cells which play a key role in relapsed AML after chemotherapy. **Material and Methods**The anti-CD123 scFv isolated from our phage display library, was PCR amplified then fused in tandem via a flexible (Gly4Ser) 3 linker. Following tree steps of cloning, expression in E.coli Bl21and purification by IMAC. The purified biscFv, was characterized by SDS–PAGE, western blotting and ELISA. **Results:** The construction of biscFv was confirmed by colony PCR and sequencing. Following expression in E.coli and purification, the purified biscFv, which had a molecular weight of ~56 kDa was confirmed by westernblot. Furthermore, ELISA, demonstrated that biscFv could bind to recombinant human CD123 with higher avidity than of its scFv counterpart.

Conclusion: On the basis of the results, it is apparent that the construction of bivalent scFv, can lead to the generation of tumor-targeting agents with improved efficacy. Thus, it may have a significant impact on the design of future antibody-based molecules. Also, this anti-CD123 biscFv, may become a good candidate for targeting LCSs which is considered incurable with conventional therapy. In this regard, further characterization remains to be done.

11170O

A Web-Based Tool to Identify Immuno-Proteins

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High-throughput immuno-genomics, immuno-proteomics, immuno-metabolomics and vast amount of experimentally generated data turned immuno-biology into a data-rich science. Most of the treatment methods and pharmaceutical drugs act via targeting proteins in human body and affect their behavior in biological systems. Druggability of components in drug discovery used to describe a biological target that is known to or predicted to bind with a drug or nutraceutical molecule. Druggable proteins could be defined as proteins which have the ability of interacting with small molecules or antibodies. Considering the necessity of druggable proteins' identification, an online Database of all druggable and reactive proteins has been designed (http://drugminer.org/). Drug Miner predicts proteins and Fc-fusion proteins that have the potential of interaction with drugs. This prediction is performed based on machine learning techniques. User friendly interface of DrugMiner would let the researchers to survey their search including: search for druggable immuno-proteins, their function, etc. In addition, it provides availability of submitting protein to check whether it can interact with drugs or not. All proteins with capability of interaction with drugs were identified as druggable proteins. Then those with immunological function were filtered and collected from Universal Protein Resource (UniProt), finally with the aid of DrugMiner a list of immune system related proteins prepared that are potentially druggable. The existing entries in the DrugMiner database, provide an opportunity for researchers minimize their experimental task in terms of time and finance since it eliminates redundant steps of any experiment. This database is promisingly recommended as initial step for protein- and drug-related experiments.

12415O

Development and functional evaluation of anti-ROR1 scFv on a panel of B- CLL cells

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Introduction: Receptor tyrosine kinase ROR1, an embryonic protein involved in organogenesis, is expressed in certain hematological malignancies and solid tumors, but is generally absent in adult tissues. Limited evidence exists demonstratin that blocking of ROR1 in tumoral cells leads to growth inhibition and apoptosis of cancer cells. Therefore, in the study reported here we aimed to isolate a specific human scFv against ROR1 using phage display technology**Material and ethods:** A human semi-synthetic Tomlinson I + J phagemid library was used for isolating

a specific scFv against ROR1. The library was infected with KM13 helper phage then phage particles displaying various scFvs fusion with surface proteinIII were panned against ROR1 up to five rounds. Enrichment of the eluted phage was measured by polyclonal phage and monoclonal phage ELISA, respectively. Then, positive scFvs were expressed in a soluble form in Escherichia coli HB2151 and tested for positive scFvs by using scFv-ELISA. **Results:** After the five biopanning rounds several specific clones were isolated which among them one phage clone (ROR13) with higher affinity was purified for further analysis. The reactivity of scFv isolated with ROR1 was confirmed by ELISA and western blotting. **Conclusion:** In summary, we have isolated a human scFv against ROR1 with high affinity and further characterization is needed to definitely pinpoint the potential diagnostic and therapeutic uses of scFV ROR1.

Poster Presentations:

7534P

Characterization of anti-CD47 scFv selected from murine antibody library by phage display technology

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Introduction: Overexpress of CD47 protein in bladder cancer stem cells (CSCs) cause to escape from immune system and act as protective mechanism for survival of tumor cells. Studies show production of recombinant antibodies with desirable affinity and specificity replacing non-engineered monoclonal antibodies have significant function for more effective Targeting of biomarkers. The aim of this study was expression, purification and characterization of a novel anti-CD47 scFv, derivative of our previously constructed murine scfv library through phage display technology. **Material and Methods:** The scFv gene of selected clone was sub-cloned from phagmid pSEX81 into the pOPE101 expression vector. 6His-tagged scFv expressed as a soluble protein in priplasm of E.coli HB2151 then purified by IMAC. The purified scFv was characterized by SDS–PAGE, western blotting and ELISA.The affinity and specificity of scFv was determined by ELISA as well. **Results:** Soluble scFv was expressed as a priplasmic protein consequent of IPTG induction. After IMAC purification, SDS–PAGE and western blotting using protein L HRP conjugate, showed a specific band of scFv antibody in size of 26 kDa... In ELISA the purified scFv recognized the human CD47 recombinant protein. Cross-reactivity studies revealed that the antibody showed desirable specificity to CD47 and the resulting KA was 5.3 × 10-8M. **Conclusion:** These findings suggested that the produced recombinant scFv antibody can recognize the CD47 antigen with an appropriate specificity and affinity. By further studies, the capability of this antibody for targeting of CD47+ CSC could be evaluated. **Keywords:** CSCs, scFv, Human CD47 protein, Affinity, Library

7698P

Determination of Affinty of Anti-Alkaline Phosphatase Monoclonal Antibodies by an ELISA – based Method

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Introduction: The affinity of an antibody to its relevant antigen often has a significant impact on the performance of that antibody. This study aimed at measuring the affinity monoclonal antibody (product A1G9G3 Hybridoma clone) against Alkaline Phosphatase (ALP) by ELISA method. **Material and Methods:** After reaching the equilibrium of soluble antigen and antibody, the affinity of anti-alkaline phosphatase IgG antibody produced by A1G9G3 colon was determined using a competitive ELISA method and drawing Klotz diagram. **Results:** Antibody's fixed concentration was determined to use in a specific ELISA, dissociation constant (Kd) was achieved as 1.2 mg/ml using Klotz method by a competitive ELISA, Kd of A1G9G3 colon was estimated as 4.3x10-9 by drawing a Klotz equation. **Conclusion:** As the Kd of human natural antibodies for most antigens ranges 10-7 and 10-11 and the smaller the Kd is, the more the antibody's affinity will be, the monoclonal antibody produced by A1G9G3 colon in the Immunity Laboratory of Tarbiat Modares University seems to be suitable for most immunological measurements. **Keywords:** Monoclonal Antibody, Affinity, Klotz diagram, Dissociation Constant

9789P

A predicted hairpin structure corresponds to resistance of cloned anti-CD20 antibody 1F5 chimeric heavy-chain gene to PCR, sequencing and possibly restriction analysis

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Introduction: Formation of secondary structure such as DNA hairpins or loops may influence molecular genetic methods and PCR based approaches necessary for genetic engineering, in addition to gene regulation. **Material and Methods:** A polymerase chain reaction with splice overlap extension (SOE-PCR) was used to create fully synthetic chimeric anti-CD20 heavy and light chain genes. The chimeric genes were cloned into the pCR-Blunt II-TOPO vector following by cloning into the pBudCE4.1 expression vector. Prediction of secondary structure was performed with the Vienna RNAfold webserver. PCR and sequencing across the predicted secondary structure of chimeric 1F5 heavy chain gene was performed with multiple protocols for standard and GC-rich templates. **Results:** In our attempt to design vectors aimed to generate mouse-human chimeric antibody against CD20 (1F5), we found that the coding sequence of chimeric heavy chain gene (VH-CH) constructed by SOE-PCR was resistant to polymerase during both PCR and sequencing reactions. Furthermore, we were also unable to analysis some positive transformants by restriction enzyme digestion. **Conclusion:** Encountering such difficulties to identify the cloned anti-CD20 chimeric heavy chain gene, we found that the VH-CH sequence is highly GC-rich and predicted to form a stable secondary structure. Our findings provide a probable note for researchers experiencing technical difficulties with construction of chimeric anti-CD20 antibody 1F5 gene vectors and also with other genes and molecular biology techniques requiring PCR-based method or restriction enzyme analysis.

10786P

Bioinformatics analysis and identification of antigen-binding regions in VHH against Clostridium botulinum neurotoxin E

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Introduction: Botulinum neurotoxins result in severe and often fatal disease, botulism. Present study was designed to in silico resolving the major obstacles in the control of diseases caused by clostridium botulinum E. For their fast effect in the treatment of various diseases, the use of recombinant antibodies has attracted the researchers. Antibodies are capable of specifically recognizing and binding antigens. Identification of the antigen-binding site, commonly dubbed paratope, is of high importance both for medical and biological applications. The aim of this study was prediction of the antigen-binding regions (ABRs) and determination of conserved functional and structural amino acids in VHH against Clostridium botulinum neurotoxin E. **Material and Methods:** In this bioinformatics study, informed by the VHH sequence, the third structure was determined using phyre2 software. After determination of the third structures, the antigen-binding regions (ABRs) of VHH, given its amino acid sequence or 3D structure, predicted using Paratome web server. To determine conserved functional and structural amino acids in VHH, antibody sequence is used as an Input file in Conseq and *POOL server. That* is a machine learning application used for *functional site prediction in proteins*. **Conclusion:** The VHH against *Clostridium botulinum neurotoxin E* was previously produced and expressed in *P. pastoris.* The aim of this study was taking steps in the direction of engineering the antibody to improve its performance.

10827P

Construction, bacterial expression of a single chain antibody targeted to the human epithelial cell adhesion molecule extracellular domain

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Introduction: The epithelial cell adhesion molecule (EpCAM) is a membrane glycoprotein that is highly expressed on carcinomas with epithelial origin and therefore of potential use as a diagnostic or therapeutic agent for a variety of carcinomas. EpCAM is explored as target in antibody-based therapies. The use of genetically engineered antibodies has emerged as an alternative to the use of polyclonal and monoclonal antibodies for the treatment and diagnosis of a variety of diseases. The single chain variable fragment (scFv) is one popular type of genetically engineered antibody. The scFv is composed of the variable regions of the heavy (VH) and light (VL) chains of an immunoglobulin molecule. Escherichia coli is one of the most widely used hosts for the production of recombinant proteins. Here, we constructed scFv against the EpCAM extracellular domain and expressed the recombinant scFv protein in Escherichia coli. **Material and Methods:** A codon optimized gene expressing scFv recognizing the human EpCAM extracellular domain was synthetically prepared and cloned under the control of an IPTG inducible T7 promoter. The pET22b (+) containing a pelB signal peptide vector was simultaneously transformed and expressed in the periplasm of BL21 (DE3) E. coli strain. **Results:** Constructed plasmid was approved by sequencing reaction and restriction enzyme analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis showed an estimated 30 kDa-size protein band corresponding to the recombinant scFv. **Conclusion:** The results provided a foundation for the development of scFv-based drugs for the treatment of the majority of tumor cells with epithelial origin including colon cancer.

10846P

Production of hybridoma cell for monoclonal antibody production against vaccinal poliovirus type 2

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Introduction: Polio is an acute infectious disease which affects the central nervous system by destruction of motor neurons in the spinal cord. Monoclonal antibody is important in research, diagnosis, treatment and production and quality control of drug and biological products. So this study was performed for preparing cell hybridomas for producing the monoclonal antibodies against polio virus type 2 for research by polio virus and quality control test for oral poliomyelitis vaccine. **Material and Methods:** In the process, the preparation of the virus, injected into mice, mice serum titers by ELISA, prepared myeloma cells, immune cells isolated from mouse spleen, fusion, the hybridoma using ELISA designed, positive hybridoma cloning, cell proliferation suitable hybridoma-produced monoclonal antibody, concentrate and purify the monoclonal antibody and finally quality control tests were conducted. **Results:** Two clones among 250 clones were capable of producing monoclonal antibody against type 2 virus that the antibody was the IgG1 with Kappa chains. Western blot test demonstrated consistency band in the 26 kDa was VP3 protein neutralization formed the virus. **Conclusion:** Hybridoma cells capable of producing the monoclonal antibody were produced that were used for the polio vaccine quality control tests including: potency tests, identification harmless test of vaccine.

11021P

Construction of a Large Human Fab Antibody Phage Display Library

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Introduction: Antibodies have emerged as clinically important drug class, more than 25 antibodies approved for human therapy and more than 240 antibodies are currently in clinical trials for various diseases such as cancer, inflammation and autoimmunity. Thus availability of a reliable repertoire is critical. Antibody libraries displayed on filamentous phage surfaces provide a stable resource from which high affinity human antibodies to any given antigen can be rapidly isolated. Antibodies derived from these libraries, could subsequently be applied directly for antibody therapy or be targeted to affinity maturation in vitro. **Material and Methods:** For first time in Iran, a large non immune Fab library was constructed by phagemid vectors pCB3 and pCB4. In this way, two different libraries of light chain (Vk-Ck) and the variable regions of heavy chain (VHμ) were constructed from the peripheral blood lymphocytes, bone marrow, tonsils and spleen of healthy human donors. **Results:** The final combinatorial Fab library ($l0^9$ members) was generated by sub-cloning the variable regions of heavy chain (heavy chain library) to phagemid vector containing light chains. After sequencing and evaluating them, the library diversity was estimated 8×10^8. **Conclusion:** Generation of the large human fab antibody library can be so useful for identifying the novel human antibodies by phage display.

11025P

Isolation of a novel human scFv inhibiting EGFRvIII expressing cell line

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Introduction: Phage display is a prominent screening technique for developing novel antibodies with high affinity against almost any antigen. The EGF receptor variant III (EGFRvIII) as a tumor specific antigen is expressed in the different types of cancers including glioblastoma multiforme (GBM), breast adenocarcinoma, and non-small cell lung carcinoma but has been rarely observed in normal tissue. Since, the majority of monoclonal and polyclonal antibodies developed against EGFRvIII have cross reactivity to wild type EGFR or other non-specific proteins. Therefore, the aim of present study was to isolate a specific human scFv against EGFRvIII using phage display technology. **Material and Methods:** A human semi-synthetic Tomlinson I + J phagemid library was used for isolating a specific scFv against EGFRvIII. Screening was performed by a unique strategy to eliminate false positive clones and avoiding from the loss of rare specific phages. **Results:** After the five biopanning rounds several specific clones were isolated which among them one phage clone with higher affinity was purified for further analysis. The reactivity of scFv isolated with EGFRvIII was confirmed by ELISA and western blotting. **Conclusion:** In the present study, for the first time, a high affinity human scFv against EGFRvIII was isolated. This study can be the groundwork for developing more effective diagnostic and therapeutic agents against EGFRvIII overexpressing cancers **Keywords:** Human single chain antibody, Phage display, EGFRvIII, cancer

11037P

Localization of the extracellular subdomains of human HER2 recognized by HER2 specific monoclonal antibodies

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Introduction: Anti-HER2 antibody-based therapy is one of the main widespread strategies in patients with HER2-overexpressing breast cancer. Anti-HER2 monoclonal antibodies (mAbs) affect cancer cells differently, targeting distinct epitopes of the antigen. **Material and Methods:** Reactivity of 8 mouse mAbs specific for human HER2 molecule was studied by a sandwich ELISA. Anti-tumor activity of these mAbs was assessed by 3H-thymidine incorporation assay. The extracellular domain of human HER2 (HER2-ECD) and different subdomains of HER2-ECD including subdomains I, II, III, IV, I+II, II+III and III+IV, were cloned and expressed in CHO cell line. Each subdomain contains 30 overlapping amino acids with the adjacent subdomain. **Results:** Our results demonstrated that 3 of the mAbs detected conformational epitopes (1T0, 2A9 and 1B5) while 5 mAbs identified linear epitopes (1F2, 1H9, 4C7, 1H6 and 2A8). Two of the mAbs (1T0 and 2A8) induced anti-proliferation effect whereas 4 mAbs (1F2, 1B5, 1H9 and 4C7) enhanced the proliferation of HER2 overexpressing tumor cells. Three of the mAbs recognized subdomain I (1F2, 2A9 and 1H6), one mAb recognized subdomain I+II (1T0), and one mAb recognized subdomain III (4C7) and 3 mAbs recognized subdomain III + IV (1B5, 1H9 and 2A8). However, none of our MAbs

recognized subdomain II of HER2 alone. **Conclusion:** These data suggest higher immunogenicity of human HER2 subdomains I and IV in Balb/c mice. It seems that there was no association between subdomains specificity and anti-tumor activity of our anti-HER2 mAbs. **Keywords:** HER2, Extracellular domain, Monoclonal antibody

11298P

Isolation of Anti-IGF1R Single Chain Variable Fragment Antibodies by Phage Display Technology

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Introduction: Breast cancer is a common neoplasm and the second leading cause of cancer related deaths in women worldwide. Studies have shown that the expression of IGF1R, a tyrosine kinase receptor, is increased in several subtype of breast cancer and is involved in breast carcinogenesis through alteration in the expression of genes that contribute to cell survival and proliferation. For these reasons, it has been suggested that, IGF1-R and its signaling pathway can be a suitable target for prevention and treatment of breast cancer. **Material and Methods:** Five rounds of biopanning were performed on a phage library (Tomlinson I+J) to isolate scFvs against IGF1R. Characterization of selected scFvs was performed by means of polyclonal and monoclonal scFv-phage ELISA, soluble ELISA and PCR. **Results:** Sixty phage clones from outputs three, four and five were randomly selected and tested for IGF1R binding by monoclonal phage ELISA. Seventeen clones were identified that specifically bound to IGF1R, but not to BSA control protein. Seven positive phages were used for soluble ELISA and were expressed in soluble form in E. Coli HB2151 in presence of IPTG induction. **Conclusion:** Seven scFvs have been isolated capable of recognizing IGF1R. These scFvs were shown to possess high binding affinity for IGF1R, which can be tested as potential immunotherapy agents for treatment of breast cancer. **Keywords:** IGF1R, Breast cancer, Phage Display

12404P

Rapid and simple digesting human IgG with pepsin and one step purification F(ab')2 in large scale

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Introduction: Immunoglobulin fragments have advantages over whole antibody molecules in several experimental procedures including immunohistochemistry and in vivo studies because they eliminate non_specific binding between Fc portions of antibodies and Fc receptors and penetrate tissue more efficiently due to their smaller size, inaddition these fragments showed better therapeutic impact as an anti venom therapy. However, experimental conditions for the production of F(ab')2 are variable, difficult, time-consuming and only, proper for few scale. Here we report optimal digestion time, pH, and enzyme to antibody weight ratio for pepsin digestion of human IgG into F(ab')2 antibody fragments in large scale and optimal method of purification. **Material and Methods:** Digestion method for human IgG were determined in different time, pH and pepsin to antibody weight ratio and purification

managed with sephadex G-100. **Results:** The best digesting way was obtained in 1 hr with a pH of 3.2 and 1:40 pepsin to antibody weight ratio and purification with sephadex G-100, respectively.and the purity of F(ab')2 fragment assessed by SDS-PAGE; indicating the purity of approximately 90%.Conclusion: Present methode demonstrate the optimal condition for in large scale digestion of human IgG. **Keywords:** Human IgG, F(ab')2 ,pepsin digestion

12405P

A Simple Method For Production Of F(ab')2 Prepared By Pepsin Digestion Of Rabbit Anti Human IgG

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Introduction: The use of F(ab')2 fragments may in certain immunological test be advantageous for the use of intact IgG antibodies. Non-specific binding through the Fc part of the IgG molecule could be avoided and IgG fragments have shown great promise for immunotherapy. The aim of present study is production of Rabbit anti human F(ab')2 fragment, but the first step in generating such product is production and purification of Rabbit's polyclonal antibody against human IgG. **Material and Methods:** First, production and purification Rabbit's polyclonal IgG against human IgG was performed. Rabbit's polyclonal IgG was cleavaged by pepsin digestion and production of F(ab')2 fragments. Then, we reported optimal digestion time, pH, and enzyme to antibody weight ratio for pepsin digestion of Rabbit IgG into F(ab')2 antibody fragments. The digestion products were examined by electrophoresis, and the combination of pepsin concentration, incubation time and pH provided the most efficient digestion of the antibody into dimeric antigen-binding fragments F(ab')2. F(ab')2 fragments Were purified by G-100 gel filtration chromatography and conjugated with FITC. **Results:** We described a simple protocol for the preparation of Rabbit F(ab')2 fragment. Optimal conditions were determined to be 30min with a pH of 4.0 and 1:40 pepsin to antibody weight ratio. **Conclusion:** According to the results of this study, the protocol, comparing to the previous protocols was time and cost beneficial. **Keywords:** F(ab')2 fragment. Polyclonal antibody. Purification. Pepsin digestion. Rabbit IgG

12406P

Isolation and characterization of Fully Human ScFv Antibodies against Gastrin Receptor from Tomlinson J Library

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Introduction: Gastric/gastrointestinal cancers are associated with highmortality worldwide. It has been demonstrated that gastrin/cholecystokinin-B receptor (CCK-BR) as a membrane of G-protein coupled receptor (GPCR) superfamily play key role in progression of gastric tumors. Therefore, CCK-BR can be considered as potential target for immunotherapy. However, production offunctional monoclonal antibodies (mAbs) against GPCR seems to be challenging, in partdue to its low level of expression, less accessibility for antibody binding and

preserving intact structure during purification. In this investigation, to tackle this problem, we implemented phage display technology (PDT) to isolate recombinant scFv antibodies recognizing native structure of CCK-BR. **Material and Methods:** In the current study, 10 specific scFv clones previously selected against CCK-BR, using solution phase biopanning, were prepared for expression and purification. After confirming expression and purification by SDS-PAGE electrophoresis and Western blotting, binding specificity of the scFvs antibodies to CCK-BR protein was evaluated with Western blotting. **Results:** All scFv antibodies were expressed and purified successfully. Expression yield of scFvs ranged from 0.3 to 1.34 mg per liter of culture. Specificity analysis of the selected scFvs revealed that 8 of 10 scFv clones detected denatured form of CCK-BR protein in western blotting. **Conclusion:** In this study, we have report a diverse panel of scFv antibody fragments specific to CCK-BR. Theseresults suggest that the selected scFv antibody fragments can be used as potential reagents for research diagnosis, imaging, targeting and immunotherapy of cancers overexpressing CCK-BR.

12407P

Expression optimization of scFvs clones Isolate from Phage-antibody library

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Introduction: Antibodies used for many research, diagnostic and therapeutic application. Phage antibody display technology is an extremely powerful platform to isolate recombinant antibody fragments against any antigen through incubation of engineered phages antibody library with a given immobilized antigen, which known as "biopanning". **Material and Methods:** ELISA screening assay after biopanning is necessary step to identify antigen-specific antibody fragment clones. Therefore, we applied different culture conditions supplemented with sucrose, media (2xYT, LB and their phosphate buffered, and TB) and Triton X-100 to increase production of soluble single- chain fragment variable (scFv) in 96-well Microtitre plates (MTPs) in order to increase numbers of positive hits. For this purpose, four scFv antibodies previously isolated from Human single fold scFv libraries I + J (Tomlinson I + J) against the second extracellular loop (ECL2) of gastrin/ cholecystokinin B receptor (CCKB-R) were tested. **Results:** Among tested media, TB and 2xYT resulted in high level expression of three functional scFvs out of four. The addition of 50 mM sucrose increased the functional yield for one of four scFvs, whereas the production of the other three were not altered. Density 0.1% Triton X-100 increased the functional expression of the two scFvs tested. **Conclusion:** In the current research it was proposed that using enrich media such as TB and 2xYT or addition of sucrose and Triton X-100 can be increased yield of functional scFvs, thereby improving ELISA screening.

12408P

Isolation and Production of ScFv Monoclonal Antibody against Glycine-Extended Gastrin

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Introduction: Glycine-extended gastrin 17(G17-Gly) is one of the most important factors in the pathogenesis of colorectal cancer (CRC). It has been demonstrated that recombinant monoclonal antibodies specific to gastrin peptide has encouraging results ingrowth inhibition of CRC *in vivo* and *in vitro* .In this work, we used phage display technology to isolate antibodies against G17-Gly as a growth promoting agent for CRC. **Material and Methods:** The human synthetic phage antibody library was used for the isolation of single-chain Fv fragment antibody (scFv)against biotinylated G17-Gly via solution phase biopanning in which specific phage antibodies are captured by streptavidin-coated magnetic beads and nonspecific phage binders are removed by washing. Five rounds of selection were performed and the enrichments of specific phages were confirmed by polyclonal phage ELISA. For identification of the biotinylated G17-Gly specific phage monoclonal scFv, ELISA screening was done for rounds 4 and5. PCR and DNA sequencing were used to determine scFv clone diversity. **Results:** Polyclonal phage ELISA showed successful enrichment of the antigen-specific phages from round 1 towards5.ELISA screening resulted in 24 scFv clone shaving positive reaction with the antigen. Alignment of nucleotide sequences for the positive colons revealed 2 diverse clones. **Conclusion:** The present study provided that phage display technology could be successfully applied to isolate scFv antibody fragments against G17-Gly through solution phase biopanning.

12412P

Isolation of single chain variable fragment (scFv) specific for Frizzled 7 (Fzd7) receptor from human single fold scFv libraries

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Introduction: Antibody phage display (APD) is a powerful technique for antibody selection, APD provide an invaluable system for its low- price, rapid and continual production of specific antibody fragments directed against targets and develops a simple detection method. Wnt signaling and one of its receptors named Frizzled 7 (Fzd7) is usually activated in a variety of human cancers. Studies have revealed that overexpression of several Wnt pathway genes, such as Fzd7 lead to cancer progression. In addition, Fzd7 could be a promising biomarker and a potential therapeutic target for cancer. Inhibition of Fzd7 and its ligand could decreases metastasis rate along with cancer cells proliferation. In the present study we isolated single chain variable fragments (scFvs) by phage display method against Fzd7 receptor. **Material and Methods:** We used semi synthetic antibody libraries (Tomlinson I + J) by Repetitive affinity selection procedure instead of immunization process to isolate scfv against Fzd7 receptor. The antibodies were characterized and their validity was checked by enzyme-linked immunosorbent assay (ELISA) and colon polymerase chain reaction (PCR) respectively. **Results:** After five rounds of panning, seven individual scFvs were obtained. The specific reactivity of selected scFvs was confirmed by ELISA. **Conclusion:** We have isolated seven scfvs which were reacted with Fzd7. Given the critical role that Fzd7 and Wnt pathway play in tumor progression, these scFvs have significant potential for therapeutics purposes.

Mucosal Immunology

Oral Presentations:

7601O

The association between HLA-DQ2.5 and severity of clinical symptoms in patients with celiac disease

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Introduction: Celiac disease (CD) is an autoimmune disorder and triggered by gluten, protein that found in wheat, barley, rye and other grains. The presence of HLA-DQ2 and HLA-DQ8 alleles is the most important genetic factors that implicated in the pathogenesis of celiac disease. Previous studies showed that there is correlation between HLA DQ alleles and severity of the mucosa. Some patients, carriers HLA-DQ2.5 allele, has important role in severity of clinical symptoms. Therefore, the aim of this study was to demonstrate the correlation between clinical symptoms and frequency of HLA-DQ2.5. **Material and Methods:** 75 celiac disease patients were included and HLA-DQ2 alleles were genotyped by Real-time PCR using SYBR Green as a low-resolution method. Alleles were examined to predict the HLA-DQ2 haplotypes including HLA-DQB1*02, HLA-DQA1*05 for HLA-DQ2.5. Then correlation between HLA-DQ2.5 and clinical sign were evaluated. **Results:** The results showed that most patients (66.7%) with severe clinical symptoms like diarrhea carried HLA-DQ2.5 risk alleles and this correlation was statistically significant (P=0.001). But no statistically significant correlations were between this haplotype and the other clinical symptoms (P>0.05). **Conclusion:** The results showed that many patients with typical presentation carried high HLA risk allele (HLA-2.5) which can participate in celiac pathogenesis and made severe clinical symptoms. **Keywords:** Celiac disease, clinical symptoms, diarrhea, HLA-DQ.

7710O

Variations in number, phenotype, and activation of the small intestinal lymphocyte-filled villi during weaning in the rats: a wonderful lymphoid organ, which may be the extra-thymic site for T cell development and oral tolerance

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Introduction: Lymphocyte filled villi (LFV) are unique lymphoid organs in the mucosa of the rat small intestine and are distinguished by the presence of packed lymphocytes occupying the entire lamina propria (LP). LFV are present in both normal and athymic nude rats. LFV may be sites of primary extrathymic T-cell differentiation in the rat small intestine. The goals of this study were to study changes in the number, phenotype and activation status of lymphoid cells in LFV, and to compare these cells with those in the epithelium and LP compartments. **Materials and Methods:** The jejunal small intestine was snap frozen, and the cut sections were stained either by the immuno-peroxidase or immuno-alkaline phosphatase technique using different monoclonal antibodies. **Results:** Total number of LFV cells were expanded approximately 2-fold during the weaning phase. Nevertheless, no detectable Ki-67 staining was evident over this time. α/ßTCR+ T-cells appeared in LFV in the third week of life. Intriguingly, there was an early increase of NK cells (at day 19) that remained greater than the proportion of α/ßTCR+ T-cells. LFV cells also contained a much higher proportion of IL-2R+ bearing cells in suckling rats compared to the iIELs and LPLs. **Conclusion:** A high proportion of IL-2R+ expression by LFV cells, an early T-cell activation marker, and low expression of α/ßTCR suggested that LFV may be primary sites for T-cell differentiation and maturation in the gut. Furthermore, they could be also involved in the deletion of antigen-reactive α/ßT-cells and could therefore be involved in oral tolerance.

10976O

Influence of Administration Route on Omp31 immunogenicity against *Brucella melitensis*

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Introduction: Brucellosis is a group of closely related zoonotic bacterial diseases caused by the members of the genus *Brucella* (*B*). *B. melitensi* 31 kDa outer membrane protein (Omp31) is a promising candidate for a subunit vaccine against brucellosis. This study surveys immunogenicity of Omp31 alone and with Freund's adjuvant (Omp31-IFA) and N-trimethyl chitosan (TMC/Omp31) nanoparticles, as well as the effect of Omp31 immunization route on immunological responses and protection. **Material and Methods:** After expression and purification, the recombinant Omp31 (rOmp31) was loaded onto TMC nanoparticles by ionic gelation. Particle size and loading efficiency of the nanoparticles were determined. Omp31-IFA was administered intraperitoneally while TMC/Omp31 nanoparticles were administered orally and intraperitoneally. Antibody detection, cytokine and protection assay were performed. Finally, immunized mice were challenged with virulent *B. melitensis*16M.**Results:** The results showed that intraperitoneal (i.p.) immunization by Omp31-IFA and TMC/Omp31 nanoparticles induced Th1 and Th1-Th2 immune responses, respectively whereas oral immunization with TMC/Omp31 nanoparticles elicited a

mixed Th1-Th17 immune response. Moreover, oral immunization increased IgA levels in feces. **Conclusion:** Altogether, our results indicated that TMC nanoparticles were able to elicit Th17 immune responses in oral administration of Omp31. Also, Omp31 when administered orally confers more protection against *B. melitensis*, which may be due to the induced Th17 response.

11185O

Upregulation of PD-L1 and PD-L2 in women, but not men, with ulcerative colitis

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Introduction: Ulcerative colitis is an idiopathic disease. The aim of this study was to evaluate whether or not the gene expression level of PD-L1 and PD-L2 in patients with ulcerative colitis differs from normal individuals. In this study, the samples were collected by colonoscopy from the margin of colon ulcer tissue. **Material and Methods:** Patients whose ulcerative colitis had been confirmed by colonoscopy and histopathology, were considered as test group (n= 50). The negative samples for this disease were considered as control group (n=50). For experimentation, total RNA was extracted from each sample and converted into cDNA. Afterwards, the gene expression levels of PD-L1 and PD-L2 in the test group were evaluated relative to the control group in accordance with TaqMan® method. **Results:** The results showed that no significant difference was observed between men in the test and control groups for PD-L1 and PD-L2 (P = 0.2 for both of them). In the women group, the obtained P-values for PD-L1 and PD-L2 were 0.002 and 0.05, respectively. Moreover, PD-L1 and PD-L2 gene expression in women in the test group were 21.31 and 8.88-fold that of the control group, respectively. **Conclusion:** Given that PD-L1 and PD-L2, especially PD-L1, in the women test group were highly expressed relative to the women control group, so the expression level of these genes can play an important role in the diagnosis of this disease in the early stages. These genes can be considered for therapeutic aims in women with ulcerative colitis disease in the future.

Poster Presentations:

11227P

Investigation of the Relationship between Helicobacter pylori and the Presence of the Gene of Clostridium difficile Toxin and Fluctuations in IgA

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Introduction: Helicobacter pylori is known as one of the most common reasons in nutrition disorders worldwide. Clostridium difficile is one of the causes of nosocomial diarrhea and anaerobic infections, which its prevalence is

associated with increased inflammation. Given the role of the bacteria in many diseases, this study aimed at evaluating antibody titers of IgA in the serum of patients with Helicobacter pylori and its relationship with the presence of Clostridium difficile toxin gene in stool samples of patients. **Material and methods:** In the present research 40 cases with positive test of stool for HPSA and 40 cases with negative test of stool were considered as control group. After culturing cases in CCFA, Clostridium difficile was separating and deriving DNA. Then PCR was done for two gene (tcdA, tcdB). After testing 2 cc from blood of them, the IgA rate of serum was measured. **Results:** Of 40 cases patients, 23 cultures (57.5%) were positive for Clostridium which 18 patients (45%) had toxin A, which 2 had high IgA, and 3 patients (7.5%) had toxin B as well as 2 patients (5%) both toxins, which had no high IgA. Of 40 controls, 18 samples (45%) were positive for Clostridium culture which 6 (15%) had toxin A, 3 (5%) had toxin B and 7 (17.5%) had both toxins. 1 individual having toxin A was high for IgA, which had a history of H. pylori infection as well. None of those having toxin B or both toxins, had no high IgA. **Conclusion:** The level of IgA in patients with positive Helicobacter pylori stool antigen test and those with a previous history of H. pylori infection having part A of Clostridium difficile toxin was observed greater extent than those with negative test.

11278P

Lung Function in Middle-Aged Men: Physical Stress and Mucosal Immunology Moderator Gene Expression

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Introduction: Increase of TGF-α gene expression leading to increased mucus production in the respiratory tract is a symptom of many common illnesses that caused to change the lung function. The aim of this study was the examination of lung function in middle-aged men as for physical stress and mucosal immunology modifiy gene expression. **Material and Methods:** The volunteers in this research were twenty-four inactive healthy middle-aged men (age: 40-50 yr, body fat: 26.25%) that randomly divided into control (n=12) and exercise (n=12) groups. The exercise group performed eight weeks HIIT exercise training 3 days a week (30 min/day) on the treadmill at 60-90% of heart rate reserve. 24 hours before and after the exercise protocol, participants' blood samples, spirometry parameters: forced expiratory volume in first second (FEV1), peak expiratory flow (PEF), maximal voluntary ventilation (MVV), forced vital capacity (FVC) and transforming growth factor-α gene expression were measured. Bio Easy Master Mix Kit and Real-time PCR method were used for TGF-α gene expression. Our data have been analyzed by independent t-test on SPSS software (v.22). **Results:** In the exercise group after 8 weeks high intensity interval training, FEV1 ($p<0.002$), PEF ($p<0.004$), MVV ($p<0.003$) and FVC ($p<0.001$) levels increased significantly. Also TGF-α gene expression in the exercise group compared to the control group showed significant decreased ($p<0.001$). **Conclusion:** The present study showed that HIIT can reduce the TGF-α gene expression as a mucosal immunology moderator and led to lung function improvement in middle-aged men.

Nanoimmunology

Oral Presentations:

9767O

Dendrosomal Curcumin Suppresses Metastatic Breast Cancer in Mice by Changing M1/M2 Macrophage Balance in the Tumor Microenvironment

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Introduction: Curcumin, a lipid-soluble compound extracted from the plant Curcuma Longa, has been found to exert immunomodulatory effects via macrophages. However, most studies focus on the low bioavailability issue of curcumin by nano and microparticles, and thus the role of macrophages in the anticancer mechanism of curcumin has received little attention so far. We have previously shown the potential biocompatibility, biodegradability and anti-cancer effects of dendrosomal nano curcumin (DNC). **Material and methods:** In this study, twenty-seven BALB/c mice were equally divided into control as well as 40 and 80 mg/kg groups of DNC to investigate the involvement of macrophages in the antitumor effects of curcumin in a typical animal model of metastatic breast cancer. At the end of intervention, the tumor volume and weight were significantly reduced in DNC groups compared to control (P<0.05). **Results:** Histopathological data showed the presence of macrophages in tumor and spleen tissues. Real-time PCR results showed that DNC increased the expression of STAT4 and IL-12 genes in tumor and spleen tissues in comparison with control (P<0.05), referring to the high levels of M1 macrophages. **Conclusion:** Furthermore treatment with DNC decreased STAT3, IL-10 and arginase I gene expression (P<0.05), indicating low levels of M2 macrophage. The results confirm the role of macrophages in the protective effects of dendrosomal curcumin against metastatic breast cancer in mice. **Keywords:** Dendrosomal curcumin - macrophage - breast cancer - BALB/c mice

10820O

Surveyon the Effect of Chitosan Nanoparticles with ArtesunateDrug – Iron on the cells of Breast Cancer of Mouse - 4T1 (In Vitro) and Tumor Growth (In Vivo) and the Amount of SecretionCytokines IFN-ɣ and IL-4(Ex Vivo)

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Introduction: Artesunate drugis an herbal medicine that is anticancer which has little side effects on healthy cells. Toincrease drug deliveryand biocompatibility Fe304andpolyethylene glycol and chitosan andfolic acid was added. **Material and method:** That nanoparticle synthesized with co-precipitationwas checked with SEM method andFTIR method. Cell viability was checked by MTT method. In studying of vivo, the study wasdone on breast cancer cells of mice Balb/C which were injected subcutaneous the tumor 4T1and size change of tumor was checked by MRI method. The amount of IFN-ɣ and IL-4 Cytokines were checked by Elisa method. The tissue of tumor and around of it and the liver of mice were checked with Histopathology. Also,the survivals of mice werechecked. **Results:** The made-nanodrug is in form of nano and the size of 234 nanometer and it has the positive charge which has all mentioned factors(Chitosan, poly Chitosan, polyethylene glycol, folic acid,Artesunate drug.Surveys showed the combination ofArtesunate and nanoparticles have favorable effect on the death rate of cell lines 4T1and tumor size.And it increases the amount of IFN-ɣ and decreases the amount of IL-4, synthesized nano-drugcauses apoptosis and necrosis and decreases Angiogenesis and Mitosis and the survivals of mice are increased and it also decreases Mastaz and it does not have the side effects and toxicity. **Conclusion:** The results showed that applying conjugated nanoparticles with Artesunate drug has more effects on decreasing of breast cancer tumor. **Key Words:** Artesunate drug, Fe304, polyethylene glycol, Chitosan, folic acid, breast cancer cell lines 4T1, MTT and Cytokines IFN-ɣ, IL-4

10840O

Immunological comparison of two delivery systems in vaccination against canine leishmaniasis

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Introduction: Visceral leishmaniasis (VL) is a fatal disease caused by the intracellular protozoan parasite Leishmania infantum. Dogs are the primary reservoir of this parasite and the vaccination of dogs could be an effective method to reduce transmission to humans. For vaccine development against VL it is necessary to use an appropriate delivery system to promote a proper antigen-specific immune response. **Material and methods:** In this study, we compared two vaccine delivery systems, namely electroporation and cationic solid–lipid nanoparticle (cSLN) formulation, to administer a DNA vaccine harboring the L. donovani A2 antigen, and L. infantum cysteine proteinases CPA and CPB without its unusual C-terminal extension. The protective potential of these two vaccine delivery systems was evaluated against L. infantum challenge in outbred dogs. **Results:** Our results show that administration of pcDNA-A2-CPA-CPB-CTE GFP as a prime-boost delivered by either electroporation or cSLN formulation protects dogs against L. infantum challenge. Partial protection in vaccinated dogs is associated with significantly ($p<0.05$) higher levels of IgG2, IFN-γ, and TNF-α and with low levels of IgG1 and IL-10 as compared to the control group. Protection was also correlated with a low parasite burden and a strong delayed-type hypersensitivity (DTH) response. **Conclusion:** This study demonstrates that both electroporation and cSLNs can be used as efficient vaccine delivery systems against visceral leishmaniasis.

11084O

Construction and characterization of myelin oligodendrocyte glycoprotein coated PLGA nanoparticles as a tolergenic vaccine in experimental autoimmune encephalitis

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Introduction: Several methods have been used to date the treatment of autoimmune diseases through the induction of antigen-specific tolerance in T cells, but noone have been proved successful. Various studies have shown that apoptotic splenocytes to which peptides or autoantigen peptides are covalently bound, are capable of inducing antigen-specific and stable tolerance in autoimmune diseases. **Material and Method:** The present study seeks to use a simple and inexpensive method to produce Poly (lactic-co-glycolic acid) (PLGA) nanoparticles that replace splenocytes for carrying antigens and induce antigen-specific tolerance and ultimately act as a tolerogenic vaccine. PLGA nanoparticles were produced using the Water/oil/water (W/O/W) method and the nanoparticles larger than 500 nm were separated by centrifuge and filtration paper with distinct cut off. The MOG peptide was covalently bound to the PLGA nanoparticles in the presence of EDCI and was injected intravenously to the C57BL/6 mice one week before the development of the experimental autoimmune encephalo-myelitis (EAE) model. **Result:** The results showed that the intravenous injection of $PLGA_{500}$-MOG_{35-55} one week before the development of the EAE model, delays the incidence of syndromes and their severity. In other words, only the intravenous injection of $PLGA_{500}$-MOG_{35-55} can induce antigen-specific tolerance, as the subcutaneous challenge of smaller nanoparticles containing MOG peptides showed different effects. A reduced delayed-type hypersensitivity response and spleen lymphocyte proliferation were observed in the mice primed with $PLGA_{500}$-MOG_{35-55}. **Conclusion:** The present study proposes a simple, inexpensive, effective and safe method for preparing MOG conjugated PLGA nanoparticles with immune tolerance properties.

11133O

Downregulation of CD73 in 4T1 Breast Cancer Cells through siRNA Loaded Chitosan-Lactate Nanoparticles

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Introduction: The immunosuppressive factors in tumor microenvironment enhance tumor growth and suppress anti-tumor immune responses. Adenosine is an important immunosuppressive factor which can be secreted by both tumor and immune cells through the action of two cell surface ecto-nucleotidase molecules CD39 and CD73. Blockade of the adenosine generating molecules has emerged as an effective immunotherapeutic approach for treatment of cancer. **Material and methods:** In this study, CD73-siRNA encapsulated into chitosan-lactate (ChLa) nanoparticles (NPs) was applied to suppress the expression of CD73 molecule on 4T1 breast tumor cells, *in vitro*. ChLa NPs were generated through ionic gelation of ChLa by tripolyphosphate (TPP). **Results:** SiRNA loaded NPs

had about 100 nm size with a poly-dispersive index below 0.3 and a zeta potential about 13. The results showed that ChLa NPs with Ch 50 kDa exhibited the best physicochemical features with the high siRNA encapsulation capacity. Synthesized NPs were able to fully bind with siRNA, protect them against serum and heparin degradation, and promote the transfection process. While the NPs exhibited low toxicity during 72 hr cell culture, the transfection of Ch-plasmid expressing green fluorescent protein (pEGFP) NPs was efficient in 4T1 cells with a transfection rate of 53.6% as detected by flow cytometry. In addition, CD73-siRNA loaded ChLa NPs could efficiently suppress the expression of CD73 as assayed by real-time polymerase chain reaction and flow cytometry. **Conclusion:**CD73-siRNA loaded ChLa NPs may be considered as a promising therapeutic tool for cancer therapy, however, further *in vivo* investigations are necessary. **Keywords:** CD73, siRNA, nanoparticle, cancer, treatment

Poster Presentations:

7544P

Administration of Zinc oxide nanoparticles improved Neutrophil function in rats with diabetes mellitus.

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Introduction: Anti-diabetic activities of zinc oxide nanoparticles (ZnO) were investigated in diabetic rats compared to zinc sulfate (ZnSO4) with particular emphasis on Neutrophil function (phagocytosis, killing and NBT reducing). Neutrophils phagocytes represent an important first line and effector function in the control of bacterial and fungal infections in diabetics. **Material & Methods:** In this study 120 male Wistar rats were divided into two healthy and diabetic groups, randomly. Each major group was further subdivided into five subgroups and then orally supplemented (gavage) with various doses of ZnO (1, 3, and 10 mg/kg) and ZnSO4 (30 mg/kg) for 56 consecutive days. Blood samples were tested for neutrophil phagocytosis, serum opsonisation power, and NBT reduction. **Results:** In diabetic rats a decline in the neutrophil phagocytic index, serum opsonisation index and the percentage of neutrophils participating in phagocytosis were observed. The data also revealed that diabetic neutrophils could reduce more NBT dye at their basal level. Application of ZnO nanoparticles at dose 3 mg/kg in diabetic rats was decreased NBT reduction in the neutrophils, significantly increased phagocytic index of the neutrophils, improved opsonisation of the serum and the percentage of opsonisation in comparison with healthy rats were found. Zinc sulfate had little effects and Zno nanoparticles at dose 10 mg/kg had more adverse effects on neutrophil function in diabetic rats. **Conclusion:** This study demonstrated the malfunction of neutrophils in diabetes mellitus and the improvement of neutrophil phagocytic function in rats with diabetes mellitus following the oral administration of ZnO nanoparticles. **Keywords:** diabetes mellitus, Neutrophil, Nitroblue tetrazolium, Phagocytosis, ZnO nanoparticles

8716P

Chitosan nanoparticle mediated co-delivery of siRNA/drug for synergic anti-lung cancer therapy

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Introduction: Chitosan (CH) is a linear polysaccharide obtained from deacetylation of the chitin and many studies focused on these polymer applications have been published such as drug delivery systems. The non-histone chromatin-binding protein HMGA2 is expressed generally in the predifferentiation mesenchyme, but it is also overexpressed in tumors of epithelial tissues. Expression of HMGA2 and its down-regulators; vimentin, MMP-9 in epithelial cells resulted in epithelial–mesenchymal transition (EMT), that has been implicated in the metastatic tumor cells. **Material and Methods:** In this study we investigated dual delivery of anticancer drug doxorubicin and the siRNA of HMGA-2 to enhancethe treatment effects. For this purpose, we carried outreal time PCR and MTT assay (cytotoxicity assay). **Result:** Our results revealed that co-delivery of Dox and siRNA of HMGA-2 by chitosan nanoparticles significantly inhibited lung cancer cells growth. Also, delivery of siRNA significantly silenced HMGA-2, vimentin, and MMP9 mRNAs, but led to overexpression of E-cadherin mRNA. **Conclusion:** Our results showed that the delivery of HMGA2 siRNA and appropriate anti-cancer drug (Dox) could lead to effective lung cancer therapy.

10973P

Isolation of Exosomes from Pro-inflammatory Mesenchymal Stem Cells Supernatant

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Introduction: Exosomes are small vesicles secreted by most cell types in culture. Exosomes could be involved in intercellular communication, allowing exchange of antigen or major histocompatibility complex and (MHC)-peptide complexes between antigen-bearing cells and antigen-presenting cells. Former findings demonstrated LPS changes the stimulation of specific Toll-like receptors that affects the immune modulating responses of multi-potent mesenchymal stem cells. The purpose of this study was to describe several centrifugation and ultracentrifugation steps for purifying exosomes of LPS-stimulated MSCs. **Material and Methods:** After isolation of mesenchymal stem cells from bone marrow of rats, these cells stimulated with 10 ng/mL LPS for 1 h and then the conditioned medium collected and ready for several centrifugation and ultracentrifugation. The first steps are designed to eliminate large dead cells and large cell debris by successive centrifugations at increasing speeds (2000 × g for 20 min then 10,000 × g for 30 min). At each of these steps, the pellet is thrown away, and the supernatant is used for the following step. The final supernatant is then ultra-centrifuged at 100,000 ×g for 70 min to pellet the small vesicles that correspond to exosomes. The pellet was washed in a large volume of PBS, to eliminate contaminating proteins, and centrifuged one last time at the same high speed. **Results:** Electron microscopy of negatively stained

exosomes revealed cup-shaped membrane vesicles of 50 to 90 nm for LPS-stimulated MSCs. **Conclusion:** These findings give simple and reliable methods for isolating exosomes of pro-inflammatory MSCs for clinical and laboratory applications that have potential of affecting immune modulating responses. **Keywords:** Mesenchymal Stem Cell, Lipopolysaccharide, Exosome, Isolation, Conditioned Medium

11017P

A Novel Immunosensor Nanomaterial for Ultrasensitive and Selective Electrochemical Diagnosis of Breast Cancer-Related Carbohydrate Antigen 15-3 as Biomarker

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Introduction: In recent years, sensitive and reliable detection of tumor markers has been posed as an essential issue. One of the most important tumor markers is the carbohydrate antigen 15-3 (CA 15-3). This antigen is important in the diagnosis of breast cancer. Different types of methods including enzyme-linked immunosorbent assay, radioimmunoassay, enzyme immunoassay, chemiluminescence immunoassay, and so on, have been applied to boost this field. **Material and methods:** Nitrogen-doped grapheme aerogel was synthesized and used for fabrication of a new electrochemical nano-immunosensor for ultrasensitive detection of the CA 15-3 as breast cancer biomarker. This immunosensor showed a very low detection limit at 0.008 U/mL. Moreover, it worked very well over a wide range of 0.05-20 U/mL. This perfect ability can be attributed to 3D porous frameworks of Nitrogen-doped aerogel grapheme by which multidimensional electron transport pathways provide. **Results:** this immunosensor can be considered as a promising tool for clinical research and this label free method can easily be adapted for the detection of other disease biomarkers and cells. A low detection limit of 0.008 U/mL was determined for this immunosensor, showing a promising label free tool for clinical applications. **Conclusion:** these methods are complicated label processes and time-consuming separations, so such methods are unlikely to meet the increasing clinical demands for ultrasensitive and selective detection of CA 15-3. In addition, these techniques are not applicable in the case of ultra-low biogenic concentrations. As a result, these methods should be replaced by alternative methods by rapid and sensitive detection of CA 15-3. One of the most promising methods for mitigating the drawbacks in the field of cancer diagnostic is electrochemical immunosensors.

11039P

Inhibitory effect of Gold nanoparticles conjugated with interferon gamma (IFNγ) and methionine on breast cancer cell line

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Introduction: To develop a gold nanoparticles complex conjugated with interferon-gamma (IFN-γ) and methionine along with application of hyperthermia, near-infrared laser beams were used for the treatment of cancer cells. **Material and Methods:** Gold nanorods (10 nm) were conjugated with IFN-γ and methionine using carbodiimide family and characterized after purification by dialysis bags. Breast cancer cells were cultured and incubated with gold nanorods in different concentrations followed by irradiation with near-infrared laser beam. Then, samples were evaluated for their viability in order to determine the effect of treatment and variables by MTT assy. **Results:** Zetasizer results confirmed the conjugation of gold nanorods with methionine and IFN-γ. The median percentage of cell viability in 0.30 μg/mL. Concentration of gold nanorods was 82%. The cell viability reached 85% at the same concentration of gold nanorods, which existed in the assayed complex. The results of MTT assay suggested that the 0.60 μg/mL concentration of gold nanoparticles complex was toxic for tumor cells ($P < 0.05$). After exposure to hyperthermia, the viability of cells at 6 min decreased to 77% in 0.30 μg/mL concentration of gold nanorods complex. **Conclusion:** The size and concentration of gold nanorods were not cytotoxic. However, their presence during irradiation of near-infrared laser increased the number of dead cells during the treatment of cells. **Keywords:** Gold nanorods, Breast cancer cell line, interferon gamma, near-infrared laser.

11199P

Comparison between the change in concentration and size of copper oxide nanoparticles in the cytotoxic properties on B92cell line

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Introduction: In recent studies, cytotoxic effects of copper oxide nanoparticles (CuO NPs) have been documented. Here, we investigated whether the size and concentration of the CuO NPs could change the cytotoxic effect of CuO NPs against B92 rat glial cell line. **Material and Methods:** The B92 cells (1×105 cells/100 μl/96-well plate) were incubated for 24 h with a serial dilution of CuO NPs (0, 5, 10, and 20 μgr/ml) at 3 different sizes (20, 60 and 120 nm). At the end of incubation, the survival of cells was determined by MTT methods. **Results:** The CuO NPs had cytotoxic effects against B92 cell line in a dose-dependent manner. Nevertheless, the size of the CuO NPs had any significant effect on the vitality of B92 cell line. **Conclusion:** It seems that the concentration of the CuO NPs has a greater effect on the cytotoxicity of nanoparticles compared to their size. **Key words:** Copper oxide nanoparticles, B92, Cytotoxicity.

11212P

Effects of IL17RB siRNA and doxorubicin dual delivery by chitosan nanoparticles on cytotoxicity, gene expression, apoptosis and migration of MDA-MB361 Breast cancer cell line

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Introduction: Breast cancer is known as one of the most common cancer among women and accounts for nearly 30% of all the detected cancers to date. The expression of Interleukin 17 Receptor B (IL17RB) rises considerably in breast cancer cells. **Material and methods:** In this study, carboxy-methyl dextran (CMD) chitosan nanoparticle

(chNPs) platforms were designed to encapsulated IL17RB siRNA and anticancer drug (doxorubicin (DOX)) and then the efficiency of the simultaneous delivery of siRNA/drug on viability and gene expression of MDA-MB361 cell lines (malignant metastatic breast cancer cell line) were investigated. **Results:** suggested that DOX-siRNA-CMD-ChNPs had about 50 nm size; with polydispersity index and zeta potential about 0.3 and 11.8 mV respectively. Morphology of loaded ChNPs was assessed through the utilization of scanning electron microscopy (SEM), moreover Fourier transform infrared spectroscopy (FTIR) was used to confirm the conjugation of DOX-siRNA-CMD-chNPs. The chitosan nanoparticles obtained from this study were stable against serum and heparin as well as had high efficiency for siRNA and drug encapsulation (73% and 75%). In this study the efficacy of DOX-siRNA-CMD-chNPs through the utilization of MTT technique, in reducing the viability of cells as well as down regulation of NF-KB and BCL2 genes expression by qRT-PCR, have been proved. In conclusion, the results indicated that delivery of IL17RB siRNA and DOX could be considered as an effective co-delivery system for the treatment of breast cancer. **Conclusion:** The patients suffering from breast cancer with the over expression of IL17RB are associated with poor prognosis and short survival. The activation of the signaling pathways of IL17RB/IL17B triggers a substantial increase in the cell growth, proliferation and invasion through the activation of NF-KB as well as the up-regulation of the expression of BCL2 (anti-apoptotic factor)

11247P

Co-delivery of siRNA and doxorubicin to colorectal cancer cells (HT-29) by chitosan nanoparticles to enhance therapeutic effects

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Introduction: Colorectal cancer is second leading cause of cancer-related mortality in the developed countries. Many genes have been detected in colorectal cancer as an epithelial to mesenchymal transition marker including high-mobility group A2 (HMGA2), E-cadherin as well as vimentin which controlled by let-7 as a regulatory factor and lead ultimately to cancer development. **Materials and Methods:** Carboxymethyl dextran (CMD) chitosan nanoparticles (ChNPs) was used to evaluate the efficacy of co-delivery of siRNA/drug on viability and gene expression of HT-29 cell lines by MTT assay and real-time PCR respectively. Characteristic of ChNPs was determined by dynamic light scattering and zeta sizer. Scanning electron microscopy and Fourier transform infrared spectroscopy were assessed to study of morphology and ChNPs/siRNA/DOX/CMD conjugation. Stability of loaded ChNPs was determined against serum and heparin. Loading capacity of ChNPs for siRNA and drug was examined by electrophoresis and centrifugation respectively. Content of released siRNA and drug were assessed by UV-Vis spectrometry. **Results:** ChNP/siRNA/DOX/CMD had about 174 nm size; moreover polydispersity index and zeta potential were 0.3 and 11.8 mV respectively. The ChNPs had high efficiency for siRNA and drug encapsulation (78% and 75%) and were stable against serum and heparin. MTT assay demonstrated ChNPs/siRNA/DOX/CMD was more effective to induce tumor cell death. Furthermore, ChNPs/siRNA/DOX/CMD could significantly reduce the expressions of HMGA2, vimentin and increase E-cadherin expression. **Conclusion:** Our results revealed that dual delivery of a key gene siRNA and doxorubicin has great impact on the treatment of colorectal cancer. **Keywords:** HMGA2, siRNA, doxorubicin, nanoparticle, colorectal cancer.

11257P

IGF1R siRNA and Doxorubicin co-delivery inhibit growth and gene expression of lung cancer cell line

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Introduction: Lung cancer is the most common cause of cancer- related death in the world. The insulin-like growth factor1 receptor (IGF 1R) is an attractive novel target for anticancer therapy which it is overexpressed in many cancers, including lung cancer. IGF 1R signaling involves the activation of various intracellular signaling pathways which implicated in progression, invasion and migration of tumor cells. There are several new therapeutic approaches involved the monoclonal antibodies and tyrosine kinase inhibitors or antisense molecule that target IGF-1R. In the study reported here, we investigated the effects of dual delivery of IGF-1 siRNA and Doxorubicin ((DOX) anticancer drug) on growth and gene expression of A-549 lung cancer cell line. **Materials and Methods:** we utilized chitosan nanoparticles (ChNPs) platforms to encapsulated IGF1R siRNA and DOX that provide siRNA/drug co-delivery system. We investigated efficiency of this system on viability of A549 cell line by utilization of MTT technique and gene expression by qRT-PCR. **Results:** Our results showed that ChNPs containing siRNA/DOX had optimum size with polydispersity index about 0.3 and zeta potential 11.8 mV. Morphology of loaded ChNPs was assessed by scanning electron microscopy (SEM) and conjugation of chNPs with siRNA/DOX investigated by Fourier transform infrared spectroscopy (FTIR). In addition, our data revealed that simultaneous delivery of IGF-1 siRNA and Doxorubicin silenced MMP-9 gene expression and caused A-549 cell death in vitro. **Conclusion:** In conclusion, our results showed that combination of IGF-1R silencing and Doxorubicin delivery could lead to cytotoxicity in A-549 cells and downregulation of Epithelial-to-mesenchymal transition (EMT) markers.

11258P

Encapsulation of pcDNA/IFN-Lambda 1 in nanospheres of PLGA as a new adjuvant for DNA vaccines

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Introduction: Expression plasmid of cytokines have been studied extensively as candidate adjuvants for DNA vaccines in preclinical models and are now entering early-phase clinical trials. Recently type III IFNs considered as a natural adjuvant for a protective immune response. It seems rationally that encapsulated pcDNA/ IFN-λ1 in PLGAas compared with naked pcDNA/ IFN-λ1 results in a strong augmentation in immune responses because of attractive properties of PLGA as a delivery system. **Material and Methods:** The eukaryotic expression plasmid pc DNA 3.1+ that expressed the IFN-Lambda1 gene encapsulated in PLGA nanoparticles (pIFN-λ1-PLGA-NPs) were prepared by a double emulsion-solvent evaporation method and optimal preparation conditions of the pIFN-λ1-PLGA-NPs were determined. The expression and bioactivity of IFN-lambda1 confirmed in vitro in our study. **Results:** Under the optimal conditions, the pIFN-λ1-PLGA-NPswere produced in good morphology with a mean diameter of 500-700 nm, with encapsulation efficiency of 30±0.5% and a Zeta potential of +2.1 mV. **Conclusion:** The successful encapsulation of pIFN-λ1 in PLGA which has attractive properties such as biodegradability and FDA approval in drug delivery systems, provides a new insight for development of novel nanoadjuvants for DNA

vaccines in infection disease and immunotherapy. **Keywords:** Interferon Lambda 1, PLGA, Adjuvant, Encapsulation, DNA vacci ne.

11303P

The effects of photodynamic therapy by nano drug zinc phtalocyanine in skin cancer cell line

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Introduction: Photodynamic therapy has been considered as a treatment procedure for cancer. **Material and methods:** In this therapeutic method, light-sensitive nano drug was injected into the target cells and then put interest area exposed to visible light consistent with the absorption spectra nano drug used. Then light-sensitive nano drug was exposed to visible light. Reactive oxygen species and free radicals will be produced which leads to cell destruction of the target tissue. Skin cancer Sw872 cell lines were cultured in 96-well plates and the amount of cell death was measured by MTT technique. **Results:** After the treatment of skin cancer cells through therapeutic methods photodynamic therapy, viability of the cells from 100 percent to 10 percent of live cells decreased. **Conclusion:** The obtained results indicated the high efficiency of this therapy in removing skin cancer cells. **Keywords:** cancer, photodynamic therapy, sw 872

12356P

The PLGA Nanoparticles and Specific Immunotherapy

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Introduction: Prevalence of Allergic diseases has been increased during last century. Specific Immunotherapy (SIT) is one of the most effective ways in shifting Th2 response to Th1 which results in treatment of allergic patients. Classical IT has several undesirable effects that can be named local allergic reactions, occasional anaphylaxis and, even, near fatal reactions and above all, phobia from needle to administrating *subcutaneous* injection and its consequent hazards. Nowadays biodegradable nanoparticles open new windows in SIT issue that they reduced the bad effects of classical SIT. These Biodegradable nanoparticles as a delivery system can be a good alternative to traditional methods. The most preference of this system is to overcome all oral vaccination related barriers as well as it's resistant to acidic conditions of the stomach and gut hydrophobic and this is while Particle itself is not toxic and has the ability to decompose into simpler elements. PLGA, poly (glycol acetic acid) is one of the biodegradable polymers that routinely used for allergy immunotherapy. The main justification for applying PLGA in immunotherapy is to induce Th1 mediated response which itself is also the targeting of immunotherapy **Material and methods:** This study was a review study that obtained from NCBI and Google scholar references.

Numerous original and review papers have been used during period of 2010-2015. To find these papers we used several key words such as SIT, Biodegradable Nanoparticles and PLGA .**Result:** The our results show that using this nanoparticles sublingually/intranasal or subcutaneously could be more effective than classical methods owing to its controlled antigen release and ability to prevail over biological barriers .**Conclusion:** Nanoparticles are good and compatible delivery systems to target specific cells, but size and polymeric derivative should be considered in regarding type of treatment. **Keywords:** SIT, Biodegradable Nanoparticle, PLGA

Oral & Dental Immunology

Oral Presentations:

7620O

Correlation between gingival expression of STAT1 and periodontal disease

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Introduction: Periodontal diseases are among the most prevalent infections in humans; the disease is characterized by the inflammatory responses to the oral bacteria and could be seen in about 10 percent of the adult population. A variety of inflammatory cytokines by involving the signaling pathways implicated in the pathogenesis of this disease. Wnt5a was expressed in several inflammatory diseases such as atherosclerosis, rheumatoid arthritis, and periodontitis. Signal Transducer and activator of Transcription-1 (STAT)-1 is a transcription factor that plays a key role in the process of intracellular signal transduction through the JAK-STAT pathway in response to inflammatory cytokines. So the aim of this study was to evaluate the correlation between gingival expression of STAT1 and periodontal disease. **Materials and Methods**: For this purpose, gingival tissue samples were collected from 20 individuals with clinically healthy gingiva and 25 patients with moderate to severe chronic periodontitis and 25 patients with aggressive periodontitis. After homogenizing the gingival tissues, RNA was extracted by RNA isolation kit. After synthesis of cDNA, expression of STAT1was evaluated by Real-time PCR. **Results*:*** Higher expression of STAT1 was found in chronic and aggressive periodontitis in comparison with healthy cases ($P<0.05$). In addition, higher expression of STAT1 was found in chronic periodontists compared to aggressive periodontitis ($P<0.05$). **Conclusion:** Altogether, the findings of this study concluded that expression of STAT1 was associated with decreasing severity and progression of periodontal disease. Therefore, STAT1 may be considered as an important target for future therapies. **Keywords**: chronic periodontitis, aggressive periodontitis, STAT1

11071O

Correlation between pulpal neuropeptides and various kinds of dental caries

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Introduction: It has been assumed that neuropeptides have some role in progression of inflammation of dental pulp. So the aim of this study was to determine the correlation between the presence and concentration of neuropeptides in dental pulps and different degrees of dental caries. **Materials and Methods:** Pulpal tissues were collected from 91 extracted teeth consisted of 15 healthy teeth without caries; 15 teeth with superficial caries; 21 teeth with moderate caries; 20 teeth with deep caries; and 20 teeth with deep complicated caries. Pulpal samples were homogenized and cultured for 72 hours. Then enzyme-linked immuno-sorbent assay (ELISA) was used to detect the Substance P (SP) and Calcitonin gene-related peptide (CGRP) in supernatant fluids. Statistical analysis was made by ANOVA, Tukey post Hoc and Pearson correlation coefficient tests. **Results:** There were significant differences regarding CGRP and SP concentrations between various kinds of dental caries ($P<0.001$) so that the levels of both SP and CGRP were as follows; deep complicated caries> deep caries> moderate caries> superficial caries> healthy teeth. **Conclusion:** It can be concluded that with progression of carious lesion, the concentrations of SP and CGRP would be increased. Also the CGRP concentration was more than SP which may be related to its regulatory role, in order to inhibit additional destructive changes in dental pulp.

11074O

Correlation between TLR2 and TLR4 expression and dental periapical lesions

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Introduction: Regarding to the importance of pathobiology of pulpal and periapical inflammation, innovations in treatment methods is the most interesting aspect in these diseases and it cannot be possible until obtaining enough knowledge about its immuno-pathogenesis. So the aim of this study was to evaluate relationship between chronic dental periapical lesion and TLR2 and TLR4genes expression. **Materials and Methods:** This study was historically cohort. The periapical lesions were collected during surgery or teeth extraction. After periapical lesion extirpation and homogenization of samples, quantitative Real time PCR was used for evaluating TLR2 and TLR4 genes expression. Data analysis was made by REST software (QIAGEN, 2009). **Results:** TLR2 and TLR4 genes expressions were significantly higher in periapical lesions greater than 10 mm ($P<0.01$). Of course the expression of TLR2 was higher than TLR4. **Conclusion:** Despite the predominance of gram negative bacteria in periapical lesions, the expression of TLR2 has been increased. It can be due to some of endodontopathic bacteria using TLR2 in order to trigger more inflammatory responses.

12420O

Evaluation of the synergistic effect of IL-4 and integrin αVβ5 on Monocytes apoptosis in giant cell granuloma patients

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Introductin: It is now well established that IL-4 has a central role in the development of monocytes to multinucleated giant cells (MGCs) by inducing the expression of integrin on the surface of monocytes. The aim of this study was to evaluation if IL-4 and β5 integrin can potentially induced the expression of anti-apoptosis factors in the peripheral blood samples of patients with giant cell granuloma. **Material and method:** Monocytes were isolated from peripheral blood samples of patients with giant cell granuloma and healthy controls using human Monocyte Isolation Kit II. Isolated monocytes were then cultured in the absence or presence of IL-4 and integrinβ5 (10 and 20 ng/mL), and following Tunel test and DNA fragmentation assay was performed to determine apoptosis. **Result:** Both IL-4 and integrin β5 inhibited apoptosis of monocytes in a dose-dependent manner. According to the statistically analyzed, when IL-4 and integrin β5 were combined, they had synergistic effects at low doses. **Conclusion:** It was concluded that both IL-4 and β5 integrin have a synergistic effect on inhibition of the apoptosis of isolated monocytes of peripheral blood of patients with giant cell granuloma. We demonstrated that IL-4 and β5 integrin could promoted the formation of MGC *in vitro* through inhibition of monocyte apoptosis. **Keywords:** IL-4, integrin β5, Apoptosis, giant cell granuloma

Poster Presentations:

11004P

Immuno-Biomarkers in Dental Stem Cells

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Introduction: There are different types of stem cells that have capacity of developing into various cell types in the body. One of the stem cell sources that have been isolated and identified recently, are Dental-derived Stem Cells. Like mesenchymal stem cells, they are self-renewal and can differentiate into many cell types. They are easily accessible and useful in repairing injuries and tissue regeneration. These characteristics have made them a proper choice for cell-based therapies in the future.In this review article we aimed to summarize Immuno-modulatory

properties of well-known sorts of dental derived stem cells. **Materials and methods:** Search for published articles was conducted from 2005 to December 2015. Relevant articles, according to the aim of this study, were identified, reviewed, and classified in six groups. **Results:** For isolation of stem cells from other residual cells, some markers are useful for separating them from other cells. Recognition of immuno-biomarkers is one of the applying methods nowadays. Dental-derived stem cell markers are various and each of them shows special properties. For example, Nanog and Nestin were shown in SHED (stem cells from human exfoliated deciduous teeth). Their expression were proved during neural differentiation. In addition, Oct4 and Scleraxis are useful in classification of them. **Conclusion:** Using specific immuno-biomarkers is recommended for selection of favorable stem cells in research and therapeutic purposes due to the different performance.

11073P

Correlation between IL-17A and IL-22 concentrations and periodontal diseases

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Introduction: Periodontal diseases are characterized by inflammatory destruction of periodontal connective tissues surrounding the teeth. Although the presence of pathogenic micro-organisms is required to trigger this process, the progression of the disease is believed to rely heavily on the production of host derived cytokines which releases in response to bacteria and/or their metabolic products. Interleukin (IL)-17 plays an important role in inflammation and certain autoimmune diseases. However, IL-17 is also an important regulator of host defense through granulopoiesis and neutrophil trafficking. Interleukin (IL) 22 is a type II cytokine that is produced by immune cells and acts on non-immune cells to regulate local tissue inflammation. And it is a member of the interleukin-10 cytokine family, which is involved in anti-microbial defenses, tissue damage protection and repair, and acute phase responses. The aim of this study was to determine the total amount and concentration of the cytokine IL-17 and IL- gingival crevicular fluid (GCF) from healthy individuals, patients with gingivitis and chronic periodontitis patients before phase-1 periodontal treatment. **Materials and Methods:** For this purpose, a total of 66 subjects, 22 males and 12 females were recruited from the Department of Periodontology School of Dentistry, Shahid Beheshti Medical University of Tehran, Iran. The Chronic periodontitis group included 22 subjects, 12 females and 10 males ranged in age from 27 to 69 (mean age 46 years).The gingivitis group included 12 females and 10 males ranged in age from 17 to 56 (mean age 30 years). Healthy group was with no evidence of clinical attachment loss, clinical inflammation and sulcular bleeding. These patients ranged in age from 21 to 42 (mean age 26 years). Sites were gently dried with an air syringe, and a sterile paper strip (Periopaper, OraFlow, NY, 11787, USA) was inserted into the gingival crevice, until mild resistance was felt, and was kept there for 30 seconds. The samples were immediately frozen at -20°C and stored until analysis. ELISA technique was used for the measurement of IL-17A, IL-22. **Results:** The concentration of IL-22 was significantly higher in chronic eperiodontitis ($P<0.001$) group followed by gingivitis group ($P<0.05$). There were no statistical differences between IL-17 concentrations in all groups. **Conclusion:** It is concluded that IL-22 probably participates in eliminating the inflammatory effects of bacteria which regulates the immune responses.

11075P

Salivary soluble CD44 levels in periodontal disease

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Introduction: Patients with periodontitis have elevated circulating inflammatory markers that can be correlated to the severity of disease. Several studies have shown that CD44 levels were raised in subjects with systemic inflammatory conditions, such as rheumatoid arthritis and bronchitis. Since patients with periodontitis have elevated circulating inflammatory markers that can be correlated to the severity of diseasronic periodontitis. **Materials and Methods:** A total of 80 patients were collected from Dental School of Shahid Beheshti University of medical sciences. They were divided into 2groups: Group 1 - healthy, Group 2 moderate to severe chronic periodontitis. 0.5 ml un-stimulated salivary sample collected at baseline by an Insulin syringe. After adding PBS, was centrifuged at 1500 rpm for 30 minutes. Then it was stored at -80°C. The sCD44 levels were analyzed using ELISA.T- test was used to compare the concentration of sCD44 between healthy and periodontitis groups. **Results:** No significant difference was found regarding sCD44 concentration between healthy and diseased groups. **Conclusion:** It is concluded that soluble CD44 levels issue inflammation or destruction. Of course, more studies are needed in order to confirm this hypothesis.

11077P

Differences in TGF-beta and IL-10 concentrations before and after periodontal surgery

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Introduction: Salivary biomarkers are potentially important for determining the presence, risk, and progression of periodontal disease. Of course, there isn't enough data about salivary anti-inflammatory cytokines and periodontal healing. However, clinical translation of biomarker technology from lab to chairside requires studies that identify biomarkers associated with the transitional phase between health and periodontal disease (i.e., chronic periodontitis). **Materials and Methods:** Fifty participants (25 with chronic periodontitis with mean age of 49.2 ± 15, and 25 healthy subjects with mean age of 34.75±18, 3) were enrolled in this study. Salivary samples were collected at baseline and 1 week and 1 month later after phase II of periodontal treatment (Surgery) by insulin syringe. Salivary concentrations of TGF-beta and IL-10 were measured by ELISA method at baseline, 1 week and 1 month after periodontal surgery. Statistical analysis were made by ANOVA and t test. **Results:** Concentrations of TGF-beta and IL-10 were the highest in 7 days after surgery ($P<0.01$) but in 1 months after surgery there is not any significant difference regarding TGF-beta between baseline and 1 month after surgery but the concentration of IL-10 was still higher than 7 days after surgery ($P<0.05$). **Conclusions:** It was suggested that TGF-beta has prominent role in healing and suppression of inflammation in first days of healing but IL-10 can act for longer periods of times.

12357P

Measurement and correlation of Soluble VEGF-R3, TNF-α, TGF-β and IL-17A/F Levels in Saliva of Patients with Minor Aphthous

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Introduction: Recurrent aphthous stomatitis (RAS) is one of the most common mucosal ulcerative of oral cavity. The role of immune system, especially cytokines in immuno-pathogenesis of aphthous stomatitis was not highly considered. The aim of this study was to evaluate the levels of salivary cytokines in patients with RAS in two clinical stages, ulcerative and healing period**. Material and Methods**: In this case –control study, 18 patients with RAS (case group) and 18 healthy individuals (control groups) who were matched for age and sex, were selected. In both ulcerative and healing stages, unstimulated saliva of patients with RAS and healthy controls were collected. Levels of salivary cytokines, including VEGF-R3, TGF-β1, TNF-α, and IL-17A/F at each stage was determined by ELISA procedure and result were compared to the control group. **Results:** The levels of salivary of VEGF-R3 and TGF-β1 were significantly reduced in ulcerative and healing stages. In addition, inflammatory cytokines including TNF-α and IL-17 A/F were significantly increased in both stages compared to control group. **Conclusion:** The findings of this study showed that the reduction of VEGF-R3 and TGF-β1 cytokines and increasing of inflammatory cytokines such as TNF-α and IL-17 A/F are effective in the pathogenesis of minor aphthous particularly in ulcerative stage**.** **Keywords:** Cytokines, Minor Aphthous, Saliva

Psychoneuroimmunology

Oral Presentations:

7531O

Dopamine receptor blocking by chlorpromazine ameliorate animal model of multiple sclerosis via change in T cell polarization

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Introduction: Recent studies indicated that dopamine changes in the brain of mice with experimental autoimmune encephalomyelitis. Moreover, it is cleared that dopamine could regulate the cells of immunity system. The present study was carried out to investigate the therapeutic effect of chlorpromazine, as a dopamine antagonist, on experimental autoimmune encephalomyelitis (EAE), an animal model of MS, and its effects on T-helper cells responses. **Material and Method:** EAE was induced by guinea pig spinal cord homogenate and complete Freund's adjuvant in Wistar rats. Animals were placed in two therapeutic groups (n=7 per group). Treatment with phenytoin (50 mg/kg-daily) was initiated in treatment group at day 12 when the treatment group developed a disability score. EAE control received vehicle alone with the same schedule. Signs of disease were recorded daily until the day 36 when mice were sacrificed. Splenocytes were tested for proliferation by MTT test and cytokine production by ELISA. **Results:** Chlorpromazine administration after the occurrence of clinical symptoms significantly regressed the clinical features of EAE. Chlorpromazine significantly inhibited the production of pro-inflammatory IL-17 and simultaneously decreased the levels of anti-inflammatory IL-10 in treatment group compared to control EAE rats. Lymphocyte proliferation was significantly decreased in treatment group compared to control group. **Conclusions:** This pharmacological approach may be as a useful strategy to control MS. **Keywords:** Multiple sclerosis; Experimental autoimmune encephalomyelitis; chlorpromazine; lymphocyte response.

9777O

Asthma Induces Anxiety Behavior in the Mouse

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Introduction: Asthma is an important national health priority because of its high and increasing prevalence, high morbidity and mortality, and direct and indirect costs. While some epidemiologic studies had shown asthma and wheezing to be associated with depression and anxiety. The aim of this study was identifying any association between asthma and anxiety in an animal model. **Materials and Methods:** Twenty male mice Balb/c strain with 20-25 g body weight were divided randomly to control and allergic groups. Asthma was induced by 2 intra-peritoneal injection of 20 µg ovalbumin (OVA) and 20 mg aluminum hydroxide (as adjuvants) in animals. After 14 days, they were challenged with 100 mg OVA and 10 ml saline during 30 minutes. Elevated plus maze (EPM) was used for showing anxiety behavior in mice. Data were presented as means ± SEM and the means of 2 groups were compared by using t test. A P value smaller than 0.05 was considered statistically significant. **Results**: In EPM test, the number of enter in close arms (15.00±0.90), time spend in close arms (228.2 ±6.25), showed an increase compared to the control group (P< 0.05). **Conclusion:** This study clearly showed that asthma may produce anxiety in the animal model used.

11263O

A comparative study on the effects of metal ions on the aggregation of Beta-Amyloid peptides and astrocyte-mediated inflammation

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Alzheimer's disease (AD) is the most common neurodegenerative disorder. One of the hallmarks of AD is the extracellular accumulation of amyloid plaques in the brain that are mainly composed of *Amyloid* β-(*1-40*) and β-(*1-42*). *Amyloid* β (Aβ) plaques are surrounded by activated astrocytes and microglia which participate in the inflammatory processes. The inflammation induced by Aβ has an important role in AD. Metal imbalance is also the leading cause for AD, owing to the fact that Aβ aggregation takes place in the synaptic cleft where Aβ, Cu (II) and Fe (III) could be found together in abnormally high concentrations. Recent studies showed that metal dyshomeostasis is involved in aggregation, inflammation and reactive oxygen species (ROS) production, key events in the development of the pathology in AD. In addition, at the early stages of AD, small diffusible oligomers (or protofibrils) activate microglia leads to inflammation, particularly early inflammatory responses. On the other hand, fibrillar Aβ showed less increase in pro-inflammatory molecules sustaining the chronic inflammation associated with the disease. In this work, the formation of Aβ40 and Aβ42 fibrils or oligomers were examined in the presence and absence of the additional metal ions, Fe(III) and Cu(II) by using fluorescence spectroscopy and atomic force microscopy. It was found that Aβ42 may be more oligomerogenic than Aβ40 and may have higher effects in inflammation at early stage of AD and Cu (II) induces oligomeric formation. Fe (III) was found to be involved in fibril formation. So it may be more involved in sustaining the chronic inflammation associated with the AD. **Key words:** β-amyloid peptide (Aβ), Cu (II) ion, Fe (III) ion, Inflammation, Astrocyte

Poster Presentations:

10784P

Relationship between the pattern of anger in depressed patients with serum levels of IgA and NK-cell percentage

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Introduction: There are conflicting findings about relationship between depression and anger with immunological indicators. We aimed at investigating the relationship between anger patterns with immune system in depressed patients. **Material and Method:** 35 Patients with major depressive disorder according to DSM-IV criteria were selected. Then, the Hamilton Depression Scale and Spielberger Anger questionnaires were used to determine the severity of depression and" anger expression pattern ", respectively. The control group without previous history of mental illness was also selected. In patients with moderate depression, serum IgA levels and NK-cell percentage were measured. **Results:** Mean difference of all types of "anger expression pattern", including; "state-trait anger" ,"anger expression out", "anger expression in", "anger control out" and "anger control in", in both study and control groups, was statistically significant ($p<0.05$). Differences in mean serum levels of IgA in both groups were not significant ($P = 0.9$), but the mean percentage of NK-cell in both groups was significant ($P = 0.04$). There was no significant relationship between IgA levels and percentage of NK- cell with all types of "anger expression pattern" in both groups. But, only in the control group, IgA had significant correlation with Anger Control out ($P = 0.04$). **Conclusion:** Moderate depressed patients versus control group had higher Spielberger scores in all types of anger expression pattern except anger control-out and anger control-in. It was found no evidence supporting the relationship between anger expression pattern with IgA levels and NK-cell percentage and it seems that depression itself causes reduced NK-cell and increased IgA levels.

10791P

Assessment of changes in the immune system in stressful situations and its relationship with the hardiness

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Introduction: The interconnectedness of physical and mental health has long been considered and many studies have been conducted in the theory of behavioral variables influence on the immune system that show a positive relationship with the hardiness of physical and mental health and cause to reduce the destructive effects of stress in the people health. The aim of this study was evaluation of the changes in the immune system in stressful situations and its relationship with tenacity. **Material and Method:** In this descriptive analytical study, 65 medical students of Jundishapur University were selected randomly and enrolled to the study. The information was collected by Kubasa questionnaire and blood sample were collected in two condition, normal (2 months before the exam) and stressful situation (one hour before the exam). Standard complete blood cell count (CBC) and percentage of natural killer cell (CD56 marker) was measured by flow cytometry to evaluate leukocytes. Data were analyzed by paired samples t-test and Pearson's correlation coefficients. **Results:** Stressful situations caused significant reduction of neutrophils and monocytes and increase of eosinophils ($P < 0.05$), and had no effect on NK cells ($P < 0.05$). Hardiness had positive relationship with the number of neutrophils and significant negative correlation with eosinophils ($P < 0.05$). **Conclusion:** Stress has caused the debilitation of immune system and the main phagocytic cells in innate immunity. However, the study showed that people with more hardiness could have better prepared immune system in stressful situations. **Keywords:** hardiness, immune system, leukocyte, medical student.

10810P

Evaluation of serum level of monocyte chemotactic protein-1 in intracranial aneurysm

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Introduction: Ruptured intracranial aneurysms (ICAs) are most common non traumatic cause of subarachnoid hemorrhage (SAH) that is associated with life threatening complications such as vasospasm, infarction and hydrocephalus (HCP). In the present study, the role of monocyte chemotactic protein-1 (MCP-1) in the pathogenesis of ruptured ICAs was investigated. **Materials and Methods:** Sera levels of MCP-1 in SAH patients having ICAs (n=22), were compared with sera levels of MCP-1 in SAH patients without ICAs (n=22) and normal age and sex

matched blood donors (n=22). Also MCP-1 level in patients with ICAs was measured for evaluation of treatment in MCP-1 level. **Results:** Mean serum level of MCP-1 in patients with ICAs was 188.2 pg/ml compared to 331.4 in normal controls (p=0.000). Serum level of MCP-1 was elevated with decrease in Glasgow Coma Scale (p=0.078) and worsening in H&H score (P=0.089). **Discussion:** The data indicated no elevation of serum MCP-1 level in patients with intracranial aneurysms compared to normal controls. However, due to the fact that sera levels of MCP-1 were positively correlated with H&H score, MCP-1 might affect the severity of the disease. **Key word:** MCP-1, Intracranial aneurysm, Subarachnoid hemorrhage.

10817 P

The Relationship between inflammatory markers and delirium in adult patients admitted in intensive care unit

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Introduction: Delirium is a common complication in elderly patients after cardiac surgery. The pathophysiology of delirium has remained poorly understood. Several conditions associated with delirium are characterized by activation of inflammatory cascade. The purpose of this study was to find out the correlation of sera levels of pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines with delirium following coronary artery bypass graft. **Materials and Methods:** In an exploratory observational study, 380 patients were enrolled, including 20 patients with delirium, which have undergone coronary artery bypass graft surgery (CABG). Serum Samples were taken, postoperatively, before, during and after delirium. Delirium was diagnosed by using the confusion-Assessment Method-ICU (CAM-ICU). IL-6 and IL-10 were determined by Enzyme Linked Immuno-Sorbent Assay (ELISA). **Results:** In delirious patients, plasma levels of IL-6 were higher in delirious stage than post delirium period (101.9±57.7 pg/ml vs 59.3±38.01 pg/ml respectively; p=0.002). There was no difference in serum IL-10 levels in pre-delirious, delirious and post delirious period. **Conclusion:** IL-6 may contribute to the pathogenesis of post coronary artery bypass graft delirium by affecting cognition through hippocampal dysfunction. Further studies are needed to elucidate the relationship of other cytokines with pathogenesis of delirium. **Keyword:** Delirium, IL-6, IL-10, Coronary artery bypass graft.

11241P

Several Immunological Mechanisms implicated in the Pathogenesis and Progression of Alzheimer's disease

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Introduction: Alzheimer's disease (AD) is a progressive neurological disorder, featured by accumulation of extracellular amyloid beta (Aβ), formation of intraneural fibrillar tangles, and microglia activation along with synaptic and neuronal loss. It has been suggested that neuroinflammation and activation of microglia may be involved in the pathogenesis of AD. Herein, current evidence regarding the immunological basis for neurodegeneration in AD was conducted. **Materials and Methods:** Articles related to Alzheimer's disease and neuro-inflammation were searched on Medline, PubMed website, EMBASE, Cochrane database of systematic reviews, and Google Scholar databases. The studies were published from 2005 to 2016 were then placed under scrutiny. **Results:** An increasing line of evidence suggested that accumulation of amyloid beta is associated with initiation of immune responses and increased concentration of pro-inflammatory cytokines such as IL-6, IL-1β and TNFα. Microglia bind to Aβ via cell-surface receptors, including TREM2, CD33, CD14, CD47, CD36, Toll- like receptors (TLR2, TLR4, TLR6, TLR9) and α6β1 integrin. These interactions lead to microglia activation and fortified production of pro-inflammatory cytokines. Perpetuation of microglia activation gives rise to chronic neuroinflammation, which in turn results in neuronal degeneration. Moreover, there are reports showing that CCL2, CCL3, CCL4 might be involved in the activation of microglia. **Conclusion:** Several immunological mechanisms including increased activity of microglia, continuous production of pro-inflammatory cytokines causing chronic inflammation, and changes in the structure and function of microglia were implicated in the destruction of neurons and progression of AD. **Keywords**: Alzheimer's disease, Cytokines, Immune system, Inflammation

Research & Development & manufacturing

Oral Presentations:

10830O

Effect of different stabilizers on stability of polyclonal antibody

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Introduction: Antibodies are important biological molecules that have wide range of application in the field of diagnosis and treatment. In order to maintain functional groups of antibodies such as antigen binding sites and keep those in optimal stability, there are different approaches. Adding different polysaccharides, amino acids and fatty acids and oils or BSA as stabilizers were investigated in different researches. The aim of this study was the evaluation of protective effect of some common or uncommon additives as stabilizers. **Materials and Methods:** In this study, the protective effects of BSA, Almond oil, Glycerol, Triton X100, Cyclodextrin, CMC, sucrose and glycine were examined in different separated groups. After selection of best effectors their complex effects were evaluated to set up a good stabilizer formulation. Stability of antibodies was evaluated in different temperatures by accelerating aging time method and other environmental under constant conditions. Stability of the treated antibodies was analyzed by ELISA method. **Results:** The obtained results showed that BSA 1%, sucrose 5% and Glycerol 2% had the best effects on maintaining the antibody reactivity, also Triton effect was acceptable. Almond oil and CMC and Cyclodextrin had no considerable protective effect. **Conclusion:** Nowadays, application of appropriate formulation for enhancing antibody stability because of its crucial roles is very important. It was concluded that obtained formulation on the basis of stabilizer effects of BSA, sucrose and glycerol have considerable protection effects on antibody reactivity and stabilize the binding positions of molecule. **Key words**: antibody, stabilizer, ELISA, specific reactivity.

11325O

Production of Rabbit anti-HSA and conjugation of it with supermagnetic nanoparticles (SPMNs)

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Introduction: Conjogated antibody with SPMNs is widely used in different magnetic based immunoassays. Production of anti-HSA conjugate with SPMNs could be used for assay of HSA in biological fluids.The protocol of conjugation is notably quick with high efficiency, too. **Materials and methods:** Rabbit anti- HSA was produced by S.C injection of HSA emulsion to the animals. The Immunoglobulins fraction was percipitated by amonium solphate. IgG fraction was purified by DEAE- sepharose. The purity was analyzed by SDS-PAGE.The SPMNs was prepared using copercipitation of $FeCl_2$ and $FeCl_3$ with amonium hydroxide (28- 30% V/V). Then they have been silica coated with TEOS (tetraethoxysilane). Rabbit anti- HSA activated by Sodium periodate. Finally, the silica coated SPMNs added to Rabbit anti- HSA in Sodium bicarbonate buffer. Then, SPMNs was removed by a magnet from the reagent and the supernatant was analysed with Bradford method. **Results:**The findings indiacate that 15µg of antibody was conjogated with 1mg of SPMNs. **Conclusions:** In this study, we have tried to summarize the most commonly used techniques for the production of congugated antibody with SPMNs, as well as to provide the conjugate that could be used in dignostic test and etc.

12344O

Cell-SELEX-Based Selection and Characterization of a G-quadruplex DNA Aptamer against Mouse Dendritic Cells

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Introduction: Targeting of dendritic cells (DCs) by aptamers increases antigen capture and presentation to the immune system. Our aim was to produce aptamers against DC molecules using the cell-SELEX procedure. **Materials and Methods:** For this purpose, 18 rounds of cell-SELEX were performed on mouse macrophage J774A.1 and CT26 as target and control cells, respectively. The selected aptamers were truncated and their binding to mouse macrophages, and immature and mature DCs analyzed. **Results:** Two macrophage-specific aptamers, Seq6 and Seq7, were identified. A truncated form of Seq7, Seq7-4, 33 nucleotides in length and containing the G-quadruplex, bound macrophages and immature DCs with kD values in the nanomolar range. **Conclusion:** We anticipate that Seq7-4 has potential as a therapeutic tool in targeting of mouse macrophages and immature DCs to efficiently improve different immunotherapy approaches.

Poster Presentations:

7639P

Aptamers: new tools in immunology

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Introduction: Aptamers are a new class of molecular probes that can be use for diagnosis, imaging tissue and targeting therapy. They are generated from nucleic acids library that may be RNA or DNA. The method that using for selection of Aptamer against a specific target is SELEX. SELEX is abbreviation of "Systematic Evolution of Ligand by Exponential enrichment", that is a repetitive process. In fact Aptamers are nucleic acid version of antibody and show high binding affinity, having dissociation constants between in the nanomolar (nM) and picomolar (pM) range, and having high selectivity towards their targets. Aptamers have significant advantages over antibodies. In general they have more heat stability than Aptamer, and have a longer shelf life. They are produce by simple and inexpensive process. Unlike antibody, production of Aptamer do not need animals. More over since synthesis of Aptamers are chemically, batch-to- batch variation do not seen. **Material and methods**: This was a review study that searched from pubmed and google scholar. Original and review papers using in the time period of 2010 to 2015. English keyword of: Aptamer, Antibody and immunology were search. **Result**: Our finding indicate that Aptamers can be powerful and useful substitutes for antibody and they can be used in target delivery, immunodiagnostic, and immunotherapy. **Conclusion**: Aptamers are suitable tools in biotechnology that can be used in immunology too. But critical point in produce of this molecules is their affinity to target. **Key words**: Aptamer, Antibody, Immunology

7696P

Investigation of different additives on the reactivity of anti- HBsAg antibodies against recombinant HBsAg

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Introduction: Antibodies have wide applications in diagnosis and treatment. In order to maintain optimal stability of various functional parts of antibodies such as antigen binding sites, several approaches have been suggested. Using additives such as polysaccharides and polyols, is one of the main methods in protecting antibodies against aggregation or degradation of the formulation. The aim of this study was to evaluate the protective effect of various additives on the specific reactivity of monoclonal antibodies (mAbs) against recombinant HBsAg (rHBsAg) epitopes. **Materials and Methods**: To estimate the protective effects of different additives on the stability of antibody against conformational epitopes (S3 antibody) and linear epitopes (S7 and S11 antibodies) of rHBsAg, heat shock at 37°C was performed in liquid and solid phases. Environmental factors were considered to be constant. The specific reactivity of antibodies was evaluated using ELISA method. The data were analyzed using SPSS software by Mann-Whitney nonparametric test with the confidence interval of 95%. **Results**: The results showed that 0.25 M sucrose, 0.04 M trehalose and 0.5% BSA had the most protective effect on maintaining the reactivity of mAbs (S3) against conformational epitopes of rHBsAg. Results obtained from S7 and S11 mAbs against linear characteristics showed minor differences. The most efficient protective additives were 0.04 M trehalose and 1 M sucrose. **Conclusion:** Nowadays, application of appropriate additives is important for increasing the stability of antibodies. It was concluded that sucrose, trehalose and BSA have considerable effects on the specific reactivity of anti rHBsAg mAbs during long storage.

10982P

Aptamers are Nucleic acid Version of Antibody

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Introduction: Aptamers are single-stranded oligonucleotide molecules (DNA, RNA) which are able to form exclusive secondary and tertiary structures andbind the high specificity and affinity to their targets. Aptamerlibrary containing about 10^{13} - 10^{15} different oligonucleotides. The aim of this study was to perform a systematic review paperto determinethe characteristics of the aptamerand benefit of replace them with antibodies. **Material and Method:** This review systematic article the base of study original and review papers from the database of PubMed and Google Scholar is published in the time period from Dec 2011 to May 2015 by in vitro selection of aptamer, targeting by aptamer andSELEX procedure key words. **Result:** Aptamersas a nucleic acid version of the antibodyand in the many cases can be anappropriate replacement to antibodies. Aptamers and antibodies the usually bind to targets of similar affinity. (Affinity in aptamer and antibody between nanomolar and picomolar). Selection of aptamer from a nucleic acid library perform by the component chemistry method of Systematic Evolution of Ligand by Exponential enrichment (SELEX). Aptamers have the advantages to antibodiesSuch as, stability to heat, the possibility of synthesize in vitroto lower cost and half-life is increasing. **Conclusion:** Aptamers as a nucleic acid version of the antibody and in the many cases can be an appropriate replacement to antibodies. Aptamer can be used in targeting molecules, diagnostic tests and treatment in the medical field. **Key word:** Aptamer, Antibody, SELEX

11305P

Purification of bovine serum albumin using immunoaffinity chromatographic

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introduction: Albumin protein is a main blood protein and has many applications, including: the use of therapeutic proteins, vaccines and enzymes as stabilizers. Also, the laboratory albumin protein is used as standard in protein assays. In our country this product is imported. The aim of this research was the purification of albumin from bovine serum via affinity chromatography using produced polyclonal antibody. **Materials and methods:** The polyclonal antibody was produced against BSA in rabbits. Thus, the pure BSA was injected to three white rabbits. For evaluating the antibody production, double diffusion, single radial and ELISA tests were conducted. Antibody purification was performed by ion exchange chromatography and protein G affinity chromatography. The purity of antibody was evaluated by SDS-PAGE. Then the purified antibody was attached to the CNBr-activated Sepharose and finally used for purification of albumin protein from bovine serum.
Results: The titer of anti-bovine albumin determined by ELISA, was 256000. Its purity (up95%) was confirmed by SDS_PAGE. Purified bovine albumin by affinity chromatography showed a single bond with a molecular weight of 66 KD. **Conclusion:** Affinity chromatography using produced polyclonal antibody would be an economical and safe method for purification of BSA. **Keywords:** Bovine serum albumin; polyclonal antibody; Purification, Immunoaffinity Chromatography

11306P

Purification of human serum albumin using Affinity Chromatography

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Introduction: Albumin is the most predominant protein in plasma, which is synthesized in liver and cause 80% of plasma colloid osmotic pressure. The annual demand for human albumin is 500 tons in the world and its request is the most biomedical solutions. There are different procedures for production of albumin among which, the Cohn method is the most common used one. Accordingly, in this research, to facilitate albumin purification and improving its purity, immunoaffinity chromatography was applied. The aim of this study was purification of human serum albumin (HSA) using immunoaffinity chromatography which included immobilized polyclonal antibody. **Material and Method**: After immunization of rabbits, passive immune diffusion and indirect ELISA tests were applied for

assessment of polyclonal antibody production against human serum albumin (HSA). Purification was performed by ion exchange chromatography (IEC) and protein G affinity chromatography. SDS-PAGE analysis was conducted to evaluate the purity of anti-HSA IgG. The produced anti-HSA antibody was used for albumin purification from human serum. Western blotting (WB) analysis was performed for functional assessment of produced HSA. **Results**: The purity rate of albumin was approximately %98, which compared to Cohn method, is approximately similar. WB analysis confirmed the functionality of produced HSA. The used immunoaffinity chromatography is a unique single phase method for HSA purification. **Conclusion**: Affinity chromatography using produced polyclonal antibody would be a convenient and economical method for purification of HSA. **Keywords**: Human Serum Albumin (HSA); Purification; Immunoaffinity Chromatography; Polyclonal antibody

11323P

Production of Goat anti-HBsAg and its conjugation with supermagnetic nanoparticles (SPMNs)

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Introduction: Conjugated antibody with SPMNs is widely used in different magnetic based immunoassays. HBsAg is the surfase and major antigen of HBV. Production of anti-HBsAg conjugate with SPMNs could be used for evaluating the HBsAg in biological fluids. The protocol of conjugation is notably quick with high efficiency, too. **Materials and methods**: Goat anti- HBsAg was produced by S.C injection of HBsAg emulsion in animals. The Immunoglobulin fractions were percipitated by amonium solphate. IgG fraction was purified by DEAE- sepharose. The purity was analyzed by SDS-PAGE.The SPMNs was prepared using copercipitation of FeCl2 and FeCl3 with amonium hydroxide (28- 30% V/V), then being silica coated with TEOS (tetraethoxysilane). Rabbit anti- HSA was activated by Sodium periodate. Finally, the silica coated SPMNs were added to rabbit anti- HSA in sodium bicarbonate buffer. Then, SPMNs were removed by a magnet from the reagent and the supernatant was analysed with Bradford method. **Results**: The findings indiacated that 15µg of antibody was conjugated by 1mg of SPMNs. **Conclusions**: In this study, we have tried to summarize the most commonly used techniques for the production of conjugated antibody with SPMNs, as well as provide the conjugate that could be used in diagnostic test and etc.

11326P

The effects of photodynamic therapy by nano drug zinc phtalocyanine in normal cells HFFF2 cell line

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Photodynamic therapy (PDT) is a minimally invasive approach, in which a photosensitizer compound is activated by exposure to visible light to produce cytotoxic oxygen and free radicals, which selectively destroy cancer cells. The light sensitive compounds are used as photodynamic therapy nano drug that were accumulated in cancer cells

more than normal cells, yet part of the nano prescribed medicine would have adverse effects on normal cells. To minimize these adverse effects, the performance and behavior of cancer cells compared to normal cells to lyser will be discussed during photodynamic therapy. Normal cells of HFFF2 cell line were cultured in 96-well plates and the amount of cell death was measured by MTT techniques. The treatment of normal cells through photodynamic therapy and viability of the normal cells compared to IC50 cancer cells, were 30 percent. The obtained results indicated the high efficiency of this therapy. **Keywords**: normal cells, photodynamic therapy, HFFF2

12421P

Purification of Hyaluronidase Enzyme from Medical Leech (Hirudo Medicinalis) using immunoaffinity chromatography

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Introduction: Hyaluronidase (Orgelase) is an enzyme found in the saliva of leeches who can digest hyaluronic acid and is also known as a developer factor. It open the way for other active ingredients found in the saliva of leeches to penetrate into deep tissue. The aim of this study is the purification of leech hyaluronidase using produced polyclonal antibody. **Material and Methods**: The rabbit polyclonal antibody was prepared against the hyaluronidase enzyme. Thus the pure enzyme was injected to rabbit and to evaluation of antibody production, ELISA and double Diffusion tests were done. Antibody purification was done by ion exchange chromatography and protein G affinity chromatography. Then the purified antibody was attached to the CNBr-activated Sepharose and finally it used for purification of hyaluronidase from leech saliva. After the purification, purity of fractions was assessed by SDS-PAGE electrophoresis. **Results**: SDS-PAGE analysis showed the purity of protein was up to 98%. Also the single band with a molecular weight of approximately 60 KD is related to leech hyaluronidase. **Conclusion:** Affinity chromatography using produced antibody would be an economical and safe method for purification of hyaluronidase from leech saliva extract. **Keywords**: Hyaluronidase; Immunoaffinity Chromatography ; leech saliva extract (LSE); Polyclonal antibody

Tolerance & Autoimmunity

Oral Presentations:

7644O

Simulation of mating-like tolerant-state by allogeneic seminal vesicle fluid in CNS of male Lewis rat

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Introduction: Central Nervous System (CNS) and fetus have long been regarded as examples of immune privilege sites. Thymus-arisen FoxP3 regulatory T cells (Treg) are one of the most important immuno-regulatory mechanisms in CNS and pregnancy. Multiple sclerosis is an inflammatory disease of CNS caused by increased Th1/Th17 response and reduced frequency and/or function of Treg. Tregs critically affect female reproductive tissue upon mating due to exposure to seminal vesicle secretion. Such an effect of seminal vesicle secretion on Treg expansion prompted us to investigate whether and how intra- CSF administration of seminal vesicle secretion affects experimental autoimmune encephalo-meyelitis (EAE), the most widely used animal model of MS. **Material and methods:** EAE was induced in male Lewis rats using guinea pig spinal cord and complete Freund's adjuvant. Intra-CSF administration of seminal vesicle fluid was conducted on day 7 and brain and spinal cord of the animals were removed on day14. Expression of mRNA for IFN-γ, IL-4, IL-17, and FoxP3 was determined in brain and spinal cord using real-time PCR where β-actin was used as reference gene. **Results:** Administration of seminal

vesicle secretion led to significant increase in IL-4 and FoxP3 expression and decrease in IFN-γ, IL-17 expression within CNS. It was concomitant with significant amelioration of clinical sign. Interestingly, seminal vesicle secretion from syngeneic male rats had not such a healing effect. **Conclusion:** The results suggested that seminal vesicle secretion contains all the required ligands for activation of Tregs pool and has anti-inflammatory effect simulating a pregnancy-like tolerant-state in organs other than reproductive organ. **Keywords:** EAE, Mating, Allogenic, CNS, FoxP3, Tregs, Seminal Vesicle Fluid, Tolerance

7695O

Investigation of the Human FCRL 1, 2, and 4 Gene Expressions in Patients with Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a common autoimmune disease characterized by inflammation of the synovial joints. The Fc receptor-like (FCRL) molecules have recently been shown to contribute to the pathogenesis of certain autoimmune disorders. In this study, the expression levels of FCRL 1, 2 and 4 in peripheral blood mononuclear cells from RA patients was investigated using real-time polymerase chain reaction (PCR). The mRNA of these molecules was detected in 44.4% (FCRL1), 53.3% (FCRL2) and 31.1% (FCRL4) of patients. Comparatively, 31.1% (FCRL1), 51.1% (FCRL2) and 26.6% (FCRL4) of controls expressed these genes. There were no significant differences in FCRL 1, 2 and 4 positivity between the patients and controls. Analysis of gene expressions in FCRL positive patients demonstrated that FCRL1 and FCRL2 were expressed at a relatively similar level in patients compared to normal subjects ($P>0.05$). However, there was a lower FCRL4 gene expression in patients compared to controls ($P<0.001$). Clinical and paraclinical evaluations indicated no significant differences between FCRL positive and FCRL negative patients. However, FCRL positive patients had a significant positive correlation between FCRL2 expression and the erythrocyte sedimentation rate (ESR; $P<0.001$, $R=0.66$), anti-cyclic citrullinated peptide (CCP) antibody ($P=0.033$, $R=0.44$) level and disease activity score (DAS28, $P=0.016$, $R=0.49$). There was a negative association with age ($P=0.009$, $R= -0.52$). In conclusion, it was observed a lower levelin FCRL4 mRNA and association of FCRL2 expression with inflammatory markers and disease activity, which suggested the contribution of these molecules to RA pathogenesis.

7713O

Histopathological investigation of neurodegeneration associated with anti-gliadin immune responses in the central nervous system of mice

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Introduction: Even though the exact cause of neurological disorders associated with gluten sensitivity is not definitely understood, recent findings suggested the involvement of immune-mediated mechanisms. Evidence pointed to the important role of anti-gliadin immune responses. The present experimental study was conducted to investigate the histopathological features of *anti-gliadin immune responses in* the central nervous system (CNS) of mice. **Material and methods**: 6–8 week old female C57BL6mice were divided into two groups. Subsequently mice were immunized subcutaneously with complete freund's adjuvant (CFA; 400µl) or peptic-tryptic (pt)-gliadin (400µg), emulsified in CFA (400µl). Boosters containing the same amount of antigen were injected on days 7 and 14.After 21 days, mice were sacrificed and CNS tissues were harvested. H&E and LFB staining methods were conducted on the prepared sections. Moreover, Immunohistochemistry procedure was performed to explore the presence of extravasated antibodies. **Results:** *In mice recieved CFA, perivascular edema was the only histopathological manifestation. However in mice immunized with pt-gliadin in CFA,* the microscopic lesions were included the perivascular edema, microgliosis and acute neuronal necrosis in the cortex, cerebellar *Purkinje cell layer, and ventral horn of spinal cord. Furthermore, extravasation of blood-born anti-gliadin antibodies along with selective targeting of Purkinje cells were observed in mice immunized with pt-gliadin in CFA.* **Conclusion:** Our findings indicated that without immune cell infiltration into the CNS, the immune responses directed against gliadin peptides may contribute to BBB breakdown, extravasation of serum anti-gliadinIgG, purkinje cell targeting, gliosis and acute neuronal necrosis in the cortex , subcortical and cerebellar Purkinje cells. **Keywords:** Gluten sensitivity, neurological disorder, Gliadin, CNS

9726O

Serum cytokine profiles in Behcet's disease

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Introduction: Behcet's disease (BD) is a recurrent multisystem auto-inflammatory disorder in which the autoimmune responses have been considered as the major etiological factors. Although serum levels of cytokines related to innate immunity and T helper-1 (Th1) cells have been evaluated thoroughly in BD patients, there are limited data on the Th2- and Th17-related cytokines. **Material and methods:** In the present study, the serum levels of sixteen cytokines related to innate immunity (TNF-α, IL-1α, IL-1β, IL-6, IL-8, IL-12 and IL-15) as well as Th1 (IL-2, IFN-γ and TNF-β), Th2 (IL-4, IL-5,IL-10 and IL-13) and Th17 (IL-17 and IL-23) cells in the sera of 44 Iranian patients with BD and 44 healthy controls have been investigated using cytokine array technique. **Results**: The serum levels of innate immunity-related cytokines, IL-1α (p=0.0001), IL-1 β (p=0.0006), IL-6 (p=0.0005), IL-12 (p=0.0067), IL-15 (p=0.0007) and TNF-α (p=0.033) were statistically higher in BD patients than healthy controls. In the case of Th1-related cytokines, IL-2 and IFN-γ were significantly higher in patients (p=0.046 and p=0.017, respectively). From Th2-related cytokines, only IL-13 was statistically higher in patients than controls (p=0.0001). Generally the sum of Th1-related cytokines were higher and Th2-related cytokines were lower in BD patients than healthy controls (p=0.013 and p=0.024, respectively). Both Th17-related cytokines, IL-17 and IL-23, were higher significantly in BD patients (p=0.037 and p=0.035, respectively).**Conclusion:** The results indicated that cytokines perturbation such as overexpression of innate immunity- as well as Th1- and Th17- related inflammatory cytokines play a pivotal role in BD susceptibility. **Key words:** Innate immunity, Th1, Th2, Th17, Cytokine profiles, Behcet's Disease.

9778O

IL-33 and TSLP gene expression in colonic lesions of ulcerative colitis

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Introduction: Ulcerative colitis (UC) is a chronic inflammatory bowel disease of the colon with unknown precise etiology. Here, IL-33 and TSLP (Thymic stromal lymphopoietin) gene expression were characterized in patients with ulcerative colitis and normal individuals. **Material and methods**: Gene expression analyzed using real time PCR. The samples were collected by colonoscopy from the margin of colon ulcer tissue. Patients whose ulcerative colitis had been confirmed by colonoscopy and histopathology, were considered as test group. In addition, the samples that were negative for this disease were considered as control group. **Results**: The results showed a specific increase of IL-33 in active UC (n=20) compared to controls (n=20) with significant difference ($p<0.0014$) but TSLP expression in patients with active UC (n=33) significantly reduced ($p<0.0001$) in compared to control group (n=33). **Discussion:** IL-33 is involved in inflammation of UC as a pro-inflammatory cytokine. This result showed up-regulation of IL-33 during active colitis. Blocking IL-33 pathway could be therapeutic strategy in treatment of UC. TSLP is required to protect the gut from inflammation and preserve intestinal hemostasis. Inflammation in patients with active UC correlates with a reduced expression of TSLP. This study showed the importance of anti-inflammatory role of TSLP to protect against colitis. **Key words:** Ulcerative colitis, gene expression, TSLP, IL-33

10834O

Study of regulatory T cells in Ulcerative colitis

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Introduction: Regulatory T (Treg) cells take part in immune homeostasis and play a pivotal role in maintaining peripheral tolerance. The aim of this study was to evaluate the frequency and function of Treg cells in active and untreated ulcerative colitis (UC) patients. **Material and Methods:** 32 subjects with newly diagnosed UC and 31 age-matched healthy controls were included in this survey. The frequency of Tregs was analyzed by flow cytometry using CD4, CD25, CD127 and FoxP3 markers. Surface expression of $CD4^+$, $CD25^+$ and $CD127^{low}$ markers were used for isolation of a relatively pure Treg population. Suppressive activity of Tregs was determined by measuring their ability to inhibit the proliferation of T responder cells. **Results:** UC patients had a lower frequency of $CD4^+$ $CD25^+$ $CD127^{low}$ $FoxP3^+$ Treg cells. Additionally, Treg cell mediated suppression was lower in UC patients compared to controls. The frequency and suppressive capacity of Tregs and MFI of FoxP3 were inversely correlated with disease activity. **Conclusion:** The results suggested that $CD4^+$ $CD25^+$ $CD127^{low}$ $FoxP3^+$ Treg cells may contribute to immuno-pathogenesis of UC, and the assessment of Treg cell frequency and function may have clinical value.

11092O

The optimization of human Th17 cell development using pro-inflammatory cytokines independently of TGF-β

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Introduction: T helper-17 (Th17) cells are a main subset of CD4+ T helper cells with a critical role in pathophysiology of several inflammatory disorders. Due to discrepancies that exist among different studies which have tried to clarify critical factors in human Th17 cell differentiation, we aimed to identify the optimal condition for Th17 cell development. **Material and Methods**: CD4+ T cells were isolated from peripheral blood samples. Purified cells were treated with Th17 polarizing cytokines (IL-1β, IL-6 and IL-23 with or without TGF-β) followed by analysis of IL-17A, IFN-γ, Foxp3 and CD25 by flow-cytometry and detection of IL-17A, IL-21, IL-22 and IL-10 in cell culture supernatants by ELISA. Also, the effects of selective inhibitors of TGF-β signaling pathway (SB-431542 and A83-01) on Th17 cell polarization were determined. **Results**: We found that combination of pro-inflammatory cytokines (IL-1β, IL-6 and IL-23) but TGF-β could not be applied as the best condition for human Th17 cells development through high expression of IL-17 and low expression of IFN-γ, Foxp3 and also production of IL-17, IL-22 and IL-21. It is shown that TGF-β is a negative regulator for this issue through induction of Foxp3 expression. Blocking of the TGF-β signaling pathway by applying its selective inhibitors is efficient for Th17 cell differentiation. SB-431542 and A83-01 couldn't inhibit the production of IL-17, IL-21 and IL-22 and restricted Foxp3 expression in differentiating Th17 cells. **Conclusion**: It was concluded that human Th17 cells could be differentiated in the presence of pro-inflammatory cytokines and TGF-β seems to be a negative regulator. **Keywords:** Th17 cell, IL-17, TGF-β, Foxp3, Pro-inflammatory cytokines

11141O

The effect of carbenoxolone on diabetes through the induction of HSP 70 and increased IFN-γ

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease in which an inappropriate self-directed immune response destroys insulin-producing β-cells in the pancreatic islets leading to dysregulated blood glucose levels. Since the heat shock proteins (HSPs) have cytoprotective effects, in this study, we evaluated the effects of carbenoxolone as an hsp70 inducer in animal model of autoimmune Diabetes in C57BL/6 mice. **Materials and Methods:** For this purpose, 40 pure male mice C57BL/6 were randomly divided into 4 groups, and autoimmune diabetes was induced by streptozotocin (Stz) in 3 groups of mice. Among these three groups, one group was considered as diabetic control. The other two were treated with intraperitoneal injection of 50 mg/kg carbenoxolone, as in one group, the drug was administered before and after the induction of diabetes (7 doses) and in the other group the drug was injected only after induction of diabetes (5 doses). Then, the amount of hsp70, fasting blood glucose, IFN-γ, IL-10 and IL-17 cytokines, and the frequency of regulatory T cells were evaluated. **Results:** The results showed that carbenoxolone increases hsp70, IFN-γ and IL-17, and decreases IL-10 production and causes increasing fasting blood sugar and which are statistically significant ($p<0.05$). **Conclusion:** carbenoxolone can cause hsp70 induction and then by increasing IFN-γ production and inhibition IL-10 production in mice with autoimmune diabetes causes toxic effects on pancreatic islet beta cells and increases the severity of the disease. **Keywords:** Carbenoxolone, Cytokine, Hsp 70, Fasting Blood Sugar

Poster Presentations:

6503P

Does genetic variants of IL-32 affect susceptibility to multiple sclerosis?

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). The etiology of MS is unknown but, environmental and genetic factors play a key role in the development of the disease. Interleukin-32 (IL-32) is a cytokine inducing crucial inflammatory cytokines such as tumor necrosis factor-α (TNFα) and IL-6 and IL-1β. It was hypothesized that single-nucleotide polymorphism (SNP) within the IL-32 promoter would be associated with susceptibility to or outcomes in patients with multiple sclerosis. **Material and Methods**: A case control study was performed of 304 subjects including 132 MS patients and 172 sex- and aged matched healthy controls. For each individual, the IL-32 rs535721922 polymorphic genotypes were determined by restriction fragment length polymorphism and polymerase chain reaction (RFLP-PCR) analysis. Statistical analyses were performed to determine whether any demographic or behavioral aspects, risk factors, or a particular IL-32 genotype were associated with MS risk. **Results:** In overall, the results showed no significant differences in allele and genotype distributions of IL-32 between MS patients and controls (P=0.064 and P=0.0384, respectively). On the other hand, frequency of TT genotype and T allele were 19.7% and 13.4%, in MS and controls, respectively. **Conclusion:** The results, for the first time, showed that rs535721922 polymorphism in IL-32 gene probably has not an impact on individual susceptibility to MS. **Keywords**: IL-32, multiple sclerosis, polymorphism

7559P

Protein-protein interaction network (PPIN) and blood transcriptome analysis reveal risk genes with high centrality in Type 1 diabetes

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease caused by the activation of lymphocytes against pancreatic B-cells, and it is required to identify efficient therapeutic markers. Systematic investigation of differentially expressed genes in the human protein-protein interactions (PPIs) network can provide important biological information for revealing the potential therapeutic targets for T1D.**Material and Methods:** In this study, differentially expressed genes identified by statistical analysis of gene expression profile of peripheral blood mononuclear cells (PBMCs) from newly diagnosed type 1 diabetic children which was prepared from Gene Expression Omnibus (GEO). Then, these genes were mapped to PPIs data to construct related subnetwork. Centrality (degree, betweenness and closeness centrality) analysis of sub-network was conducted by Cytoscape software. Functional modules were identified by Cluster ONE algorithm. **Results:** By statistical analyzing of gene expression profile, 1024 and 1443 genes were determined as up-regulated and down-regulated genes, respectively. After mapping these genes on the PPI network, the sub-network was constructed with 949 nodes and 1776 edges. By topological analysis of the subnetwork, high degree nodes (hub) and high between-ness nodes (bottleneck) were determined. Immune response, genetic information processing and metabolism were the main class of involved biological processes. Then, 7 hub-bottleneck proteins were identified which involved in functional modules and 7 proteins which had more centrality measures in the subnetwork as candidate markers. **Conclusion:** The results provided new insight in T1D treatment by discovering of network-based biomarkers and could be considered as potential therapeutic targets for T1D.**Key words**: Type 1 diabetes, Gene expression, PPIs network.

7568P

IL-27 Gene Polymorphisms in Iranian Patients with Behcet's Disease

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Introduction: Behcet's Disease (BD) is a chronic systemic inflammatory disease of unknown etiology, principally characterized by relapsing periods of a broad range of clinical symptoms. Cytokines play fundamental roles in the pathogenesis of BD. Polymorphisms within cytokine genes have been found to play a pathogenic role in the development of autoimmune/inflammatory disorders. Interleukin 27 (IL-27), a new pro/anti inflammatory cytokine, is a great candidate for chronic inflammatory disease studies. The purpose of this study was to investigate a possible association between polymorphisms in the IL-27 gene and susceptibility to BD. **Material and Methods:** Fifty Iranian patients with BD and one hundred healthy individuals were examined for rs153109A/G and rs181206T/C IL-27 gene single nucleotide polymorphisms using RFLP-PCR and ARMS-PCR, respectively. Allele and genotype distributions were compared between groups using chi-square or Fisher's exact test. **Results:** Frequencies of the rs153109AA genotype and rs153109A allele were statistically higher in BD patients compared to the control group ($p = 0.034$ and $p = 0.011$, respectively). The genotype and allele frequencies of rs181206 T/C polymorphism in BD patients were not significantly different from those of healthy controls. **Conclusion:** Present findings demonstrated, for the first time, that IL-27 gene rs153109 A/G SNP may be involved in susceptibility to BD in the Iranian population.

7575P

Kinetic of T cell response in the central nervous system (CNS) during experimental autoimmune encephalomyelitis (EAE)

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Introduction: Multiple sclerosis (MS) and its animal model EAE are regarded as an autoimmune disease of the CNS. In both diseases, the pathology and clinical symptoms of EAE suggest dynamism in type/intensity of T cell responses including Th1, Th2, Th17, and naturally occurring regulatory T CD4+CD25+FoxP3 (Treg) cells. **Material and Methods:** In this study we analyzed CD4+ T cell responses during the course of the disease from 0, normal state, to 5, peak of the disease. To do so, EAE was induced in Lewis rats using guinea pig spinal cord and complete Freund's adjuvant. Expression of mRNA for IFN-γ, IL-4, IL-17, and FoxP3 was determined in brain and spinal cord using real-time PCR where β-actin was used as reference gene. **Results:** In the brain and spinal cord, IFN-γ and IL-17 expression showed an increasing trend in animals from 1 to 5 comparing to normal animal while showing a ring-form trend. However, IL-17/ IFN-γ ratio was maximum at score 2. IL-17/IFN- γ ration was more than 1 in animals scored 1 whereas inversed in animals scored 0, 4, and 5. There was no significant difference between IL-17 and IFN-γ level in animals scored 2 and 3. Level of IL-4 and FoxP3 expression was markedly down-regulated from score 1 through 5 compared with normal animal. Interestingly, IL-17/IL-4 ratio was more than 1 in animals scored 1 through 5 whereas inversed in normal animals. **Conclusion:** Our results illustrated a harmony of T cell response with different role at different time at the same stage.

7576P

Assessment of MxA (myxovirus resistance protein A) gene expressionas marker of biological activity of interferon-betain patients with Multiple Sclerosis receiving CinnoVex, Rebif, and Betaferon

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Introduction: The identification of factors that can effect on the efficacy of drugs in relapsing-remitting multiple sclerosis (MS) is important. The treatment with IFN-β induces production of neutralizing antibodies (NAbs) in some patients. NAbs bind to IFN-β, reduces bioactivity and clinical efficacy of the drug and subsequently worsening the disease.Determination of MxA (myxovirus resistance protein A) gene expression as marker of biological activity of interferon-betain patients with Multiple Sclerosis receiving CinnoVex,Rebif, and Betaferon. **Material and method**: The MxA gene expression or in vivo IFN-β bioactivity was measured by a real-time PCR assay in peripheral blood mononuclear cells (PBMCs) of 31 patients with RRMS receiving IFN-β for at least 12 months. Expanded Disability Status Scale (EDSS) scores and relapse were analyzed using the results of NAbs assay. **Results:** Of the 31 RRMS patients receiving IFN-β, 25 (80.6 %) showed NAbs after 12 months of treatment. However there was no significant difference in the number of relapse (P = 0.1) and EDSS (P = 0.9) among three groups. **Conclusion:** NAbs inhibit the MxAgene expression. MxA gene expression explain the relationship between anti-IFN-β antibodies and bioactivity in patients with MS receiving IFN-β. MxA can be integrated with clinical and imaging indicators to guide individual treatment decisions. Lack of MxA bioactivity, a switch to a non-interferon-

beta therapy should be considered. **Key words:** Multiple Sclerosis, Interferon-beta, neutralizing antibodies, bioactivity, MxA

7584P

*IL-23R*eceptor Gene rs11209026 and rs1004819 SNPs in Multiple Sclerosis

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Introduction: Multiple sclerosis (MS) is a neurodegenerative disease characterized by inflammation in the central nervous system (CNS), leading to complete disability . Th17 cells express IL-23 receptor (IL-23R) and IL-23 play a key role in the development of Th17 cells. The aim of this study was to investigate the association of single nucleotide polymorphisms (SNP) in *IL-23R* gene, including rs11209026 and rs1004819, with multiple sclerosis (MS). **Material and Methods:** The blood samples collected from 135 MS patients and 135 healthy subjects as a control group. Genomic DNA was isolated from whole blood using a routine salting out. IL-23 receptor genotyping at positions rs11209026 and rs1004819 was conducted by PCR-RFLP technique. **Results:** No significant differences were observed between patients and controls regarding the genetic variations at rs11209026 and rs1004819. Although G allele of rs11209026 variant increases in control compared to MS patients but it was not statistically significant. Statistical analysis also showed no significant differences between patients with RRMS, SPMS, PPMS and PRMS patterns regarding the frequencies of genotypes and alleles at SNPs rs11209026 and rs1004819 in *IL-23R* gene**.** **Conclusion:** The data indicated that there is no association between IL23R polymorphism and MS susceptibility or severity of the disease.

7622P

Investigation of Relationship between the Release of Fecal Genes of lactobacillus and FBS Level in People with Diabetes

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Introduction: Insulin resistance and diabetes type 2 have steadily increased in the past few decades. In addition to genetic and environmental factors, intestinal microbes may also have an important role in the disease process of diabetes. Infection is a major cause of death in diabetics. Chronic hyperglycemia impairs the body's defense mechanism and natural intestinal micro flora may also be affected by the diseases and even lead to its progression. The purpose of the study was to investigate the relationship between the normal intestinal micro flora (lactobacillus) and FBS levels in diabetic patients. **Material and methods:** Of 45 diabetic patients and 45 healthy individuals referred to Ibn Sina medical laboratory, blood and stool samples were collected. Fasting blood sugar (FBS) levels were measured in the study population, which 45 cases had normal FBS and 45 cases had normal FBS. DNA extraction and PCR related to genes of lactobacillus were performed from stool subjects. **Results:** Means of FBS were 175 mg/dl in diabetic group, and 96 mg/dl in healthy subjects. Also, in the investigation of releasing the genes

of fecal lactobacillus, about 89.1% of diabetic patients and 60% of healthy people had lactobacillus genes in their feces, which represents an increase of fasting blood sugar and release more lactobacillus genes in diabetic patients compared to healthy subjects. FBS levels and PCR in fecal lactobacillus genes had statistically significant difference (P≤0.022). **Conclusion:** FBS increased fluctuations were associated with higher incidence of lactobacillus bacteria that this accompaniment is effective in disease progression and complexity of pre-awareness of diabetes. **Keywords:** FBS, Lactobasillus, diabetes type 2

7645P

Comparison of T cell response pattern in lymph node draining central nervous system and testis during EAE

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Introduction: Multiple sclerosis (MS) is a demyelinating inflammatory disease of the central nervous system. Experimental autoimmune encephalomyelitis (EAE) is a widely used model for MS. It is known that MS affects women more than men and this has been attributed to sex hormones, and/or sex-linked gene, and more robust immune responses in females. However, it is more severe in males and sexual behaviors/fertility is changed in males affected by MS. The aim of this study was to compare T cell response in testis and deep cervical lymph node (DCLN) draining central nervous system. **Material and methods:** Therefore, EAE in male Lewis rats using guinea pig spinal cordand complete Freund's adjuvant. The animals were evaluated for weight loss and clinical sign of EAE. **Results:** Expression of mRNA for IFN-γ, IL-4, IL-17, and FoxP3 was determined in testis and DCLN in animals scored 1 through 5 using real-time PCR where β-actin was used as reference gene. Our result indicated a similar pattern of IFN-γ and FoxP3 expression with an increasing trend in testis and DCLN. **Conclusion:** However, the pattern of IL-17 and IL-4 was different. IL-17 and IL4 did not change in animals scored 1 though 5 in the testis, whereas they showed an increasing trend in DCLN. This indicates, firstly immune system attacks testis along with CNS. Secondly, there may be molecular mimicry between CNS and testis and the differences may be due to different microenvironment and/or regulatory mechanisms.

7646P

Comparison of T cell-mediated immune surveillance and in CNS and deep cervical lymph node

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Introduction: Central Nervous System (CNS) has long been assumed as immune privilege site. It was initially thought that CNS is out of reach of the immune system because of the barriers. However, it was later found that the

privilege is not absolute and the immune system has access to CNS. More investigations gave birth to the concept of immune surveillance. Indeed, CNS is physiologically subjected to immune-regulatory mechanisms and is continuously monitored by blood-borne T cells to establish immune surveillance. CNS lacks an obvious lymphatic system, however, it has been suggested that deep cervical lymph nodes acts as drainage lymph nodes of CNS. **Material and methods:** In this study, male Lewis rat immunized with complete Freund's adjuvant and T cell pattern was compared between CNS and deep cervical lymph node. To do so, expression of mRNA for IFN-γ, IL-4, IL-17, and FoxP3 was determined in the brain, spinal cord, and deep cervical lymph nodes, corresponding to Th1, Th2, Th17, and FoxP3^{+} regulatory T cells (Tregs), using real-time PCR where β-actin was used as reference gene. **Results:** Expression of IFN-γ and IL-17 were significantly higher in brain and spinal cord rather than deep cervical lymph nodes. Inversely, IL-4 and FoxP3 expression was significantly higher in deep cervical lymph nodes rather than brain and spinal cord. **Conclusion:** Our results suggest different homing pattern of effector T cells between CNS and deep cervical lymph nodes.

7660P

Evaluation effect of garlic extract on Foxp3 gene expression and suppression capacity of rats thymocytes

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Introduction: Two different effects of anti- tumor and anti- inflammatory roles for garlic extracts have been shown. Probably, the anti-tumor effect of garlic is related to the regulation of immune system responses. The aim of this study was to evaluate the garlic extract on Foxp3 gene expression (regulatory cells transcription factor) and suppression ability of rats' thymocytes. **Material and Methods**: Thymocytes were cultured with 0, 1, 10 and 100 µg of alcoholic and aqueous extract of garlic with/without of 10 µg of Phytohemagglutinin (PHA) for 48h. The quantitative expression of Foxp3 gene was detected using Real-Time PCR. To bioassay of T-regulatory function, the treated thymocytes were co- cultured with peritoneal macrophages for 72h. Release of nitric oxide (NO) by macrophages was detected in the supernatant of co-culture by Elisa. **Results:** Analysis variance of data showed that both alcoholic and aqueous extract decreased expression of Foxp3. In the presence of PHA, 1 µg of both extract decreased expression of Foxp3 and 0, 10 and 100 µg of both extracts decreased the expression of Foxp3 during absence of PHA. Treated thymocytes with alcoholic extract compared to aqueous extract increased release of NO from macrophages; indicates that thymocytes suppression capacity is declined. **Conclusion:** This study showed that the anti-tumor effects of garlic extract probably is related to decrement ofFoxp3geneexpression and thymocytes suppression capacity; which may increase the other immune cells responses against tumor.

7697P

Serum OX40 levels in patients with NeuroMyelitisOptica (NMO)

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Introduction: Neuro-Myelitis Optica (NMO) is an autoimmune inflammatory disease of the CNS disturbing spinal cord and optic nerves specified by the presence of pathogenic serum autoantibodies against aquaporin 4 (AQP4) in the majority of patients. The role of Tcell is ambiguous. OX40 (CD134) is a member of the tumor necrosis factor receptor family and is expressed selectively on activated T lymphocytes which increases in several autoimmune diseases. The aim of the study was to evaluate serum OX40 levels in patients with NMO and control group. **Material and method:** The study involved 19 patients with NMO and 19 controls. Serum OX40 levels were determined by the enzyme-linked immunosorbent method (ELISA). **Results:** The present study showed that there was no significant difference between OX40 levels in the serum of patients with NMO compared with controls ($P > 0.05$). **Conclusion:** Serum OX40 levels could not be as a diagnostic or treatment marker for NMO.

7711P

Determination of expression of miR-146a and miR-155 and its correlation with regulatory T cell in Systemic Lupus Erythematosus patients

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the presence of pathogenic autoantibodies. Dysregulated number and function of regulatory T cells are implicated in the pathogenesis of SLE. MicroRNAs are small noncoding RNAs that regulate the expression of the genes involved in immune responses regulation. These molecules are concerned as anticipant biomarkers for diagnosis, prognosis and treatment of SLE. The purpose of this study was to investigate the expression of microRNAs and its association with regulatory T cell in systemic lupus erythematosus patients. **Material and Methods:** In the current study, 20 healthy controls and 20 patients with SLE were studied. Their PBMCs were isolated, and regulatory T cells were analyzed by flow cytometr. MiRNAs were extracted, cDNA synthesized and the gene expression of miR-146a, and miR-155 were assessed by Real-Time PCR method. **Result:** In SLE patients the expression level of miR-146 has decreased compared to controls (1.83±2.1 versus 4.01±5.26), while the expression of miR-155 has increased significantly compared to controls (11.07±4.51 versus 7.77±3.72) ($p<0.001$). Decreased expression of miR-146a had positive correlation with regulatory T cells ($p=0.029$). **Conclusion:** MicroRNAs could control cellular events such as cell growth, and differentiation. They have influence on the regulatory T cell population. It seems that by influencing regulatory cells these molecules can control immune responses in SLE patients. **Keywords:** Systemic Lupus Erythematosus, MicroRNA, Regulatory T cell

8705P

Influence of vitamin D3 on the expression of miR-146a and miR-155 in patients with systemic lupus erythematosus

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune inflammatory disease with multiple organ manifestations. The causes of lupus are unknown, although recent studies have expressed that micro-RNAs are involved in the pathogenesis of lupus. Immuno-modulatory role of vitamin D3 has been considered in SLE. The purpose of this study was to investigate the effect of vitamin D3 in vitro treatment on the expression of some mi-RNAs including MiR-146a, and MiR-155.**Material and Methods:** Twenty SLE patients and 20 healthy subjects were enrolled in the current study. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured in the presence and absence of vitamin D3 (50 nM). Mi-RNAs were extracted and cDNAs were synthesized and gene expression levels of MiR-146a, MiR-155 were evaluated by Real Time PCR, Taq-Man method. Finally the results were analyzed by SPSS software (ver. 16).**Results:** Real Time PCR analysis showed that vitamin D treatment significantly down-regulated the expression level of MiR-146a in SLE patients compared to control group (2.9±3.2 vs 8.7±1.2) (P= 0.025), while the expression of MiR155 was up-regulated (7.8±1.9 vs 6.9±4.3) (P=0.048). **Conclusion**: The findings showed that vitamin D3 could regulate the expression levels of miR-146a and miR-155 in SLE patients. It seems that vitamin D could be effective for prevention and treatment of lupus patients by controlling the expression of miRNAs. **Keywords**: lupus, micro RNA, vitaminD

8719P

Expression of TLRs 3, and TLR8 and their correlation with autoantibodies profile in systemic lupus erythematosus patients

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Introduction: Systemic lupus erythematosus (SLE) is a severe systemic autoimmune disease with heterogeneous clinical manifestations. Recent experimental and clinical studies have placed new emphasis on the role of Toll-Like Receptors (TLRs) in the pathogenesis of SLE. TLRs are important innate immune receptors for the identification and clearance of pathogens. TLRs by deleting apoptotic cells play an important role in the control of the production of pathogenic autoantibodies and development of clinical features of SLE. **Material and Methods**: In the current study, Peripheral Blood Mononuclear Cells (PBMCs) from 20 SLE patient and 20 healthy controls were separated. RNAs were extracted using Tripure, cDNAs were synthesized, and the expression levels of TLR3, and TLR8 genes were assessed by Real-Time PCR method. Auto-antibodies profile was screened on serum by using a commercial kit. **Results:** The expression level of TLR3 was up-regulated in SLE patients compared to controls (5.96±2.47 VS, 5.05±3.44), while the expression of TLR8 was down-regulated (0.17±2.89 VS, 3.18±5.34) (p=0.03). Ninety percent of SLE patients were ANA positive, and anti-SSA was the most prevalent autoantibody, followed by anti-dsDNA (59.1%) and anti-nucleosomes (57.9%). The findings showed that the decreased expression of TLR3 was significantly correlated with the presence of anti-nucleosomes autoantibodies in SLE patients (p=0.03). **Conclusion:** The results showed that TLR3 and TLR8 have differential expressions in SLE patients compared to controls. It was

also showed that TLR3 may play an important role in the pathogenesis of SLE by power on the production of some auto-antibodies. **Key words:** systemic lupus erythematosus, autoantibodies, TLR3, TLR8

9787P

Influence of Probiotics on Regulatory T Cells and Related Cytokines in lupus-like syndrome induced murine model

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune inflammatory disease. Production of autoantibodies and activated T cell against self-antigens are hallmarks of the disease. The etiology of SLE is not fully understood, but decrease of T regulatory (Treg) cells may be one of the significant factors in the pathogenesis. It's shown that some probiotics such as lactobacillus delbrueckii and lactobacillus rhamnosus may induce regulatory T cells production and function, so can have useful role in SLE remission. The aim of this study was to evaluate the effect of these tolerogenic probiotics on Treg cells and related cytokines. **Material and Methods:** Lupus-like disease was induced in Balb/c mice. Then they were treated with the probiotics. Then, mice were killed, the spleens were extracted to analyze the percentage of Treg cells by flow-cytometry. RNAs were extracted and cDNA was synthesized. The expression levels of IL10, Foxp3 and TGF-β genes were assessed by Real-time PCR method. **Results:** The findings showed that probiotics could modulate regulatory T cells and their related molecules Foxp3 and TGF-B in lupus like induced mice model. This study is in progress and the full data will be presented and discussed in the congress. **Conclusion:** Probiotics can modulate immune responses in lupus like induced mice model. **Keywords:** Treg cells, SLE, Probiotics, Autoimmune disease

9821P

The role of leukemia inhibitory factor in autoimmune demyelination patients

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Introduction: Leukemia inhibitory factor (LIF) is a glycoprotein from the interleukin-6 *family* of cytokines that has been identified in different mammals.LIF regulates these complex functions by binding to its specific receptor β subunit. The receptor (LIFR) of this cytokine is expressed in different cells including hepatocytes, neurons, and lymphocytes. Autoimmune injury to oligodendrocytes elicits an endogenous response in the central nervous system,

which initially limits the acute injury of oligodendrocytes and myelin. It was determined by multifocal inflammatory demyelination, axonal loss and neurodegeneration. Auto reactive T helper 1 (Th1) and Th17 cells are thought to be the main effector cells in autoimmune demyelination, Loss of suppressive function of circulating CD4+ Tregs is demonstrated in autoimmune demyelination patients. **Material and methods:** This review focused on possible mechanisms underlying the associations between LIF and autoimmune demyelination, with emphasis on LIF which plays a key role in neuroin flammationation. We searched in pub med, science direct and Google scholar. **Results:**Although the clinical utilization of LIF in clinical setting is hurdled by many limitations, in experimental autoimmune encephalomyelitis animal model, administration of LIF was found useful in ameliorating the clinical symptoms by preventing demyelination, which suggests its possible role in autoimmune diseases by increasing Tregs and limiting Th1 development in these patients by supporting the expression of FOXP3.**Conclusion:** In conclusion, LIF is associated with an increase in Tregs which leads to modulating the Immune response and limits Autoimmune Demyelination. Providing further motivation for the use of LIF as a novel treatment for autoimmune diseases is required.

10811 P

Asingle-nucleotide polymorphism inthe interleukin-12B gene (rs3212227) was associated with multiple sclerosis in patients from southeast of Iran

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Introduction: The polymorphisms in the *IL-12B* gene, encoding the IL-12 P40 subunit, were associated with several autoimmune and inflammatory diseases.The aim of this investigation was to evaluate the association of a single nucleotide polymorphism (SNP), rs3212227, in *IL-12B* gene with multiple sclerosis (MS) in patients from southeast of Iran. **Material and Methods:** Blood specimens were obtained from 140 MS patients and 140 healthy subjects as a control group. The genomic DNA was extracted and the SNP rs3212227 determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. **Results:** The frequencies of the AA, AC and CC genotypes at SNP rs3212227 were 55.0%, 17.9% and 27.1% in MS patients and were 61.4%, 29.3% and 9.3%, in healthy subjects, respectively. The frequency of CC genotype at rs3212227 was significantly higher in MS patients as compared with healthy control group ($P<0.001$). However, the AC genotype was less prevalent in MS patients than in healthy control group ($P<0.02$). The frequencies of A and C alleles at SNP rs3212227 were 63.09% and 36.07% in MS patients and 76.07% and 23.9%, in healthy control subjects, respectively. The frequency of C allele was significantly higher whereas the frequency of A allele was lower in MS patients than healthy control group ($P<0.001$).**Conclusion:** These results represented that the presence of CC genotype and C allele at SNP rs3212227 in *IL-12B* gene were associated with susceptibility to MS disease whereas the presence of AC genotype and A allele may confer a protection against disease. **Keywords:** Multiple sclerosis; IL-12; gene polymorphism

10812P

Investigation of the mRNA expression of regulatory T cells-specific transcription factor (FOXP3) in the peripheral blood mononuclear cells from patients with multiple sclerosis

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Introduction: The regulatory T (Treg) cells activity was regulated by master transcription factor FOXP3 and they have important role in the establishment of self tolerance. The aim of this study was to determine the expression of Treg cells-specific transcription factor FOXP3 in peripheral blood mononuclear cells (PBMC) from multiple sclerosis (MS) patients and healthy subjects. **Material and Methods:** The PBMC were obtained from 26 MS patients and 20 healthy subjects as a control group.The PBMC were then cultured in RPMI-1640 at 1×10^6 cells/ml in presence of 10 µg/ml MOGor without any stimulation as negative control. The PBMC were incubated at 37 °C in a 5% CO_2 incubator for 36 h. After this period, the PBMCs were collected for total RNA extraction. The mRNA expression of FOXP3 in PBMC was determined by using real time-PCR. **Results:** In healthy subjects, the fold change expression of FOXP3 were 1 ± 0.45in non-stimulated PBMCs and 0.87 ± 0.33 in MOG-stimulated PBMCs. In MS patients, the expression of FOXP3 were 0.24 ± 0.16 in un-stimulated PBMC and 1.36 ± 0.30 in MOG-stimulated PBMCs. In healthy subjects, no significant difference was observed between MOG- and un-stimulated PBMCs regarding the expression of FOXP3. In MS patients, the expression of FOXP3 in MOG-stimulated PBMCs was significantly higher than un-stimulated PBMCs ($P<0.01$). No significant difference was observed between MS patients and healthy group regarding the expression of FOXP3 in non-stimulated or MOG-stimulated PBMCs ($P<0.10$ and $P<0.28$, respectively). **Conclusion:** These results indicated the expression of Treg-specific transcription factor FOXP3 without any significant changes between MS patients and control group. **Keywords:** Multiple sclerosis, Regulatory T cells, FOXP3

10822P

Investigation serum vitamin D and CRP in pregnant women with hypothyroidism

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Introduction: hypothyroidism is due to decrease in T3 and T4 hormone and increase in TSH production with symptoms including: fatigue, depression, metabolic disorders. The most complication in pregnancy is

hypothyroidism. Vitamin D is an essential element in physiologic function of thyroid hormones and immunologic system. Therefore, the aim of study was performed to examine relationship between serum vitamin D level and CRP and anti-TPO in pregnant women with hypothyroidism. **Methods & materials:** Serum sample of 113 patients was taken and three parameters including: vitamin D, CRP, anti-TPO was done. The method of assay was ELISA for vitamin D and anti-TPO and nephelometry for CRP. Statistical analysis was done by Prism demo graph. **Results**: Our data showed that patient with positive serum anti-TPO had significant lower vitamin D, and there was no significance difference between CRP level and vitamin D. **Conclusion**: According to these evidences, there is relationship between low level of vitamin D and hypothyroidism particularly when anti-TPO is positive. But in this study did not show relationship between CRP and vitamin D level and anti-TPO. **Keywords**: vitamin D, CRP, hypothyroidism, pregnancy

10842P

Association of IL-27 (rs153109 and rs17855750) gene polymorphisms and serum Levels with risk of Systemic Sclerosisin Iranian patients

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Introduction: Inflammatory cytokines including IL-27 may play an important role in the pathogenesis of systemic sclerosis (SSc). Therefore, genetic variants and sera levels of IL-27 were studied in SSc. **Material and methods:** Two polymorphisms of IL-27p28 gene (rs153109 and rs17855750) were investigated in a total of 232SSc patients and 234 controls by PCR-RFLP method. The IL-27 serum levels also were checked by ELISA. **Results:** The rs153109-AA genotype and rs153109-Aallele frequencies were significantly higher in patients compared to controls (p=0.007 and p=0.002, respectively). However, no significant differences were observed in genotype and allele frequencies of rs17855750 between patients and controls. In addition, after categorization of patients into rs153109-G and rs153109-non G carriers, rs153109-AA genotype was more frequent in patients with kidney involvement compared to cases without involvement (p=0.03). Moreover, AA carriers frequency was higher in CRP^{+}male patients compared toCRP^{-} male patients (p= 0.03). On the other hand, after patients stratification into rs17855750-TT versus rs17855750-TG+GG carriers, late onset disease was observed in patients with rs17855750-TT genotype (p=0.03). Furthermore, haplotype analysis showing AG haplotype was associated with increased SSc susceptibility (p=0.001) while GT was a protective haplotype (p=0.007). IL-27 serum levels showed an insignificant higher levels in patients compared to controls (p=0.08). Furthermore, IL-27 serum levels were higher in patients with increased FTP (>1 cm) compared to those with normal FTP (<1 cm, p=0.003).**Conclusion:** Functional SNP in IL-27p28 promoter (rs153109) and IL-27 levels may be associated with susceptibility and clinical manifestations of SSc. **Key words:** Interleukine-27, polymorphism, systemic sclerosis.

10843P

Association of IL-26 gene polymorphisms (rs6581801 and rs10784694) and its serum level with risk of Multiple Sclerosis in Iranian patients

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Introduction: IL-26 is a novel pro-inflammatory cytokine and may be important in the pathogenesis of Multiple Sclerosis (MS). In the present study, association of rs6581801 and rs10784694 in *IL-26* gene with susceptibility to and clinical manifestations of MS were investigated. **Material and methods:** 265 MS patients and 265 controls were genotyped using RFLP-PCR method. Serum level of IL-26 was measured in 44 MS patients and 44 controls by ELISA. **Results:** rs10784694 and rs6581801 did not show any significant association with susceptibility to MS. However, the frequency of rs10784694 GG genotype was significantly lower in female patients compared to controls (p=0.03). In addition, rs6581801CC genotype was associated with higher progression index in male patients. Haplotype analysis showed that CA and CG haplotypes were significantly associated with higher and lower risk of MS (p<0.0001 and p=0.02, respectively). IL-26 serum level was not different between patients and controls, though significant higher level of IL-26 was observed in male patients compared to male controls (p=0.04). Furthermore, male patients who were rs6581801C allele carrier showed significantly higher level of IL-26 compared to non-C carriers (p=0.03). **Conclusion:** The results indicated that *IL-26* gene polymorphisms might be associated with susceptibility to MS in a gender biased manner. **Keyword:** Multiple sclerosis, Interleukine-26, Polymorphism

10849P

Association of IL-32 gene polymorphisms (rs9927163 and rs12934561) and its serum level with risk of Type 1 diabetes in Iranian patients

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Introduction: IL-32 is a pro-inflammatory cytokine and may be important in the pathogenesis of autoimmune disease such as Type 1 diabetes (T1D). In the present study, association of rs9927163 and rs12934561 in *IL-32* gene as well as IL-32 sera levels with susceptibility to T1D were investigated. **Material and Methods:** 230 T1D patients and 238 controls were genotyped by PCR-RFLP method. Serum level of IL-32 was measured in 44 T1D patients and 44 age and gender matched controls using ELISA. **Results:** Analyses based on sex, revealed a lower frequency of rs9927163TT genotype in female patients compared to female controls (p=0.02). In addition, rs12934561CT genotype frequency was higher in male patients compared to those of male controls (p=0.001). Genotype combination analysis revealing TT/CT frequency in male patients, was higher than male controls (p=0.01), while an opposite effect was observed in women (p=0.01). IL-32 levels were not different between controls and patients with disease duration of less than three months (p=0.6), though levels of IL-32 extremely reduced in patients with disease duration more than three months compared to controls (p=0.005). **Conclusion:** This study, for the first time, provided evidence of a gender-biased association between polymorphisms in *IL-32* gene and susceptibility to T1D. However, functional studies are needed to reveal how *IL-32* polymorphisms affect T1D susceptibility. **Keyword:**Type 1 diabetes, Interleukine-32, Polymorphism, ELISA.

10904P

Effect of Lactobacillus Delbrocki on the expression levels of miR-146a, miR-125a and miR-181a on PBMCs of patients with systemic lupus erythematosus

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Introduction: Systemic lupus erythmatosus (SLE) is a chronic inflammatory autoimmune disease which mainly affects young women.SLE is described by production of autoantibodies and activated immune cells against self-antigen. MicroRNAs (miRNAs) are a new class of small group of non-coding, single-stranded RNA molecules that regulate gene expression at post-transcriptional level by degrading or blocking translation of messenger RNA (mRNA). They play important roles in the pathogenesis of various autoimmune diseases, including SLE. Probiotics showed anti-inflammatory properties in the immune system. Therefore tolerogenic probiotics could inhibit and modulate immune response in autoimmune disease. In this study, the effect of probiotic Lactobacillus rhamnosus on the expression levels of miR-146a, miR-125a and miR-181a on PBMCs of lupus patients was evaluated. **Material and methods:** 30 SLE patients and 30 healthy control were enrolled in the study. PBMCs were isolated from whole blood by Ficoll gradient centrifugation. Isolated cells were treated with probiotic Lactobacillus Delbrocki and cultured in RPMI-1640 with 10% FCS in a humidified atmosphere of 5% CO2 at 37°C for 24h. After that, total RNA containing miRNAs were extracted. cDNA was synthesized and the level of gene expression of miR-146a, miR-125a and miR-181a were analyzed by Real Time-PCR method. **Results:** The data showedthat probiotics by changing the expression rate of miRNAs could modulate the immune responses in SLE patients. This study is on progress and the complete data will be presented in the congress**. Conclusion:** Probiotics by affecting the expression rate of miRNAs could play a modulatory role in SLE patients**. Keywords:** MicroRNAs, Systemic Lupus Erythematosus, Autoimmunity, probiotic

10932P

Evaluation of the level of anti-Mumps IgG and IgM antibody in NMO and MS patients

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Introduction: Neuromyelitisoptica (NMO) is a demyelinating disease of the central nervous system that IgG reacts against aquaporin-4(AQP4; expressed on astrocytes). NMO has clinical manifestations resembling those of multiple sclerosis. The presence of AQP4 antibody in NMO patients is a hallmark factor that differentiate the NMO from MS. Viruses are inflammatory factors in white and grey matter of the brain. Inflammatory alterations facilitate transfer of autoantibody from blood-brain barrier. The aim of this study was to evaluate the presence of anti-Mumps IgG, and IgM, in NMO and MS patients. **Material and methods:** In order to examine the levels of IgG, and IgM, ELISA was conducted for 25 NMO, 30 MS, and 30 healthy individuals (Euroimmun, Germany).**Results:** 19 NMO, 16 MS, 14 healthy samples were positive for anti-Mumps IgG (P=0/09). Also, 1 NMO, 1 MS were positive for anti-Mumps IgM(P=1). **Conclusion:** It is concluded that the level of anti-Mumps IgG and IgM in NMO and MS patients were not significantly different. There was no association between the level of anti-Mumps antibody and NMO. However, the importance of viruses as an etiologic factor in autoimmune disease is still a controversial topic.

10951P

Pregnancy outcomes in patients with lupus

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Introduction: Systemic lupus erythematosus (SLE) is a chronic and multisystemic autoimmune disease caused byproducing Ab againstdifferent components of cell that occurs predominantly in women in fertile age. Pregnancy induces dramatic immune and neuroendocrine changes in the maternal body in order to protect the fetus from immunologic attack and these modifications can be affected by SLE. The association of SLE and pregnancy, mainly with active disease and especially with nephritis, has poorer pregnancy outcomes. **Material and methods:** It was written with searching keywords such as "Systemic lupus erythematosus (SLE)" and "Pregnancy outcomes" in databases include PubMed and Google scholar. **Results:** It has been estimated that women with SLE have fewer live births compared to the general population, particularly those with high disease activity. Adverse fetal outcomes in obstetric SLE include fetal loss, IUGR (intrauterine growth restriction), premature birth, premature rupture of membranes, perinatal mortality and rarely neonatal lupus. Although these patients have fewer live births with more pregnancy complications, they may have subsequent uncomplicated pregnancies after a poor outcome. A retrospective study suggests that only 4 months, not the traditional 6 months of disease quiescent SLE prior to pregnancy improves the outcomes. **Conclusion:** SLE pregnancies are considered high risk condition, and should be monitored frequently during pregnancy. Prenatal care of pregnant patients with SLE requires close collaboration between rheumatologist and obstetrician. Planning pregnancy is essential to increase the probability of successful pregnancies.

10954P

A modified murine model for systemic sclerosis

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Introduction: systemic sclerosis is an autoimmune disorder characterized by excessive accumulation of extracellular matrix in organs specially skin and lungs. Animal models are useful in providing clues to study several types of human diseases and for testing new methods of treatment. Here, we show that the induction of scleroderma murine model was successful. **Material and method:** we have induced a murine model for systemic sclerosis by daily injections of bleomycin for 4 weeks in BALB/c female mice. Mice were sacrificed on days 10, 28 and 35. Skin and lung tissues were examined by H&E and masson's trichrome staining. hydroxyproline colorimetric assay were performed to measure the collagen content of the tissues. **Results:** There was a significant increase in collagen accumulation and fibrosis on days 28 and 35. In contrast no fibrosis was seen on day 10. **Conclusion:** We induced scleroderma model which closely resembles human pathology.

10981 P

Prevalence of IA2 anti insulin auto antibody in a group of children in Birjand city, Iran

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Introduction: Type 1 diabetes is a common autoimmune disease and has been increasing during recent decades. Researches support the role of immune system particularly humoral immunity in pathogenesis of the disease and several anti-insulin antibodies have detected in patient's blood even before symptoms emerge. Regarding the predictive value of anti-insulin antibodies, the aim of this study was to evaluate the level of IA2 auto antibody in a group of children and teenagers in the Birjand city of Iran. **Material and Methods:** Demographic data, family history and risk factors were evaluated by means of questionnaire in 330 participants (mean age=10.5, range 6-15 y). IA2 level was measured by ELISA technique in duplicate. **Results:** 2 individuals (0.6%) had type 1 diabetes. Family history of diabetes was positive in 21 of fathers (6.4%), 14 of mothers (4.2) and 4 of both parents (1.2%). Mean of BMI was 22.3 ± 2.4. Seven of participants (2.1) had high level of IA2 antibody (3 boys and 4 girls) among them two had diabetes, two were obese and five had positive family history for diabetes. **Conclusion:** The result of this study showed that IA2 auto-antibody may be useful as a predictive tool for evaluation the risk of type 1 diabetes in high risk children. **Key words:** auto antibody, Diabetes, Insulin, IA2,

10986P

Assessing diagnostic value RM antibody in recognizing immuno-histochemistry colon cancer

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Introduction: Colorectal cancer is an important disease with a large morbidity and mortality and also with increasing health care costs because of widespread of the multi-model therapy and of the new drugs that continue to appear. Assessment of the level of the CEA (carcino-embryonic antigen) expression by immuno-histochemistry methods is very important, as this parameter is widely used in investigation and monitoring of colon cancer and also in many other types of cancer. In this study, the diagnostic value of RM antibody have been evaluated compared to DAKO antibody in order to detection of CEA in colon cancer and normal tissues of other cancers. **Material and methods:** Overall 88 samples involved cancerous (with CEA) as case group and (without CEA) a control group examined by immunohistochemistry methods. This test was performed by tissue section preparation and then according to protocol, tissue slid was prepared, after that RM antibody was used for one groups and DAKO antibody used for another groups. **Results:** Immunohistochemistry methods were used for analysis of 88 samples that cancerous samples had CEA and the control did not had this marker. In this study collectively 89 tissues with CEA and without CEA with immunohistochemistry methods were analyzed. The results were shown that the sensitivity and specificity of RM antibody compared to DAKO antibody were 100 and 100, respectively, on the basis of Mac-Nemar analysis the kapp coefficient was 1. ($p<0.001$) **Conclusion:** The study have shown that RM antibody could be used in diagnosis of CEA in colon cancer. The diagnostic value of RM antibody has highest sensitivity, and specificity of RM antibody was 100%. The results revealed that the RM antibody could be used in immunohistochemistry of CEA in colon cancer and other cancerous tissues. **Keywords:** RM monoclonal antibody, CEA (carcinoembryonic antigen), colon cancer, immunohistochemistry.

11062 P

Regulatory effects of various IFN-β formulations therapy on CXC chemokines CXCL1 and CXCL9 serum levels in relapsing-remitting multiple sclerosis

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Introduction: The multiple sclerosis (MS) is described as a complicated immune system disorder. The MS most frequent symptoms are recurrent myelin losing in paralleled central nervous system (CNS) inflammation. Immunity is considered as a crucial parameter in pathogenesis of MS. Chemokines, as paramount members of immune system, are involved in immune responses. Therefore, this project was aimed to examine and compare the serum levels of CXCL1 and CXCL9 in Iranian relapse remitting MS (RRMS) patients following therapy with IFN-β formulations therapy. **Material and methods:** Clinical specimens were obtained from 100 unrelated healthy controls along with 36, 13, 12 and 9 RRMS patients treated with Cinnovex™, Avonex, respectively. Specimens were taken during 2008-2009 at the Samenol Aeme special disease center, Kerman - Iran. The serum levels of CXCL1 and CXCL9 were measured by ELISA (R&D, UK), in patients and controls, immediately after specimen collection. **Results:** Analysis of the obtained data indicated the levels of CXCL1 and CXCL9, (as members of inflammatory chemokines) were significantly increased in presence of all formulations and it was more up-regulated by Avonex® and with a lesser extent by Cinnovex™. **Conclusion:** Results of this study proposed that Avonex® are much more powerful than Cinnovex™ in regulation of the immune system and the dose of Cinnovex™ probably should be increased to achieve an overt therapeutic response in RRMS patients.

11063 P

Differential systemic levels of CXCL12 and CXCL1 as angiogenesis and CXCL9 and CXCL10 as anti-angiogenesis CXC chemokine's in gestational diabetes mellitus mothers and their neonates

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Introduction: 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 levels of CXCL9, CXCL10, CXCL1 and CXCL12 in GDM mothers and their neonates. **Material and 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 levels of CXCL9, CXCL1, CXCL10 and CXCL12 were measured by ELISA in studied groups. **Results:** The results showed increased levels of angiogenesis chemokine's CXCL1, CXCL12 in parallel with decreased angiostatic chemokine's CXCL9 and CXCL10 neonates delivered from mothers with GDM. The results also showed that the levels of studied CXC chemokine's were 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 the expression of CXC chemokine's in GDM is related with the balance between angiogenesis / angiostatsis phenomenon associated with pregnancy and follows a pattern of inflammatory in pregnant women.

11068P

Investigation of the serum levels of interleukin (IL)-33 and its gene polymorphism in patients with multiple sclerosis

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Introduction: IL-33 is a newly identified cytokine that implicated in the pathogenesis of some diseases. The aim of this study was to evaluate the serum IL-33 levels and a single nucleotide polymorphism (SNP), rs1929992, in its gene in patients with multiple sclerosis (MS). **Material and Methods:** Blood samples were collected from 140 MS patients with various disease pattern and 140 healthy subjects as a control group. The serum concentrations of IL-33 were measured by ELISA and SNP at rs1929992 were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. **Results:** The mean serum levels of IL-33 in MS patients were significantly higher than control group (P<0.001). The mean serum levels of IL-33 were also significantly higher in patients with RRMS, SPMS and PPMS patterns as compared to healthy control group (P <0.006, P<0.001, and P<0.02, respectively). The frequencies of CC genotype and C allele at SNP rs1929992 were significantly higher in patients as compared to control group (P<0.001). In patients with RRMS pattern, the frequency of CT (18.6%) genotype was significantly lower than control group (29.3%, P<0.05). The levels of IL-33 in MS patients with TT genotype or T allele was significantly higher than patients with CC/CT genotypes or C allele (P<0.02). **Conclusion:** These results showed elevated levels of IL-33 in patients with MS that represents that cytokine may be involved in the pathogenesis of MS. The SNP rs1929992 may be associated with MS disease.

11097 P

Vitamin D ameliorates the pathological and clinical symptoms of experimental autoimmune encephalomyelitis

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Introduction: It has been reported that vitamin D has broad anti-inflammatory and immunomodulatory effects. The aim of this study was to evaluate the effects of vitamin D on clinical symptoms of experimental autoimmune encephalomyelitis (EAE) in a mouse animal model. **Material and Methods:** EAE was induced in C57Bl/6 mice by immunization with myelin oligodendroglial glycoprotein mixed with complete Freund's adjuvant. The mice were

intra-peritoneally administered PBS or olive oil in control groups and vitamin D (200 ng every two days) in treatment group, from day +3 to +30. The EAE clinical scores and body weight were evaluated till day 30. **Results:** In this study, the PBS- orolive oil-treated EAE mice showed the clinical symptoms of EAE at days 10 and 9, respectively. The vitamin D-treated EAE group exhibited the clinical symptoms at day 13. The data showed that the PBS- or olive oil treated EAE mice developed a serious inflammation in the CNS, whereas treatment with vitamin D significantly diminished the infiltration events. The mean of body weight in the PBS- olive oil and vitamin D-treated EAE mice was significantly lower than that observed in normal control group after 15 and 17days, for PBS and olive oil treated groups and during 17-30 days post MOG immunization for vitamin D-treated group. However, the mean of body weight in the vitamin D-treated group was significantly higher as compared to PBS-treated EAE mice. **Conclusion:** These results showed that the vitamin D-treated EAE mice exhibited mild signs of EAE, a delay in disease onset and low infiltration of the inflammatory cell into the spinal cord.

11161 P

Study of mesenchymal stem cells frequency in relapse phase of RRMS patients

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory multifocal demyelinating disease of the central nervous system (CNS) that affects predominantly young adults. The autoimmune inflammatory process is believed to be essential for the development of the disease that leads to CNS damage. Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells that can be isolated from many adult tissues. MSCs induce peripheral tolerance and migrate to injured tissues, where they can inhibit the release of pro-inflammatory cytokines and promote the survival of damaged cells. The aim of this study was to investigate the frequency of MSCs in peripheral blood of RRMS patients in relapse phase. **Material and Methods:** In this report multicolor flow cytometry assay was used to analyze the MSCs frequency in peripheral blood, as a population of CD45− CD34− CD90+ CD105+ cells in RRMS patients whose diagnosis was definite and was not corticosteroid treated within the last 6 months(n=10) compared to sex and age-matched healthy control group(n=10). **Result: :** In this report there was a significant difference in the percentage of MSCs CD45-/CD34-/CD90+/ CD105+ between the groups. Patients with RRMS were shown to have higher proportions of circulating CD45-/CD34 /CD90+/CD105+ compared to the controls. **Conclusion:** Increasedcirculating MSCs in RRMS patients might be because of mobilization by cytokine stimulation to reduce inflammation.

11163P

Determining the Prevalence of16S rRNA Genes of *Helicobacter pylori*, Campylobacter and Bifidobacteria in the Stool of Patients with Celiac Disease and Its Relationship withHLA-DQ2

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Introduction: Celiac disease is an intestinal problems associated with safety which is characterized by intolerance to gluten. The risk of celiac disease is strongly related to human leukocyte antigen-DQ2 (HLA-DQ2). The purpose of this study was to determine the prevalence of 16SrRNA genes of *H. pylori*, campylobacter and bifidobacteria in the stool of celiac disease and its association with the HLA-DQ2.**Material and methods:** Blood and stool samples were collected

from 40 patients with celiac disease and 40 healthy people. Blood samples were tested for HLA-DQ2 by HLA serological typing method. DNA was extracted from stool samples. 16SrRNA genes of *H. pylori*, campylobacter and bifidobacteria were amplified by stool PCR. **Results:** 60% (n=24) of patient group had HLA-DQ2 genotype. The results of stool PCR for 16SrRNA genes of *H. pylori*, campylobacter, bifidobacteria were 66.66%, 4.16% and 66.66% in these people, respectively. Also 10% of the control group was positive for HLA-DQ2 genotype, and the result of PCR for 16SrRNA gene of *H. pylori* in these patients was 75%. While the amplification of 16SrRNA gene of bifidobacteria was positive in all these subjects, but the 16SrRNA gene of campylobacter was not found in none of them. **Conclusion:** In people with celiac disease and positive HLA-DQ2 genotype, the prevalence of 16SrRNA genes for *H. pylori* and bifidobacteria was less than healthy individuals. In other words, the presence of rod-shaped bacteria in patients with celiac disease was lower.

11166P

The relationship between levels of IL-12 and IL-4 in diabetic patients

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Introduction: Diabetes is a common and debilitating disease that is increasing at an alarming rate due to individual lifestyle, by prevalence about 5% in Iran. The disease is diagnosed by fasting blood sugar (FBS) test. The purpose of this study was to investigate FBS fluctuations with levels of IL-12 and IL-4 in diabetic subjects. **Material and methods:** The present study had two case and control groups; there were 45 patients with diabetes in case group, whose level of FBS was more than 125. Blood samples were taken from all subjects. FBS and IL-4 and IL-12 were tested by Nephelometry and ELISA method according to the manufacturer's kit. **Results:** The average amount of FBS was 175 in diabetic group and 95 in the control group and in 55.5% of case group had IL-4 and 58.5% of people had higher IL-12. **Conclusion:** According to the obtained data, the average serum levels of IL-4 and IL-12 in patients with diabetes, whose FBS was higher than 125, was more than healthy people. Statistical analysis by T-Test showed that IL-12 levels had significant statistical relationship with fluctuations in the FBS in diabetic patients. But there was no significant relationship between the increase of FBS and IL-4 levels in people with diabetes. **Keywords:** FBS, IL-12, IL-4.

11167 P

The Relationship between Levels of Hs-CRP and IFN-Gamma in Diabetic Patients

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Introduction: Diabetes is one of the most common diseases in the world. Its prevalence has been reported in Iran about 5% and is increasing at a rate of 1% per year. The disease is diagnosed by FBS and HbA1C tests.This study aimed to evaluate the fluctuations of HbA1C through levels of IFN-gamma and Hs-CRP in people with diabetes. **Material and methods:** In this case-control study, the case group consisted of 45 patients with diabetes who had HbA1C level of more than 6 AU/ML and 45 healthy individuals were selected. Blood samples were collected from all subjects. The tests of HbA1C, Hs-CRP and IFN-γ were respectively carried out by Nephelometry and ELISA in accordance with the instruction of manufacturer's kit. **Results:** The average HbA1C in the case group was equal to 8.8 AU/ML and in the control group was 5.5 AU/ML. In the case group, about 84.7% of subjects had higher than normal levels of IFN-γ and about 58.7% had higher than normal levels of Hs-CRP. **Conclusion:** The data showed that the mean serum level of IFN-γ and Hs-CRP in diabetic patients with higher HbA1C was more than healthy people and there was significant relationship, statistically. **Keywords:** FBS, HbA1C, hs CRP, IL-12, IL-4, IFN- γ.

11297 P

Tolerogenic dendritic cells produced by lentiviral-mediated CD40- and interleukin-23p19-specific shRNA can ameliorate experimental autoimmune encephalomyelitis by suppressing T helper type 17 cells

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Introduction: Down-regulation of soluble or membrane-bound co-stimulatory molecules by RNAi in dendritic cells can prevent the activation of immune responses. **Material and methods:** 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 interleukin (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 myelin oligo-dendrocyte glycoprotein (MOG)35–55-specific T cells for production of IL-10 compared to CD40LV-DCs, p19LV-DCs and BMDCs transduced with control lentiviral vector (CoLV-DCs). Moreover, injection of transduced CD40+p19LV- BMDCs in EAE mice resulted in more reduction in clinical score, significant reduction in IL-17 or increased production of IL-10 by mononuclear cells derived from the lymph nodes or spinal cord compared to CoLV-DCs-treated EAE mice**. 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:** CD40, dendritic cell, IL-23p19, lentiviral vector, RNAi

12352P

Down-regulation of Immunosuppressive Molecules, PD-1 and PD-L1 but not PD-L2, in the Patients with Multiple Sclerosis

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Introduction: Programmed cell death-1 (PD-1) and its ligands, PD-L1 and PD-L2, have been regarded as important immune system regulatory molecules. The aberrant expression of the molecules has been related to several autoimmune disorders. This study is aimed to assess the mRNA expression level of PD-1, PD-L1, and PD-L2 molecules in the Peripheral Blood Mononuclear Cells (PBMCs) from Multiple Sclerosis (MS) patients. **Materials and methods:** PBMCs were isolated from the whole blood of 50 MS and 50 healthy individuals. The total RNA content of the leukocytes was extracted. Then, cDNA was synthesized from the RNA of the cells. Afterwards, quantitative analysis of PD-1, PD-L1 and PD-L2 was carried out through Real Time PCR using the TaqMan gene expression assays. **Results:** Relative expression of PD-1 and PD-L1 in PBMCs from MS patients was significantly lower compared with the healthy control group (P=0.003 and 0.012, respectively). However no significant difference was observed in the expression level of PD-L2 between patients and healthy individuals. Relative expression of PD-1 correlated with EDSS score of the patients (r=-0.763, P=0.008). **Conclusions:** Downregulation of the immunosuppressive molecules, PD-1 and PD-L1, may imply that over-activation of immune cells in Multiple Sclerosis occurs through signaling dysfunction of these molecules and PD-L2 plays no important role in this context. **Keywords:** PD-1, PD-L1, Multiple Sclerosis, Gene Expression

12353P

Evaluation ofAD-MSC (Adipose derived –Mesenchymal stem cells) as a vehicle for IFN-β delivery in Experimental Autoimmune Encephalomyelitis

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Introduction: As it is well-known IFN-β is widely used as a disease modifying agent for treatment of Multiple sclerosis. However, the therapeutic efficacy of IFN-β is limited by raising neutralizing antibodies against it after a period of systemic administration. Adipose derived –Mesenchymal stem cells (AD-MSCs) are unique ones, which have been representing a promising cell-based therapy for autoimmune diseases, particularly MS (Multiple Sclerosis) and its related animal model, the experimental autoimmune encephalomyelitis (EAE).**Material and Methods:** In the present study, AD-MSCs was used as a therapeutic vehicle and engineered with a lentiviral particle (VP) to express murine interferon beta (MSCs-VP/IFNβ) and its effects on EAE was examined. **Results:** MSCs-VP/IFNβ treated group dramatically improved the induced Treg cell production ($P<0.005$). The expression of anti-inflammatory cytokines such as IL-10 and TGF-β significantly was increased in this group compared to controls. Taken together, the results indicated that using AD-MSCs along with IFN-β as an anti-inflammatory agent in EAE, could play a critical role in down-regulating the inflammatory condition through the up-regulation of IL-10 and TGF-β and Treg cell induction, and provide evidence supporting the stem cell based therapies. **Conclusion:** On the basis of these findings, development of new therapeutic approaches as well as understanding the mechanisms by which IFN-β acts in the MS disease are crucial in finding new ways to increase the duration of IFN-β effects during treatment.

12358P

Numerical status of Circulatory Regulatory T Cells and Plasmacytoid Dendritic Cells in Type1 Diabetes

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Introduction: Type1 Diabetes (T1D) is the result of the autoimmune destruction of insulin-producing beta cells. Having considered the matter that regulatory T cells (Tregs) and plasmacytoid dendritic cells (PDCs) act as mediators of peripheral tolerance, the possible alterations of such cells were investigated in peripheral blood of patients with T1D compared to normal individuals. This comparison may lead to a better understanding of the immunopathogenesis processes involved in T1D. **Material and Methods:** 92 participants, including 49 patients with T1D and 43 healthy controls were studied. 3ml of blood was collected from all participants. After isolating PBMCs, plasmacytoid dendritic cells and 2 subtypes of Tregs, $CD4^+CD25^+FoxP3^+$ and $CD8^+CD28^-$ cells were counted by 3-color flow cytometry. The association between enumeration and T1D was studied by multivariate regression and discriminate function models. **Results:** The frequency of $CD4^+CD25^+FoxP3^+$ Tregs ($P=0.038$) and PDCs ($P=0.039$) in the peripheral blood of diabetic patients was less than that of healthy subjects. Having compared some models containing different cells and their combinations, no profound explanation was found for each subset or their combinations in T1D.**Conclusion:** The decrease of $CD4^+CD25^+FoxP3^+$cells and PDCs in diabetic patients may suggest their role in the onset or development of the disease. Therefore, it's likely that their pharmacologic stimulation direct immune responses towards tolerance and prevent the development or onset of diabetes in susceptible individuals. **Keywords:** Diabetes mellitus, plasmacytoid dendritic cells, $CD4^+CD25^+FoxP3^+$ regulatory T cells, $CD8^+CD28^-$ regulatory T cells.

12371P

Change Cytokine profile and immune cells population following exposure to the venom from Hemiscorpius lepturus scorpion venom under in vitro conditions.

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Introduction: A previous clinical study has shown increases of TNF-□□IL-1, IL-6 and IL-8 levels in serum of patient envenomed by H lepturus scorpion. The aim of the present study was to assess the changes in various cytokine levels and immune cells population following in vitro exposure to this venom. **Material and Methods:** Peripheral blood mononuclear cells (PBMC) were exposed to 1, 3 and 5□g/ml of H lepturus venom for 24 hr. Control samples were treated similarly in the absence of the venom. The cytokine levels in the supernatant media were measured by ELISA. PBMC Based on specific surface markers, identification and quantization of TC, NK, Th, activated and regulatory T cells and GITR cells were carried by flow cytometry method (BD, USA). The collected data were analyzed by Excell software. **Results:** IFN-□ and IL2 showed significant 2.5 and 10 fold increase respectively. While IL10, showed a significant increase (by 4.5 fold) and IL-5 and IL-3 by 2 fold. TNF-□, IL-12p70 increased by 4 fold. Overall trends of changes following exposure to H Lepturus venom in both whole blood and PBMCs had similar trends. Following exposure to H lepturus venom the cytometric results for PBMC showed the following changes: a reduction of CD4/CD8 ratio (from 3.14 to 2.08), reduction of CTL cells (from 1.46 % to 089 %); regulatory cells were also reduced from 1.06 % to 0.89%. While a significant increase in Th-activated cells (from 0.21 to 0.65 %) and GITR cells (from 1.94% to 3.03%). No significant changes were observed for NK and B cells. **Conclusion:** Besides its cytotoxic properties which were shown by the increase in TNF-□ and IL-12p70; this venom has direct immune suppressive and possible indirect immune stimulating actions which may be utilized in the development of novel agents that may be identified and used for treatment of different immune mediated illnesses.

12373P

Investigation of the Human FCRL 1, 2, and 4 Gene Expressions in Patients with Rheumatoid Arthritis

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Introduction: Rheumatoid arthritis (RA) is a common autoimmune disease characterized by inflammation of the synovial joints. The Fc receptor-like (FCRL) molecules have recently been shown to contribute to the pathogenesis of certain autoimmune disorders. **Material and methods:** In this study, the expression levels of FCRL 1, 2 and 4 in peripheral blood mononuclear cells from RA patients using real-time polymerase chain reaction (PCR). **Results:** The mRNA of these molecules was detected in 44.4% (FCRL1), 53.3% (FCRL2) and 31.1% (FCRL4) of patients.

Comparatively, 31.1% (FCRL1), 51.1% (FCRL2) and 26.6% (FCRL4) of controls expressed these genes. There were no significant differences in FCRL 1, 2 and 4 positivity between the patients and controls. Analysis of gene expressions in FCRL positive patients demonstrated that FCRL1 and FCRL2 expressed at a relatively similar level in patients compared to normal subjects (P>0.05). However, there was a lower FCRL4 gene expression in patients compared to controls (P<0.001). Clinical and paraclinical evaluations indicated no significant differences between FCRL positive and FCRL negative patients. However, FCRL positive patients had a significant positive correlation between FCRL2 expression and the erythrocyte sedimentation rate (ESR; P<0.001, R=0.66), anti-cyclic citrullinated peptide (CCP) antibody (P=0.033, R=0.44) level and disease activity score (DAS28, P=0.016, R=0.49). There was a negative association with age (P=0.009, R= -0.52). **Conclusion:** a lower level in FCRL4 mRNA and association of FCRL2 expression with inflammatory markers and disease activity were observed, which suggested the contribution of these molecules to RA pathogenesis.

12378P

Effect of Nigella Sativa oil on the recovery of oral and genital ulcers in Behcet's disease

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Introduction: This study demonstrates the effect of topical Nigella Sativa oil usage on improvement of oro-genital ulcers in Behcet's disease. In this double blind investigation,40 patients were enrolled in two groups. First 20 petiants, topical used Nigella sativa oil with 10% glycerin three times daily. The next 20 petiants, only used glycerin. Evaluation were done for about 4 days after 8 days of treatment. Then results were recorded and compared concurrently with clinical observations in the both groups. Nigella Sativa oil, act in recovery of oral and genital ulcers. Effectiveness of this oil is due to it's anti-inflammatory properties. **Material and Methods:**500 gr Nigella Sativa were placed under 500 bar pressure of hydraulic compressor system and oil was extracted from 0.4-micron filter paper. Then reverse thin layer chromatography was used to determine exctracted fixed oil. Dilution of fixed oil were performed by glycerin with ratio of 01:10. Results**:** Results indicate that Nigella sativa oil have an impact on the rate of oral wounds healing. So after 8 days usage of this oil, ulcers were recovered. Although it may still be some residual scarring that with consuming more oil will completely disappear. Glycerin can reduce pain in the short term but does not effect the rate of healing. although, due to the distincts location of the oral and genital ulcers and differences between sexes, results were diverse. **Conclusion:** According to the results, rate of improvement in genital ulcers is expected to be slower.

12402P

Evaluation on alteration of Oncostatin M (OSM) plasma levels in multiple sclerosis

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Introduction: Oncostatin M (OSM) is a cytokine belonging to Interleukin 6 family (IL-6), expressed by T-cells and monocytes, affecting jak1, jak2, Tyk2, STAT3 signaling pathways resulting the implication of inflammation. OSM has also got an impact on neural precursor cell activity in central nervous system (CNS). OSM active role in CNS and its secretion by infiltrated cells suggests a probable role in multiple sclerosis (MS). Thus, OSM plasma level concentrations were evaluated in relapsing-remitting multiple sclerosis (RRMS) patients compared to the healthy subjects. **Material and Methods:** As a case-control study, 60 patients with clinical symptoms of multiple sclerosis

and 30 healthy subjects without any neuro-inflammatory disease background were randomly selected for this study. Enzyme Linked Immunosorbent Assay (ELISA) procedure was conducted and the data got to the statistical analysis applying SPSS 19 (Independent t-test and Spearman Correlation tests were manipulated). **Results:** The results of this test showed that the plasma level of OSM in the group of patients (634.68 ± 658.43(pg/ml)) was lower than the plasma level of OSM in healthy people (860.57 ± 891.16(pg/ml)), but this difference cannot be significant in terms of the statistical tests (P-Value > 0.05).**Conclusion:** OSM plasma levels do not play a major role in RRMS in population-based studies, and EDSS is an independent variable from OSM plasma concentration.

Tumor Immunology

Oral Presentations:

7700O

Evaluation of IL-17RB expression and function in CD3+ and CD19+ cells in peripheral blood mononuclear cells of chronic lymphocytic leukemia patients

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Introduction: The presence of cytokines in tumor cells surrounding microenvironment greatly influence the progression or suppression of tumors. IL-17E is produced by a variety of cells including immune cells and non-immune cells. IL-17E is involved in the initiation of Th2 cells mediated immunopathogenesis. Signal transduction of IL-17E is beginning through a heterodimeric receptor complex that composed of IL-17RA/IL-17RB. Therefore, the aim of this study is to investigate the expression and biological function of IL-17RB on the CD19+ and CD3+ cells of chronic lymphocytic leukemia (CLL) patients compared to healthy subjects.**Materials and Methods:** Initially IL-17RB protein expression on T cells and B cells was determined in peripheral blood mononuclear cells (PBMC) of 5 patients with CLL and 5 healthy subjects using flow-cytometry. Then PBMC of patients and normal subjects cultured in RPMI-1640 medium supplemented with 10% human AB+ serum (HABs) with recombinant human IL-17E (IL-25) for 72 hours and expression of IL-17RB evaluated on CD3+and CD19+surfaces using flow-cytometry. **Results:** our data indicated that IL-17RB expression on CD3+ PBMCs of CLL patients were significantly lower than normal subjects. After treatment with hIL-17E we found increased IL-17RB levels in CLL CD3+and CD19+ cells compared with normal CD3+and CD19+ cells. (P value <0.05) **Conclusion:** According to our findings, IL-17RB on CD3+ and CD19+ cells of CLL patients was significantly higher than CD3+ and CD19+cells of normal subjects in the presence of IL-17E. IL-17E may have induced proinflammatory microenvironment and increased tumor cells viability by expressing inflammatory receptors such as IL-17RB.

9748O

Frequency and Functional Characterization of Exhausted $CD8^+$ T-cells in Chronic Lymphocytic Leukemia

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Introduction: Exhausted T-cells are a group of T lymphocytes with low functional properties which are induced during some chronic pathological conditions. These cells are recognized with expression of multiple inhibitory receptors such as programmed death-1 (PD-1) and T-cell immunoglobulin and mucin domain-3 (Tim-3). In this study, the phenotypic and functional properties of exhausted $CD8^+$ T-cells were investigated in chronic lymphocytic leukemia (CLL). **Materials and Methods:** Twenty-five untreated Iranian CLL patients and fifteen healthy controls were included. Frequency of $CD8^+/Tim\text{-}3^+/PD\text{-}1^+$ exhausted cells was determined by three-color flow cytometry. For functional analysis, monocyte-depleted PBMCs were applied on magnetic separation columns to positively isolate T-$CD8^+$ lymphocytes. Purified $CD8^+$ T-cells were then stimulated with PHA and PMA/ionocymin to assess their proliferative responses and cytokine production by MTT and ELISA methods, respectively. Cell cytotoxicity of isolated $CD8^+$ T-cells was determined using CD107a degranulation assay. **Results:** The proportion of exhausted $CD8^+$ T-cells was significantly higher in CLL patients compared to controls ($p=0.04$). Isolated $CD8^+$ T-cells from CLL patients showed functional defects in proliferation ($p<0.001$), cytotoxicity ($p<0.001$), and cytokines production. While the levels of IL-2, TNF-α and IFN-γ were significantly lower in CLL patients ($p<0.001$), IL-10 concentration was higher in the patients group ($p<0.001$). CLL patients with progressive clinical stages showed higher levels of exhausted $CD8^+$ T-cells. **Conclusion:** Our data indicates that $CD8^+$ T-cells in CLL patients are exhausted and exhibit defects in proliferation, cytotoxicity, and production of pro-inflammatory cytokines. Targeting immune inhibitory receptors to restore the function of exhausted T-cells could be helpful in immunotherapy of CLL.**Keywords:** Exhausted T-cells, Tim-3, PD-1, Chronic lymphocytic leukem

11251O

The chemokine CXCR7 is selectively expressed on acute B-lymphoblastic leukemia cells and mediates their trans endothelial migration

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Introduction: The function of chemokine stromal cell-derived factor (SDF)-1 and its receptor CXCR4 in the pathobiology of hematological malignancies is known, but the role of another, receptor for SDF-1, CXCR7, has not yet been defined. **Methods and materials**: Using RT-PCR, flow cytometry and immunohistochemistry analysis; we screened the expression of CXCR7 in 14 hematological cell lines as well as primary cells from patients with acute lymphoblastic leukemia (ALL). Migration of the cells was determined by chemotaxis. **Results**: Our data showed that CXCR7 was strongly expressed in 4 out of 5 tested B cell lines (Raji, NC-37, NALM6, Ramos and REH) where as 2 T cell lines (Jurkat and CEM) and 2 of 7 myeloid evaluated cell lines weakly presentedCXCR7. Moreover, CXCR7 was also strongly expressed in peripheral blood cells and bone marrow biopsies of all 9 patients diagnosed with B precursor ALL. In contrast, its expression was very weak in normal lymphocytesand even negative in T precursor ALL samples. Next we examined whether CXCR7 plays a role in the chemotaxis of these cells and found that Anti-CXCR7 antibody did not inhibit SDF-1-induced chemotaxis. However, we found that Anti-CXCR7 antibody inhibited the transendothelial migration of B cell lines and primary B precursor ALL cells in a dose-dependent manner. **Conclusion:** we demonstrated that CXCR7 is expressed on B lymphoblasts, and the SDF-1/CXCR7 axis plays a crucial role in the trafficking of these cells, indicating that CXCR7 could be a therapeutic target in patients with B precursor ALL.

11314O

CD8+ lymphocyte subsets in Tumor Draining Lymph Nodes of Bladder Cancer Patients

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Introduction: Cytotoxic CD8+ T cells, as an essential part of the adaptive immune system play pivotal roles in anti-tumor immune responses. It is well documented that cytokine expression profiles and activation statues of these cells during anti-tumor immune responses, could determine the outcome of host-tumor interaction. Therefore, in this study, we aimed to evaluate CD8+ lymphocyte subsets in tumor draining lymph nodes of patients with bladder cancer. **Materials and Methods:** 30 untreated patients with bladder cancer recruited in the present study. Mononuclear cells were isolated from draining lymph nodes using Ficoll-Hypaque and activated with PMA/Ionomycine in the presence of Golgi inhibitors. The cells were then permeabilized and stained with appropriate antibodies against CD8, IFNg, IL-17 and IL-4 markers. Data were collected on a four-color flow cytometer and analyzed by Cell Quest Pro software. **Results**: Our results demonstrated that the percentage of Tc1, Tc2 and Tc17 subsets did not show any significant difference in patients with various pathological statuses. Despite no difference in the frequency of Tc2 cells, the mean expression of IL-4 in this subset significantly increased in the patients with high histological grade comparing to those with low grade (P=0.009). Furthermore, the percentage of CD8+ lymphocytes expressing both IFNg and Il-17 was also higher in these patients (P=0.01). **Conclusion:** Taken together, our data suggested that increased expression of IL-4 by Tc2 lymphocytes, in addition to elevated frequency of IL-17 and IFNγ double-positive CD8+ inflammatory lymphocytes, may promote bladder cancer progression.**Keywords:** Bladder cancer, Lymph node, Tc1, Tc2, Tc17

12384O

Detection of CD4+CD25-FoxP3+ T cells producing Interleukin -2, IL-10 and Interferon -γ in tumor draining lymph nodes of colorectal cancer patients

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Introduction: CD4+CD25-FoxP3+ cells are a newly recognized subset of T cells which was first reported in autoimmune diseases. In our previous study we detected this subset in tumor draining lymph nodes (TDLNs) of patients with breast cancer. As little is known about their function in TDLNs of cancer patients, in this study, their frequency and also ability to produce Interleukin (IL)-2, IL-10 or Interferon (IFN)–γ in TDLNs of colorectal cancer (CRC) patients were investigated. **Materials and Methods:** Mononuclear cells were isolated from lymph nodes of 13 patients with CRC using Ficoll-Hypaque gradient centrifugation. Cells were stimulated in vitro and stained with CD25, CD4, FoxP3, IFN-γ, IL-10 and IL-2 or isotype matched antibodies and subjected to flow cytometry. **Results:** Our results showed that CD25-FoxP3+ and CD25+FoxP3+ cells comprised 5.3 ± 1.7 % and 10.5 ± 2.6 % of CD4+ T cells in TDLNs of CRC patients respectively. Of CD4+CD25-FoxP3+ cells, 13.9 ± 3.4% produced IFN-

γ, 28.9 ± 7.9% expressed IL-2 and 2.5 ± 1.1% produced IL-10. The frequency of IFN-γ or IL-2 producing cells among CD4+CD25-FoxP3+ cells was significantly higher than in CD4+CD25+FoxP3+ cells (P<0.0001) while no difference was seen between two subsets in terms of IL-10 production (P=0.18). **Conclusion:** CD4+CD25-FoxP3+ cells were presented in TDLNs of colorectal cancer patients. In comparison with CD25+FoxP3+ Treg cells, significantly higher percentages of CD25-FoxP3+ cells were capable of producing IL-2 or IFN- γ. More phenotypic and functional evidences are required to classified CD4+CD25-FoxP3+ cells as a subset of regulatory T cells. **Key words:** CD4+CD25-FoxP3+ cell, cytokine, colorectal cancer, lymph node

Poster Presentations:

3475P

Tumor Necrosis Factor-α Engineered Mesenchymal Stem Cells AffectCytokine Responses in Tumor Model

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Introduction: The importance of specific cytokines in stimulation of immune system toward anti- tumor immunity suggests possible exploitation or induction of such cytokines. Engineering the mesenchymal stem cells (MSCs) with tumor necrosis factor-α (TNF-α) could be used for enhanced cancer therapy. **MaterialsandMethods:** tumor induction in BALB/c mice followed by injecting 4T1 cells subcutaneously. Mice in each test groups were co-treated with DCs and/or (TNF-α)-MSCs,on day 7 after tumor induction. The controls included untreated, empty vector-MSCs, DCs-lipopolysaccharides (LPS) and immature DCs (iDCs) mice groups. Then, TNF-α, TGF-β, IL-4, IL-6, IL-12, IL-10 and IFN-γ cytokines levels from murine splenocytes were assessed by ELISA kit. **Results:** Comparing to the corresponding controls, our findings showed induction of T helper 1(*Th1*) induction and promoting cytokines(TNF-α, IL-12,IFN-γ, IL-6) as well as suppression of *Th2(IL-4)* and *Treg* responses(TGF-β, IL-10) in test groups, which led to a valuable anti-tumor immune response .**Conclusion:** Our study demonstrates that concomitant genetic modification of MSCs with TNF-αled to anti-tumor immunity response in presence of DCs.

7632P

Interleukin-6 (IL-6) productionandsecretion in human lung cancer cell lines

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Introduction: Emerging evidences indicated that Interleukin-6 (IL-6) is an important mediator of inflammation that attenuates immune response to various cancers. Increased sera levels of IL-6 has been reported in different types of

malignant cancers. The aim of our study was to determine the possible secretion of IL-6 by a range of lung cancer cell lines in culture media. **Materials and Methods**: Initially, two-dimensional gel electrophoresis coupled to liquid chromatography mass spectrometry were used to identify the secretome of large cell lung cancer cell lines QU-DB and Mehr-80. **Results:** we found that Mehr-80 (a neuroendocrine variant of large cell lung cancerthat was established from the pleural effusion of a 40-year-old Iranian woman), secreted IL-6 in culture media. However, QU-DB (a primary lungtumor from a 70 year-old man) did not secret IL-6. **Conclusions:** Our results indicate that IL-6 is secreted by a neuroendocrine variant of large cell lung cancer with poor prognosis. Investigation of IL-6 in secretome of other lung cancer lines by ELISA is underinvestigation. **Key words:** Interleukin-6 (IL-6), large cell lung cancer, QU-DB, Mehr-80

9745P

Increased Efficacy of a Dendritic Cell-Based Therapeutic Cancer Vaccine with Adenosine Receptor Antagonist and CD73 Inhibitor

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Introduction: On the basis of the major role of dendritic cells (DCs) in initiating immune responses, a range of approaches has been established using DCs to induce the immune response against various cancers. However, the presence of immune suppressor mediators such as adenosine in the tumor microenvironment reduces the efficacy of this method. In this study, we investigated whether blockade of the A2A adenosine receptor with a selective antagonist and a CD73 inhibitor may lead to an increased efficacy of a dendritic cell-based therapeutic cancer vaccine. **Method &Material:** Mice injected with 4T1, received bone marrow derived dendritic cell and drugs. 24 hours after the last injection, mice killed and assays were performed. Tumor growth, specific anti-tumor immune response and survival studied in case and control groups. **Result:** According to the findings, this therapeutic combination resulted in slowed tumor growth, prolonged survival of tumor-bearing mice, and improved specific antitumor immune responses. **Conclusion:** In the current study, we suggested that targeting cancer-derived adenosine, improves DC-based cancer immunotherapy outcomes.

9749P

T-CD4$^+$ Lymphocytes are Exhausted and Display Functional Defects in Chronic Lymphocytic Leukemia

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Introduction: Exhausted T-cells are a subset of both CD4$^+$ and CD8$^+$ T-cells that are developed from repeated antigen exposure during chronic infections and malignant tumors with increased expression of inhibitory molecules,

such as T-cell immunoglobulin and mucin domain-3 (Tim-3) and programmed death-1 (PD-1). In this study, the frequency and functional properties of exhausted T-CD4$^+$ lymphocytes were evaluated in chronic lymphocytic leukemia (CLL). **Method &Material** PBMCs were obtained from 25 untreated CLL patients and 15 healthy volunteers. Frequency of CD4+/Tim-3+/PD-1+ T-cells was measured by three-color flow cytometry method. For functional analysis, CD4$^+$ T-cells were positively isolated from monocytes-depleted PBMCs using magnetic bead separation column. Isolated T-CD4$^+$ lymphocytes were then stimulated with PHA and PMA/neomycin to assess their proliferative responses and cytokines production by MTT assay and ELISA, respectively. **Results:** The proportion of CD4$^+$/Tim-3$^+$/PD-1$^+$ T-cells was significantly higher in CLL patients than that of normal controls ($p<0.0015$). Isolated CD4$^+$ T-cells from CLL patients showed lower proliferative responses ($p<0.0001$) and production of pro-inflammatory cytokines compared to healthy controls ($p<0.05$ for IL-2, $p<0.0001$ for IFN-γ, and $p<0.0001$ for TNF-α). Moreover, IL-10 was highly produced by CD4+ T-cells from CLL patients ($p<0.0001$). The number and functional properties of exhausted CD4+ T-cells were associated with disease progression. **Conclusion:** In this study, CD4$^+$ T-cells from CLL patients showed functional and phenotypic defects. Since the exhaustion phase of T-cells is a reversible and transient step, so targeted therapy and blocking of immune inhibitory molecules could be a promising tool to restore the host immune responses against CLL leukemic cells.

Keywords: Exhausted T-cells, Tim-3, PD-1, Chronic lymphocytic leukemia

9764P

Generation of stable transgenic cell line expressing recombinant CD24

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Introduction: CD 24 is a small heavy glycosylated protein which is overexpressed in numerous cancerand cancer stem cells. So, CD24 can be a potential target in treatment of cancer. The considerable amount of antigen is needed for identification of new special antibody by display techniques *e.g.* ribosome and phage display. So, generation of stable and high level antigen producing cell line can be useful in immunological researches. Chromosomal integration of transgene is one of the ways to generate of stable cell lines. In this study, we used 18SrRNA gene for site specificintegration of CD24 gene by homologues recombination. **Material and Methods:**A construct coding CD24 extracellular domain and neomycin (G418) resistance gene were cloned in pEX-A plasmid which has a homologues arm (18S rRNA sequence) in both sides of multiple cloning site. The construct with homologous arms was amplified by PCR and transfected inCHO cell line by lipofectamin2000. After 72 hours G418, 400µg/ml was added to cultures and the integration and expression of the construct were verified. **Results:** The cloning of construct was confirmed by PCR and restriction analysis. Also, the homologous recombination and CD24 expression were confirmed by PCR,RT-PCR and western blot analysis, respectively.**Conclusion:**Our findings show 18S rRNA gene is a good candidate for replacement of desired gene in order to generate stable and high level gene expressing cell line.

9814P

Galectin-9 Expression Pattern in Patients with Gastric Cancer and Peptic Ulcer Diseases

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Introduction: Chronic inflammation and dys-regulation of the immune system mechanisms are now well established as primary triggers of gastric cancer. Galectin-9 (Gal-9) is well-known as stimulator and inducer of cell aggregation, cell adhesion, and apoptosis of tumor cells. Since Gal-9 was introduced as the main ligand of the immune system inhibitory receptor, T-cell immunoglobulin and mucin domain protein-3 (Tim-3), the expression pattern of this molecule was studied in biopsy sections obtained from gastric cancer and peptic ulcer patients. **Material and Methods:** Gastric biopsies were obtained from 46 patients with gastric cancer, 44 patients with peptic ulcer disease and 41 normal subjects who underwent endoscopy for evaluation of their gastric problems. Rapid urease test and H&E staining of biopsy samples were conducted for detection of Helicobacter pylori infection. Total RNA was extracted from all tissues and used for cDNA synthesis. Relative expression of Gal-9 mRNA was determined by Real-time PCR using β-actin as a housekeeping gene. **Results:** Gal-9 was similarly expressed in all three studied groups. No statistical difference was found for Gal-9 expression between gastric cancer patients and normal subjects (p=0.30) and also between peptic ulcer group and normal individuals (p=0.16). No correlation was found between Gal-9 expression and age, gender or tumor grade. In addition, Helicobacter pylori infection was not associated with Gal-9 expression (p=0.51). **Conclusion:** Similar expression of Gal-9 in gastric tissues from patients with gastric cancer, peptic ulcer and also normal individuals suggested no possible role forthis molecule on tumorigenesis and immunoregulatory mechanisms in this malignancy.**Keywords:** Gastric cancer, Peptic ulcer disease, Galectin-9, Helicobacter pylori

9825P

Effect of leukemia inhibitory factor upon NKG2D mRNA expression and presentation on NK cells

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Introduction: Natural Killer (NK) cells are one of the most important natural immune cells that play a crucial role in immune responses against microorganisms and tumors through cell surface receptors. Among these receptors, natural killer group 2, member D (NKG2D) receptor plays a key role in the cytotoxic function of the NK cells. Leukemia inhibitoryfactor (LIF) is a multi-functional cytokine secreted from cells such as lymphocytes and hepatocytes. **Material and methods:** This study aimed to evaluate the effect of LIF on NKG2D receptors expression and presentation on NK cells. For this purpose, peripheral blood mononuclear cells from healthy volunteers were isolated and the effect of LIF on NKG2D receptors expression and presentation was investigated after 12, 24, and 48 h of incubation using flow cytometery and real time- PCR. **Results**: After periods of 12, 24, and 48 hr, LIF reduced the expression and presentation of NKG2D receptors in NK cells. **Conclusion:**The results suggested that this cytokine can modulate the body's immune response through suppression of NKG2D receptors expression and presentation in NK cells.

9839P

Th22 cells in colon cancer development and progression

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Introduction: T helper 22 (Th22) lymphocytes are new players in immune response that are involved in inflammatory diseases, but their roles in the immunopathogenesis of tumor microenviroment is presently unknown. This study is planned to investigate the profile of intra-tumoral Th22 cells in colon cancer (CC) patients. **Material and Methods:** Thirty newly-diagnosed colon cancer patients were included in this study. Tumor tissues and non-tumor tissues (away from local tumor, as control sample) were minced from patients and cellsuspensions were passed through a cell strainer. After isolation, cells were cultured in the presence of cell stimulator, after 4h the cell suspensions were stained with labeled specific antibodies (CD4-FITC, IL-22-PE, IFN-γ-percep5.5 and IL-17-APC). Then, using BD flowcytometry, the expression levels of Th22($CD4^+IFN\text{-}\gamma^-IL\text{-}17^-IL\text{-}22^+$) cells were determined and flow cytometry data was analyzed with Flowjo software. **Results:** The percentage of intra-tumoral Th22 cells was significantly increased in tumor tissues compared with that in non-tumor tissues ($P < 0.001$). However, the percentage of Th22 cells was significantly higher in advanced stage III–IV of tumor versus early stages I–II ($P < 0.01$). Our results indicate that the increased expression of intra-tumoral Th22 cells were positively correlated with tumor progression and staging. **Conclusion:** This results suggesting that intra-tumoralTh22 cells may play important role during proliferation, progression and invasion of colon cancer. However, targeting Th22 cells may have potential therapeutic efficacy in patients with CC. **Keyword:** Th22 cells, colon cancer, tumor microenvironment, intratumoral

10831P

Secondary metabolites extracted from a local strain of *Streptomyces* regulate cytokine gene expression in human peripheral blood mononuclear cells

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Introduction: The secondary metabolites derived from microorganisms are important candidates for discovery of new drugs. *Streptomyces* bacteria are the potential sources of secondary metabolites with a wide variety of biological activities. *Streptomyces calvus* is one strain of this genus which can be an appropriate case to isolate novel compounds. In this study, the effects of secondary metabolites extracted from a native strain of *Streptomyces calvus* on gene expression of various cytokines in human Peripheral Blood Mononuclear Cells (PBMCs) were evaluated. **Material and methods:** Bacterial sample was inoculated in Mueller hinton broth and secondary metabolites were extracted. Cells (PBMCs) were isolated from human blood and were treated with *S. calvus* secondary metabolites for 48 h. Then, quantitative real time-polymerase chain reaction (qRT-PCR) assays for a few selected pro-inflammatory and inhibitory cytokine gene expressions were carried out. **Results:** Interleukin-2 (IL-2) and interferon-γ (IFN-γ) expression levels in PBMCs increased in response to treatment in a concentration-dependent manner and also the levels of immunosuppressive cytokine, interleukin-10 (IL-10) reduced. **Conclusion:** This *in vitro* study revealed that the secondary metabolites from *S. calvus* can successfully stimulate human PBMCs.

Therefore, these metabolites have the potential to serve as an effective immunostimulator that can be a significant case in tumor immunotherapy. **Keywords:** Human peripheral blood mononuclear cells, Interleukin-10, Interleukin-2, Interferon-γ, *Streptomyces calvus*

10967P

Investigation and Comparison of Serum Biomarkers by Mining the Autoantibody Repertoires of Lung Cancer Patients and Smokers

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Introduction: Lung cancer is the most common cause of cancer-associated mortality world wide and therapies would be more effective if well-timed diagnosis occurs. The existence of specific autoantibodies in the serum of cancer patients mayhave diagnostic and prognostic values. **Materials and Methods:** We used a random Ph.D-C7C phage display peptide library and after several rounds of panning, remarkable enrichment of phages that could specifically bind with serum IgGs of patients and smokers were observed. Then 55 clones were selected by monoclonal phage ELISA. The phages DNA were extracted and the sequence analysis of the selected clones was evaluated by Blastp in refseq_protein database. 898 proteins were investigated in the database EnrichR and then based on the studies performed in this area, classification was done. **Results:** Of the 898 proteins obtained, the 30 and 22 proteins in lung cancer patients and smokers were found that including c-Kit, HGF and IGF1 signaling pathways in patients with lung cancer and Notch, CDC42 and Wnt signaling pathways in the smokersConclusion: This is the first study investigated the serum autoantibodies of lung cancer patients and smokers that cause to identif proteins which have critical roles in the important signaling pathways of the various cancers as well as lung cancer. **Keywords:** Autoantibody, lung cancer, Phage display, panning.

11107 P

Immunomodulatory effects of Breast Cancer Adipose-Derived Stem Cells (ASCs) on Peripheral Blood Lymphocytes

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Introduction: Tumors are complex tissues of different cell types, including stromal cells, cells of the immune system and mesenchymal stem cells (MSCs). MSCs play a significant role as immunomodulators in tumors. They

affect tumor microenvironment by either producing different cytokines or modulation the function of immune system. The present study aimed to investigate the effects of adipose derived stem cells (ASCs) on IL-10 cytokine production by peripheral blood lymphocytes (PBLs). **Materials and Methods** ASCs were enzymatically isolated and characterized from breast fatty tissues of five normal individuals and five patients with pathological stage II breast cancer. The ASCs from the normal and cancer patients were co-cultured with normal PBLs for 5 days. Then the immunomodulatory effect of ASCs was analyzed by ELISPOT method by counting the IL-10 producing PBL clones. **Results:** Based on the results, the production of IL-10 was about 3-fold higher in PBLs co-culturing with breast cancer ASCs compared to those cultured alone. The mean±SEM of the number of IL-10 producing clones were 62.8 ± 8.44 and 21.78±7.66in PBLs exposing to ASCs compared to untreated PBLs, respectively (Pvalue= 0.005). In coculture with normal ASCs PBLs produced more IL-10 cytokine (mean±SEM=44.6 ± 9.52) compared to the control group (P value = 0.08). **Conclusion:** Based on the results of this study, the cross-talk between ASCs and the immune system can induce the expression of anti-inflammatory cytokines such as IL-10 in the immune cells leading to the anti-inflammatory responses in the tumor microenvironment. **Keywords:** Adipose-derived stem cell, Breast cancer, Immunomodulation, IL-10.

11124P

Comparison of the IgE level in patients with cancer and healthy subjects

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Introduction: Immunoglobulin E (IgE) is a type of antibody that plays a key role in allergy diseases and immune responses against parasites. Epidemiological studies indicated that there is an inverse association between plasma IgE levels as seen in allergic patients and the risk of cancer development. The aim of this study was to investigate the plasma IgE levels in common cancers such as breast cancer and patients with chronic lymphocytic leukemia in compare to healthy subjects. **Materials and Methods:** In this case-control study, twenty patients with CLL also twenty patients with breast cancer as the cases groups and twenty age-matched healthy subjects as the controls group were evaluated. Plasma IgE levels in both groups were measured using ELISA method. Data from both groups were analyzed and compared by kolmogorov-smirnov test and paired t-test with SPSS version 16. **Results:** The IgE levels were 9.045IU/ml in CLL patients and 35.84IU/ml in patients with breast cancer and 40.73IU/ml in healthy subjects. Our data showed that plasma IgE levels in CLL patients group were significantly less than controls group ($p<0.05$), but not seen significant difference between patients with breast cancer and controls group. **Conclusion:** According to our findings, the level of IgE in patients with CLL is significantly lower than normal subjects. However, IgE is effective in immune surveillance against tumor also active and passive immunotherapy. It seems there is an inverse correlation between serum IgE levels and the risk of developing CLL, as well as in allergic diseases of tumor immunity may be done with more intensity. While there is no correlation between the IgE level and the risk of developing breast cancer. **Key words:** Breast cancer, chronic lymphocytic leukemia (CLL), ELISA, Immunoglobulin E

11169 P

Role of Killer Bcells in cancer and antitumor immunity

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Introduction: B cells are phenotypically and functionally heterogeneous. B cells play multiple roles in tumor immunity. Accumulating literature indicate that B cells are significantly involved in antitumor responses. In this regard, B cells present tumor antigens to Tcells to generate antitumor CTLs. Upon tumor antigen stimulation, B cells can differentiate into plasma cells to produce antibodies and target tumor cells via ADCC and/or CDC. B cells migrate to tumor tissue and become TIL-B cells which may induce humoral immune response or act as killer cells in situ. On the other hand, regulatory B cells have been described to downregulate antitumor responses by producing immunomodulatory cytokine IL-10, suppressing Th1 immune responses, and enhancing Treg and Tr1 responses. In addition, B cells may act as killer cells to directly cause tumor cell lysis in the absence of antibodies. Recent studies have shown that B cells express death inducing ligands and can therefore mediate celldeath under many circumstances. **Results:** Evidence has emerged that B cells express Fas ligand (FasL),tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), programmed death ligands 1and 2 (PD-L1 and PD-L2), and granzyme B(GrB) which are potentially involved in B cell mediated direct cytotoxicity against tumor cells.**Conclusion:**This study demonstrated that the composition of immune cells subsets in peripheral blood reflects changes in the tumor microenvironment. Further characterization of B cell subsets responsible for these conflicting functions demonstrated in tumor immunity and understanding of the related molecular mechanisms iwould help develop novel clinical strategies for cancer immunotherapy. **Key words:** B cell,Killer B cell, Antitumor, Immunotherapy

11176 P

Role of NOD-like receptor family pyrin domain containing 3 (NLRP3) in colon cancer and antitumor immunity

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Introduction: NOD-like receptors (NLRs) are especially important for the recognition of sterile inflammation such as uric acids and silica. NLR- mediated innate immune systems play an important role in both antitumor immunity and tumorigenicity. For example, nucleotide-binding oligomerization domain-containing protein 1(NOD1) has a protective role against tumors, and knockdown of NOD1 promotes tumor growth in breast cancer model in vivo. NODlikereceptor family pyrin domain containing 3(NLRP3) serves as a sensor for activating the inflammasome pathway which regulates pro-caspase-1 cleavage and subsequent IL-1β activation. **Results:** NLRP3 is a negative regulator of chemical colon carcinogenesis. In a dextran sulfate sodium(DSS) and azoxymethane-induced colon cancer model, NLRP3 −/− mice showed increased colitis and colitis-associated cancer, which was correlatedwith attenuated levels of IL-1β and IL-18 at the tumor site. **Conclusion:** NLRP3 may also have a role in the promotion of tumors as in inflammation- induced skin cancers through the enhancement of inflammatory environment, which suggest a dual role forNLLRP3 in the regulation of host immunity forpro- or antitumor responses.

11182 P

The Immune system and Cancer; A review study

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Introduction: The immune system plays a dual role in cancer: it can both suppress or promote tumor growth A network of cells, signals, and organs helpprotect against foreign infectious agentsand cancer. Cells of the immune system are derived from hematopoietic stem cells (HSC) in the bone marrow. Immune cells that respond later during infection and specifically to antigens are involved in the adaptive immune response and include B and T cells. **Materials and Methods:** In this study we used several databases such as PubMed, Google Scholar and journals such as Nature and Science to overview on papers as a systemic review. Here, we focused on lymphocytes (B and T cells), cells of specific immunity, then classified the collected results in tables to use in future researches. **Results:** The immune system protects against cancer in three ways; by inducing protective immunity against cancer leading microorganisms, resolving inflammation and recognition followed by killing of tumors. The immune system can recognize tumor-specific antigens or tumor-associated antigens. We identified infectious agents as carcinogens like Epstein–Barr virus and Helicobacter pylori and other oncogenes and other agents. **Conclusion:** One-sixth of all cancers are caused by infectious agents. The function of the immune system is protecting the host from environmental agents and it is also involved in repair mechanisms. Identifying cancer protecting pathways help researcher focus on them and provided easier body health studies.

11188P

Placental microenvironment exerts differential effects on cancerous behavior of breast cancer cells with distinct phenotype

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Introduction: Development of cancers from nests of embryonic cells within normal tissues has been theorized more than a century ago. In fact, there are inconceivably enormous similarities between pregnancy and malignancy, so that pregnancy characterized by cancer like process of implanting and invading trophoblasts can be considered as a pseudo-malignant phenomenon. Despite highly invasive properties of trophoblasts, however, multiple in situ mechanisms exist that keep their invasive behavior under the tight control. In the current study. **Materials and Methods:** we evaluated for the first time the effect of placental explants conditioned medium (CM) on proliferation, adhesion, Matrigel invasion, wound healing motility, matrix metalloproteinase (MMP) activity and pro-inflammatory cytokine production in estrogen receptor -negative (MDA-MB-231) and -positive (MCF-7) breast

cancer cell lines. **Result:** Our results showed that CM significantly reduced proliferation of both cell types in a dose- and time-dependent manner ($p<0.05$-0.001), while stimulated invasion in MDA-MB-231 cells and increased MMP9 and MMP2 activity ($p<0.001$). No significant effect was observed in adhesive capacity of breast cancer cells following CM treatment. CM significantly reduced motility of MCF-7 cells at all time points (2-30 hr)($p< 0.001$), while it stimulated motility of MDA-MB-231 cells ($p<0.05$-0.001). The level of IL-6 in supernatant of MDA-MB-231 cells treated with CM was higher compared to control ($p< 0.01$). Both cell types produced significantly higher levels of IL-8 after treatment with CM ($p<0.05$). **Conclusion:** our observations implied that placental microenvironment exerts differential effects on cancerous behavior of breast cancer cells with distinct phenotype.
Key words: Placenta, Breast cancer, Metastasis, Invasion, Motility, proliferation, Pro-inflammatory cytokines

11262P

Stem Memory CD4$^+$ T cells (T_{SCM}) in tumor draining lymph nodes of patients with breast cancer

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Introduction: Memory T stem cells, T_{SCM}, have been recently recognized as a new subset of memory compartment, which compromise 2-3% of T cells population in peripheral blood of healthy individuals. These cells represent a naive T cell phenotype expressing CD45RA$^+$, CD45RO$^-$, CCR7$^+$ and CD27$^+$, but distinguish by expression of CD95 and CD122 memory markers. With stem cell like properties; self-renewality, multipotency and the ability to differentiate into different memory progenies, T_{SCM} has been regarded as special T cell memory subtype in cancer immunotherapy. In the present study, the frequency of CD4$^+$ T_{SCM} in positive and negative tumor draining lymph nodes of patients with breast cancer was investigated.**Materials and methods:** We used anti-CD4, -CCR7, -CD45RO, -CD95 antibodies to detect T_{SCM} cells in draining lymph nodes of 50 untreated patients using flow cytometry method and then compare their frequency with different clinical and pathological conditions.**Result:** The percentage of CD4$^+$$T_{SCM,}$ CD95$^+$ or CD95^{Hi} cells significantly increased in involved lymph nodes ($P=0.016$ and $P=0.47$, respectively) and patients with lymphovascular invasion ($P=0.013$ and $P=0.005$). Moreover, those cells with CD95^{Hi} expression also elevated in patients with pre-neural invasion ($P=0.046$). 11086**Conclusion:** It seems that the increasing frequency of CD4+T_{SCM} cells in patients with tumor infiltrating lymph nodes or positive invasion may be simply a reflection of continuous encountering with tumor antigens; however, it remains to be elucidated whether these cells have any specificity for breast cancer. **Key words:** Breast cancer, Lymph node, Stem memory T cells

11332 P

Investigation of natural killer (NK) and natural killer T (NKT) lymphocytes in peripheral blood of patients with benign and malignant salivary gland tumors

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Introduction: We investigated the mean percentage of NK cells ($CD3^-$ and $CD16/56^+$) as well as NKT lymphocytes ($CD3^+$ and $CD16/56^+$) in peripheral blood of the patients with salivary gland tumors (SGT). **Materials and methods:** Forty patients with benign (20) and malignant (20) SGTs (mean age of 48.32 ± 16.32) and 20 age/sex matched healthy donors were recruited. Fluorochrome-conjugated antibodies and Flow cytometry was used to investigate the cell types. Bonferroni corrected pv ($p<0.017$) was considered as significant level. **Results:** The mean percentage of NK cells was found to be significantly higher in both patients with malignant (25.21±3.35, p=0.001) and benign (20.97±1.98, p=0.001) tumors in comparison to healthy controls (11.55±1.83). However, the difference was not significant between patients with malignant and benign tumors (p=0.57). Although the mean percentage of NKT lymphocytes was not significantly different between patients with malignant SGTs and control group (8.57±1.74 vs7.89±0.99, p= 0.66), a tendency toward significant increase was found in the patients with benign tumors (11.27±1.26) in comparison to patients with malignant tumors and healthy controls (p=0.025, and p=0.06 respectively). Association study with the tumor progression factors revealed that the percentage of NKT cells, but not NK cells, was decreased in blood circulation of the malignant patients by the progression of the diseases. **Conclusion:** These data collectively suggest the role of NK cells, as well as the NKT cells, in pathogenesis of SGTs. The detailed interpretation requires more investigation on the cellular subtypes and function of the mentioned cells.
Key words: NK, NKT and salivary gland tumors

12332 P

Th17 lymphocytes in bladder cancer tumor draining lymph nodes

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Introduction Th17 ,as a pro-inflammatory$CD4^+$ T helper subset, plays a controversial role in tumor immunity. These cells and their related cytokines, particularly, IL-17,can promote tumor growth via inducing inflammation and angiogenesis. As Tumor Draining Lymph Nodes (TDLNs)are the immunologically active sites in immune responses developing lacation,defining immunological properties of Th17 cells could provide valuable information about their role in the context of cancer. Thus, in the present study, we evaluated percentage of Th17lymphocytes in TDLNs of patients with bladder cancer.**Materials and methods:** Mononuclear cells isolated from TDLNs of 30 bladder cancer patients, were subjected to surface- and intra-cellular staining for CD4, IL-17 and IFNγ– after activation with PMA/Ionomycin in the presence of Golgi inhibitors. The data were collected on a four-color flow cytometer and analyzed by CellQuest-Pro software. The frequency of Th17 cells and mean expression of cytokines was calculated in $CD4^+$ lymphocytes. Then patients with different clinico-pathological cinditions were compared with eachother. .**Results:** Our results indicated that although the percentage of Th17 cells and mean expression of IL-17 and IFNγ did not show any significant changes, an increase in the frequency of $IL\text{-}17^+IFN\gamma^+$double positive Th17 cells could be observed in patients with higher histological grades (P=0.039).**Conclusion:**Although it is not clearthat Th17 cells,which co-expressIFNγ and IL-17, represent a stable phenotype or a transitional phase, both IFNγ and IL-17areimportant mediators in inflammation; andit seems thatthese cellsplay an key role incancer progression.

12335P

Program cell Death-1 (PD-1) and Program cell Death Ligand-1 (PDL-1) expression on the tumor infiltrated lymphocytes (TILs) in patients with bladder cancer

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Introduction: Bladder Cancer is the fourth most common cancer in around the world. Chemotherapy still remain as the main treatment of these patients, however, the patients' outcome has been still reported to be poor. Recently, many evidences have emerged to indicate that immune checkpoint inhibitors including anti-PD-1 and anti-PDL-1 monoclonal antibodies could reinvigorate anti-tumor immune responses. This study aimed to investigate the PD-1 and PDL-1 expressions on infiltrated lymphocytes to bladder tumor tissues. **Materials and methods:** Tumor tissues were obtained from 34 untreated patients with bladder cancer through their surgical resection. Single cells were separated by mechanically mincing tumor tissue were then surface-stained with appropriate fluorochrome-conjugated monoclonal antibodies specific for CD45, PD-1, and PDL-1. For exclusion of nonviable cells, 7-amino-actinomycin was used. Data were collected on a four-color flow cytometer and analyzed by flowJo software. CD45 high expressing cells were considered as tumor infiltrated lymphocytes (TILs).**Results:** The mean percentage of TILs was 12.16 ± 3.47 among bladder cancer patients. The frequencies of PD-1 and PDL-1 positive lymphocytes were 3.46 ± 1.52 and 0.36 ± 0.11 in 7-AAD^{-}CD45^{Hi} live cells, respectively. However, the percentage of them showed no difference in the patients with different pathological conditions.**Conclusion:** Although the expression of PD-1 and PDL-1 could be delineated on the bladder tumor infiltrated lymphocytes, no significant association was found between the expression of these inhibitory molecules on TILs and tumor progression. However, tumor heterogeneity besides determining the ligand expression on surface of tumor cells should be considered. **Key words**: PD-1, PDL-1, TILs, Bladder cancer

12417P

SDF-1α, TGF-β and CD105 play key roles in trafficking of mesenchymal stem cells toward hepatocellular carcinoma HepG2 cells

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Introduction: The migration of mesenchymal stem cells (MSCs) toward some tumors including hepatocellular carcinoma (HCC) has been approved. Existence of MSCs in HCC microenvironment cause weak clinical prognosis via local immunosuppression and induction of proliferation and invasion of cancerous cells. As arrival of MSCs in HCC microenvironment contributes to progression of the tumor, recognition of key mediators involving in the migration of MSCs toward HCC can reinforce to development of efficient anticancer adjuvants.**Materials and methods:** The conditioned medium (CM) of HepG2 cells were collected after 24 hours of culture in DMEM medium containing 0.1% BSA. The migration of MSCs toward HepG2 CM was assessed after 48 hours of treatment with increasing concentrations of SDF-1α and antagonists of CXCR4 (AMD3100), TGF-βR (GW788388) and CD105 (SB4315) using ECM gel coated transwell plates. **Results:** Treatment of MSCs with SDF-1α increased their motility toward CM. However, the cells incubated with SDF-1α at 100nM showed the highest migration rate (1.62 fold compared to the migration of untreated MSCs; p<001). Applying the CXCR4, TGF-βR and CD105 antagonists

as monotherapy decreased the migration rate but their combination decreased the number of migrated cells more potently (4.51 fold; p<001). **Conclusion:** This study revealed that both SDF-1α and TGF-β are critically important in the migration of MSCs toward HepG2 cells. Antagonists of CXCR4, TGF-βR and CD105 exhibited a preliminary potential to be used as adjuvants for blocking the interaction between MSCs and HCC cells to alleviate the MSCs-induced proliferation and invasion and to enhance the local antitumor immunity.

Tumor Genomics & Biomarkers

Oral Presentations:

7553O

Altered expression of tumor suppressor micoRNAs may predict breast cancer response to therapy with paclitaxel

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Introduction: MicroRNAs (miRNAs) are small non-coding RNAs which have been interested as major players in the process of carcinogenesis. Several miRNAs have been identified as tumor-promoting (oncomirs) or tumor suppressor miRNAs in breast cancer. In this study we aimed to investigate the expression pattern of let-7a and miR-205 tumor suppressor miRNAs under treatment with paclitaxel. **Materials and methods**: IC50 of paclitaxel was determined for four breast cancer cell lines including MCF-7, MDA-MB-231, SKBR3 and BT-474 by MTT assay. Expression level of let-7a and miR-205 was determined in comparison with U6 before and after treatment with paclitaxel using quantitative reverse transcriptase real-time PCR.**Results**: After treatment, expression level of both let-7a and miR-205 was significantly increased in HER2 over-expressing cell line BT-474 (26.4 fold, p=0.0009 and 7.2 fold, p=0.00013, respectively). In contrary, HER2 negative cell lines, MCF-7 and MDA-MB-231, showed significant decreased expression of both let-7a (30.3 fold, p<0.0001 and 13.5 fold, p<0.0001, respectively) and miR-205 (20 fold, and 18.1 fold, p<0.0001 respectively). Controversially, SKBR-3 revealed significant decreased expression of both let-7a (1.3 fold, p=0.0007) and mir-205 (1.3 fold, p<0.0001). **Conclusion**: Our results confirmed the better response of HER2 over-expressing breast cancer to paclitaxel at miRNA level. One putative reason could be up-regulation of tumor suppressor miRNAs, after treatment with paclitaxel. On the other hand, triple-negative breast cancer cell line (MDA-MB-231) showed significant decreased expression of tumor suppressor miRNAs as sresisting mechanism againstpaclitaxel therapy. This is in agreement with bad prognosis of triple negative breast cancer.

9773O

Dtection of auto-antibody against 14-3-3 protein zeta as a potential biomarker incolorectal cancerpatient's sera

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Introduction: Colorectal cancer (CRC) is one of the most frequently detectedcancers therefoe screening this cancer is an effective way for early detection and increasing survival rate.14-3-3 protein zeta, a member of the 14-3-3 protein family that involved in numerous important cellular pathways in cancer initiation and progression. It has been shown that 14-3-3 protein zeta play a central role inpromoting tumor progression through regulating of multiple pathways including binding to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. In this study, we investigated anti-14-3-3 protein zeta autoantibodies in the sera of CRC patients as a novel biomarker for CRC detection by 2-dimentional electrophoresis and mass spectrometry techniques. **Material and methods:** Serum samples were collected from 80 CRC patients and 20 healthy controls. As the source of antigens, the protein lysate of SW48 cell line was analyzed through two-dimensional (2D) immunoblotting. Particular protein spots that reacted with sera from CRC patients in 2D immunoblotting picked up from the SDS-page and were identified by MALDI-TOF/TOFmass spectrometric analysis. **Results:** The results showed thatCRC patient's sera from stage III and IV disease could react with 14-3-3 protein zeta. **Conclusion:** We suggested that presence of auto-antibody against 14-3-3 protein zeta may be proposed as the novel serum biomarker for CRC diagnosis and prognosis. **Key word:** Colorectal cancer, anti-14-3-3 protein zeta, auto-antibody biomarker

9813O

Up-regulation of T-cell Immunoglobulin and Mucin Domain-3 in Patients with Gastric Cancer and Peptic Ulcer Diseases

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Introduction: T-cell immunoglobulin and mucin domain protein-3 (Tim-3), an inhibitory immunoregulatory receptor, has been recently implicated in tumor biology and current studies have demonstrated a strong correlation between Tim-3 expression and tumor-associated immune suppression. In the present study, the expression of Tim-3 was evaluated in patients with gastric cancer (GC) and peptic ulcer diseases (PUD) both at mRNA and protein level. **Material and methods:** A total of 133 gastric tissue biopsies including 43 gastric cancer, 48 peptic ulcer and 42 normal biopsies were collected. Additionally, the corresponding benign adjacent normal biopsies were also prepared for 6 gastric cancer patients. Infection with Helicobacter pylori was determined by rapid urease test for all patients and H&E staining was conducted for gastric cancer biopsies. Tim-3 relative mRNA expression was determined by SYBR Green based Real-time PCR using β-actin as a reference gene. Tim-3 protein expression was also studied by immunohistochemistry method. **Results:** Tim-3 was expressed at a higher level in gastric cancer (p=0.030) and peptic ulcer (p=0.022) patients compared to normal group. Moreover, among paired samples obtained from gastric cancer patients, tumoral tissues showed more Tim-3 expression in comparison with normal adjacent tissues (p=0.019). These findings were supported by detecting Tim-3 protein in both TILs and gastric tissues. **Conclusion:**

Higher Tim-3 expression in patients with gastric cancer and peptic ulcer confirms the local immunosuppression and regulatory mechanisms. Targeted immunotherapy by blocking of inhibitory receptors like Tim-3 could be a useful and promising approach in gastric cancer treatment. **Keywords:** Gastric cancer, Peptic ulcer disease, Tim-3, Helicobacter pylori

10853O

Silencing High Mobility Group Isoform I-C (HMGI-C) Enhancing Paclitaxel Chemo-sensitivity in breast adenocarcinoma cells (MDA-MB-468)

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Introduction: HMGI-C (High mobility group protein isoform I-C) protein is a member of the high-mobility group AT-hook (HMGA) family of small non-histone chromosomal protein that can modulate transcription of an ample number of genes. Genome-wide studies revealed up-regulation of the HMGI-C gene in many human cancers. HMGI-C might play a critical role in the progression and migration of various tumors. However, the exact role of HMGI-C in breast adenocarcinoma has not been cleared. **Materials and Methods**: The cells were transfected with siRNAs using transfection reagent. Relative HMGI-C mRNA and protein levels were measured by quantitative real-time PCR and Western blotting, respectively. The cytotoxic effects of HMGI-C siRNA, Pacritaxel alone and in combination with breast adenocarcinoma cells were determined using MTT assay. The migration after treatment by HMGI-C siRNA, Pacritaxel alone and in combination were detected by wound-healing, respectively. **Results**: HMGI-C siRNA significantly reduced both mRNA and protein expression levels in a 48 hours after transfection and dose dependent manner. It was observed that the knockdown of HMGI-C led to the significant reduced cell viability and inhibited cells migration in MDA-MB-468 cells in vitro. **Conclusions:** These results proposed that HMGI-C silencing and Pacritaxel treatment alone can inhibit the proliferation and migration significantly. Furthermore, synergic effect of HMGI-C siRNA and Pacritaxel showed higher inhibition compared to Mono-treatment. Altogether, HMGI-C could be used as a promising therapeutic agent in the treatment of human breast adenocarcinoma. Therefore, HMGI-C siRNA may be an effective adjuvant in human breast adenocarcinoma. **Key words**: HMGI-C (High mobility group protein isoform I-C), small interference RNA (siRNA), breast adenocarcinoma, Paclitaxel

11115O

Suppression of Snail-1 induces apoptosis and alters microRNA expression in human urinary bladder cancer cell line

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Introduction: Snail-1 is a mediator of survival and cell migration which is known as one of the important transcription factors raised in numerous cancer types.,. Snail-1 gene may has a role in recurrence of several cancers including bladder cancer by down-regulating E-cadherin and its related miRNA inducing an epithelial to mesenchymal transition and.The aim of this study was to investigate the effect of a specific Snail-1 siRNA on

apoptosis and altering EMT related miRNA in EJ-138 (bladder cancer) cells.**Material and method** The cells were transfected with siRNA using transfection reagent. The cytotoxic effects of Snail-1 siRNA, on bladder cancer cells were determined using MTT assay**.** Relative Snail-1 mRNA levels were measured by QRT- PCR,. Apoptosis was measured by TUNEL test based on labeling of DNA strand breaks. We also evaluated miR-29b, miR-21 and miR-203 expression by QRT-PCR to determine alteration in miRNA expression involved in EMT. **results:** Snail-1 siRNA significantly reduced mRNA expression levels in 48 hour after transfection at the concentration of 60 pm in bladder cancer cells. We also showed that the silencing of Snail-1 led to the induction of apoptosis and miR-21 and miR-29b depression have been shown in snail-1 suppressed group in EJ-138 cells in vitro. **Conclusions:** These results proposed that Snail-1 might play an important role in the progression of bladder cancer and become a potential therapeutic target for triggering apoptosis and suppression of EMT related miRNA in bladder cancer.**Key word:** Snail-1, small interference RNA (siRNA), Bladder cancer, apoptosis, EMT, Micro RNA

11225O

Differential Altered expression level of miR-21, miR-203, miR-10b and miR-194 in Paclitaxel-treated breast cancer cell lines

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Introduction: MicroRNAs play important roles in many cancers including breast cancer. It has been shown that miR-10b is involved in initiation of invasion and metastasis of breast cancer, and increased expression of miR-194 is correlated with reduced migration and invasion. miR-21 and miR-203 act as oncomiR in breast cancer. In this study, we investigated the expression level of four mircoRNAs before and after treatment with paclitaxel. **Materials and Methods:** Breast cancer cell lines (MCF-7, MDA-MB-231, SKBR3 and BT-474) were cultured and MTT assay was performed to determine IC50 of paclitaxel for each cell line. After RNA extraction and cDNA synthesis, expression level of miRNAs was quantitatively evaluated using Real-Time PCR. **Results:** MiR-21, miR-203, miR-10b and mi-194 were significantly increased in BT-474 after treatment with Paclitaxel (17.66, 2.09, 51.5 and 46 fold). In paclitaxel-treated SKBR-3, miR-21 and miR-203 showed significant upregulated expression (2.42 and 2.07 fold) and expression of miR-10b and miR-194 were significantly decreased (21.7 and 1.8 fold). HER2-negative cell lines, MCF-7 and MDA-MB-231 showed significant decreased expression of miR-21 (43.4 and 33.3 fold), miR-203 (10.98 and 156.2 fold), miR-10b (15.8 and 3.4 fold) and miR-194 (100 and 34.4 fold). **Conclusion**: Increased expression of oncomiRs in HER2-positive cell lines in response to paclitaxel could be compensatory mechanism to resist against chemotherapy. However, inconsistent alterations in the expression level of both oncomiRs and tumor-supressing miRNAs in HER2-positive and –negative cell lines failed to support this idea that altered expression of studied miRNAs could be useful to predict response to therapy with Paclitaxel.

Poster Presentations:

3468P

The lack of association between -670 A>G polymorphism in Fatty acid synthase gene and risk of breast cancer

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Introduction: Breast cancer is the most frequent malignancy among women worldwide. The Fatty acid synthase (FAS) gene, plays a central role in the tumor growth and its metastasis. A functional gene polymorphism in the promoter region of FAS namely -670 A>G has shown effects on the transcription activities of this gene and we aimed to investigate an association between this polymorphism and risk of breast cancer.**Materials and Methods:** Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was employed to determine FAS -670 A>G genotype in 115 patients with breast cancer and also 115 healthy individuals.**Results**:Frequency of FAS -670 AA, AG and GG genotypes was 52.2%, 39.1%, 8.7% respectively in patients. Result of genotype frequency in controls for FAS -670 AA, AG and GG was 47%, 41% and 10.4% respectively.There was no significant difference between case and controls (P=0.78).**Conclusion**: The FAS -670 A/G gene polymorphism did not appear to have any influence on breast cancer susceptibility.**Key Word**: breast cancer, FAS -670 A/G, gene polymorphism

7656P

IL-27 gene polymorphisms in acute lymphoblastic leukemia patients and itsrelation to prognosis

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Introduction: IL-27 is a member of the IL-12 family with two distinict inflammatory and anti-inflammatory functions. This cytokine has an important role in anti-cancer activity. The aim of the present study was to examine the association between IL-27 gene polymorphisms and risk of acute lymphoblastic leukemia (ALL) in children and its relation with prognosis and response to therapy. **Materials andMethods**: 164 ALL patients and 175 sex and age-matched healthy controls were enrolled in this study.The IL-27 rs153109 andrs17855750 polymorphisms were

analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results**: There was a significant difference in allele and genotype distributions of rs153109(P=0.001) and rs17855750(P=0.02) between patients and controls. The rs153109 AG genotype and allele G was significantly higher in patients than controls. A higher frequency of rs17855750 TG genotype and G allele was observed in patients compared to the healthy subjects. The rs153109 AG genotype and allele G was associated with age (P=0.027).The relation of these single nucleotide polymorphisms with ALL prognosis and response to therapy were also investigated and will be presented. **Conclusions:** The result of this study showed a significant association of IL-27 gene polymorphisms and risk of ALL development.

9771P

Down regulation of miR-143 expression in Tumor compared to Tumor Margins in Patients with Colorectal Cancer as a biomarker

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Introduction: MicroRNAs modulate gene expression by binding to mRNA and their target dependently eminent role as oncogenes or tumor suppressors, has been detected. MicroRNAs expression play a radical role in the pathobiology of numerous cancers. In this research, we examined miR-143 expression in the tumor cells and tumor margins of patients with colorectal cancer . **Materials and Method**: Tumor cells and tumor margins were separated after being biopsied from 50 patients with colorectal cancer. Total RNA content was extracted from both tissue types and then, first-strand cDNA was synthesized from the RNA of cells. Although, Quantitative analysis was carried out through SYBR Green real-time RT-PCR . For statistical analysis, SPSS (v.21) software was applied to compare the expression level of microRNAs between tumor cells and tumor margins. **Results**: The results of Real time PCR showedthat expression level of miR-143 was obviously lower in tumor cells compared to tumor margin in colon and rectum. **Conclusions**: The lower expression level revealed that mir-143 can be utilized as diagnostic biomarker in colorectal cancer. tThis investigation may introduce this microRNA as a colorectal cancer therapeutic target.**Keywords**: MicroRNA, miR-143, Colorectal Cancer, Prognostic, Biomarker

9812P

Studies on the possible association of single nucleotide polymorphisms of the MMP-1, 3 and TIMP-1, 2 genes in tumorsspecimens from Iranian patients with esophageal cancer.

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Introduction: Today it becomes evident that Matrix metalloproteinase (MMPs) and their tissue inhibitors (TIMPs) have an undeniable role in degrading and remodeling of extra cellular matrix and because of this property, in many ways have important roles in different stages of initiation and promotion of tumor tissues. **Material and methods:** In this study single nucleotide polymorphism of MMP-1(-1607 1G/2G) ، MMP-3(-1107 5A/6A) genes and their tissue inhibitors (TIMPs) TIMP1 (+372 C/T) andTIMP2 (+303 C/T) was genotyped by means of PCR-RFLP and with the DNA extracted from blood of 99 normal controls and DNA extracted from the tumor tissues of 93 patient with esophageal squamous cell carcinoma. **Results**: According to the results obtained from statistical analysis of the data by spss software; distribution of both alleles and genotypes in MMP1 were significantly different between cases and controls ($p \leq 0.003$,$p \leq 0.001$), but for MMP3 polymorphism this difference was just significant in distribution of genotypes and not alleles ($p \leq 0.001$). We did not find any significant differences in allele and genotype distribution of TIMP-1 and TIMP-2 polymorphisms between cases and controls. **Conclusion**: 52.7% of patients have 2G/2G genotype in MMP1 gene and according to its Odds ratio 3.68(1.98-6.83) this genotype is a risk factor for esophageal cancer. 5A/5A genotype of MMP3 polymorphism was observed only in patients and normal controls did not have this genotype. This genotype also whit the Odds ratio of 17.36(2.23-134.94) is a risk factor for esophageal cancer.

10833P

miR-338 Expression Profiling in Tumor and Tumor Margins in Patients with Gastric Cancer

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Introduction: Gastric cancer has the first place in cancer related deaths ranking in Iran. Emerging evidence has shown that microRNAs (miRNAs) are associated with the progression of cancer or tumorigenesis. In this survey, expression profile of miR-338 in tumor and tumor margins of patients with gastric cancer was evaluated. The miRNA was selected based on previous studies for their therapeutic potential and the identification of biomarkers for early detection of gastric cancer (GC). **Material and method**: First, RNA was isolated from cancer tissue and its marginal tissue that was collected from 40 cases who had gastric cancer. Then, first-strand cDNA was synthesized from total RNA . Afterwards, Quantitative analysis was performed by real-time RT-PCR using SYBR Green method. **Results**: Expression level of miR-338 in caner tissues was down-regulated compared to tumor margins. **Conclusion:** miR-338 may function as a novel tumor suppressor gene in gastric cancer and regulate the apoptosis of cancer cells. miR-338, hopefully, could serve as a potential biomarker and therapeutic target towards gastric cancer. **Keywords:** miR-338, gastric cancer, biomarkers

10891P

Lin28 and let7a miRNA co-expression pattern in T47D breast carcinoma cell line affected throw direct radiotherapy

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Introduction: MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression. Among these, members of the let-7 miRNA family control many cell fate determination genes. Lin28 is a specific, post-transcriptional inhibitor of let-7 biogenesis. One of the most ancient and highly conserved microRNAs (miRNAs),

the let-7 family, has gained notoriety owing to its regulation of stem cell differentiation, essential role in normal development, as well as its tumor suppressor function. Mechanisms controlling let-7 expression have recently been uncovered, specifically the role of the RNA-binding protein Lin28 – a key developmental regulator - in blocking let-7 biogenesis. Oncogenic regulation of let-7 miRNAs has been demonstrated in several human malignancies but their correlation with Lin28 has not been studied in breast cancer. Resistance to radiation therapy is a main trouble for the actual treatment of cancers. Lin28 has been shown to contribute to breast tumorgenesis; though, the association between Lin28 and radioresistance remains unknown. **Material and method**: We cultured carcinoma cell (T47D), and the miRNAs from these samples were profiled using Real time PCR analysis. In this study, we investigated the association of Lin28 and let-7 miRNA with radiation effect on gene expression level in human breast cancer cell lines. **Results**: The results showed that the expression level of Lin28 was upregulated. We further demonstrated that expression of let-7a ($p = 0.000$) was inversely correlated with those of Lin28.**Conclusion:** Taken together, these results indicated that Lin28 might be one mechanism underlying radiation resistance, and Lin28 could be a potential target for overcoming radiation resistance in breast cancer. **Keywords**: Breast Cancer, Radiation Resistance, lin28, let-7a

11009P

Comparison of protein phosphates 2A cancerous 10995inhibitor gene expression in prostate cancer cell lines.

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Introduction: Protein phosphatase 2A (PP2A) complexes function as tumor suppressors by inhibiting the activity of several critical oncogenic signaling pathways. Consequently, inhibition of the PP2A phosphatase activity is one of many prerequisites for the transformation of normal human cells into cancerous cells. The aberrant expression of cancerous inhibitor of proteinphosphatase 2A (CIP2A), a recently identified endogenous PP2A inhibitor in malignancy, by inhibiting PP2A activity, caused resistance to apoptosis. Because, the relation between CIP2A and prostate cancer was poorly understood, in this study, we investigated the CIP2A expression level in prostate cancer cell lines.**Material and Methods**: Prostate cancer cell lines (PC-3, Du-145 & LNCAP) were cultured in a normal condition. Total RNA was extracted by RNX-plus and CIP2A gene expression was examined with Real time-PCR. GAPDH was used as a reference gene**.Results:** The results showed that CIP2A expression level was elevated in prostate cancer cell lines incomparable to the normal cells ($p<0.05$). Also, CIP2A expression level in LNCAP cell line was higher than 2 other cell lines and Du-145 cell line had the least expression level compared to 2 cancerous cell lines.**Conclusion:** Due to the role of CIP2A in carcinogenesis and its over expression of CIP2A in prostate cancer cell lines and it seems that CIP2A has an important role in prostate cancer. So, CIP2A may serve as a therapeutic target in prostate cancer treatment.Key words: CIP2A, prostate cancer, cell line

11034P

Expression of immunomodulating genes in breast cancer

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Introduction- Altered metabolism of essential nutrients, such as amino acids (*e.g.* arginine and tryptophan) and lipids (*e.g.* arachidonic acid), is a mechanism that tumors use to form an immunosuppressive microenvironment in order to escape immune recognition. In this study, we investigated the expression of enzymes that are involved in metabolism of arginine [arginase 1, arginase 2 and inducible nitric oxide (iNOS)], tryptophan [indolamine-2, 3 deoxygenase (IDO)] and arachidonic acid [Cyclooxygenase-2 (COX-2)] in breast cancer tumors.**Material and Methods**: The tumor tissues were collected from patients with breast cancer and control tissues as a calibrator were obtained from healthy individuals who had mastoplasty for cosmetic purposes. Extracted RNA samples were analyzed by real-time PCR to evaluate arginase 1, arginase 2, iNOS, IDO and COX-2 genes expression while HPRT was used as a reference gene. Results- Real-time PCR analyses revealed increased expressions in arginase 2 (1.85±0.52), iNOS (5.74±1.22) and COX-2 (2.59±1.42) genes in the tumor tissues compared to the control; however the expression levels of arginase 1 and IDO genes did not differ. Conclusion- Our results suggest that arginine depletion by arginase 2 and iNOS along with production of PGE2 by COX-2 from arachidonic acid are involved in formation of tumor suppressive microenvironment. Therefore, it appears that by alteration of metabolism of arginine and arachidonic acid, the tumors can actively be involved in development of an immunosuppressive environment.

11016P

The association between allergy history and breast cancer risk: A case-control study

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Introduction: The relation of allergy history on cancer developments remains controversial. Therefore, we aimed to evaluate this association in a case-control study. **Materials Methods:** we conducted a case-control study at Cancer Institute of Iran, Iran Tehran, and enrolled 165 control and 168 breast cancer patients. All participants were interviewed in details about their history of allergic symptoms like rhinitis/conjunctivitis, wheezing and allergic diseases. Odds ratios (ORs), p-value and 95% confidence intervals (CIs) were calculated. **Results:** We found ever having asthma is inversely associated with the risk of breast cancer (OR: 0.46; 95% CI: 0.26-0.83; p-value: 0.0083) and conjunctivitis symptom has a small reduction effect (OR: 0.48; 95% CI: 0.22-1.03; p-value: 0.04). However, there was no association between risk of breast cancer and dermatit, allergic rhinitis and food or drug allergies. **Conclusion:** Our findings suggested that history of asthma and conjunctivitis were associated with a trend toward a reduced risk of breast cancer.

11051P

Key role of Dkk3 protein in inhibition of cancer cell proliferation: an in silico identification

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Introduction: Dkk3 is a member of Dkk family proteins, regulating Wnt signaling. Dkk3 plays different roles in human and mouse tumors. Dkk3 predominantly act as a tumor suppressor, however several reports revealed that Dkk3 could accelerate cancer cell proliferation. Herein, we aimed at launching an in silico study to determine Dkk3 structure and its interactions with Kremen and LRP as Wnt signaling receptors as well as EGF receptor. **Materials and methods**: Dkk3 moleculemodel was built using various softwares. Different protein modeling approaches along with model refinement processes were employed to arrive at the final model. To achieve the final complex of Dkk3 with Kremen, LRP and EGFR molecules protein-protein docking servers were employed. **Results**: Model assessment softwares indicated the high quality of the finally refined Dkk3 3D structure, indicating the accuracy of modeling and refinement process. Our results revealed that Dkk3 is capable of interacting with Kremen, LRP and EGFR with comparable binding energy. **Conclusion**: Dkk3 efficiently interacts with LRP, Kremen and EGF receptor and may be a promising protein in cancer therapy by blocking Wnt and EGFR downstream signaling.
Keywords: Bioinformatics; Wnt signaling; Dkk; EGFR; Cancer

11057 P

OCT4B1 suppression, down-regulate BCL2 gene family in human tumor cell lines

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Introduction: The OCT4B1, a new discovered variant of OCT4 is expressed more than other variants in both human cancer cell lines and tissues. New finding showed this variant has anti-apoptotic potency in mentioned cells and tissues. BCL2 family is one of the twelve gene families involved in apoptosis pathway with negative control in apoptosis recurrence. The aim of the present study was to investigate the effects of OCT4B1 silencing on several genes of BCL2 family in human tumor cell lines. **Materials and methods:** Three human tumor cell lines; AGS (gastric adenocarcinoma), 5637 (bladder tumor) and U-87MG (brain tumor) were transfected with specific OCT4B1 siRNA and a scrambled sequence as control, using Lipofectamine 2000 commercial kit. The expression rate of BCL2 gene family transcripts were evaluated, using a human apoptosis panel-PCR kit. **Results:** Expressional profile of the studied BCL2 transcripts in three cell lines is almost similar. Nineteen of twenty one studied genes in BCL2 family showed down-regulation, fourteen genes were decreased in expression more than 3 and three genes (BAD,BCL2 and BNIP3L) more than 10 folds. BCLAF1 showed up-regulation (in U87MG and 5637 tumor cell lines) and MCL1 showed unchanged gene expression. **Conclusions:** According to these results, it may be concluded that OCT4B1 suppression can lead to apoptosis in tumor cell lines via down-regulation of several BCL2 transcripts. Thus, OCT4B1 suppression effects on BCL2 may be considered as promising target genes in future studies in cancer research and therapy.

11061P
The survey of Self-renewal gene expression in bladder cancer and tumor cell lines

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Introduction: Re-expression of self-renewal genes is one of the causes of cancer. So, expressional profile of self-renewal genes including, OCT4, NANOG, KLF4, SOX2 and NUCLEOSTEMIN in two cell lines (5637 and HT1376), cancer and normal tissues of bladder cancer biopsy tissues were analyzed.**Materials and Methods:** In this experimental study, the cell lines were cultured in RPMI1640 medium. Cancer tissue samples were selected from samples referred to the pathology laboratory (fresh tissue), according to clinical symptoms and laboratory findings. Tissue edges were selected as healthy tissue. Expressional profile of interested genes was done by using specific primers and Real-Time PCR method. **Results:** The Real-Time PCR results showed that the expression of studied genes in cancer cell lines and tumor tissues were down by approximately similar pattern but unexpressed in healthy tissue.**Conclusion:** The expression of OCT4, NANOG, KLF4, SOX2 and NUCLEOSTEMIN genes were necessary for induction of self-renewal potency. The results showed that these genes were expressed in cancer tissue and cancer cell lines in compared to normal tissues. The investigation of expression pattern of mentioned genes in different tumor cell lines and tumor tissues were recommended.

11159P
Evaluation of serum chemerin in patients with breast cancer

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Introduction: Breast cancer is a major cause of cancer-related death and the most common solid malignancy in women worldwide. Chemerin is a new recognized adipokine eith inflammatory activity that initiates inflammation via chemo taxis of immature DCs and macrophages. In this study we investigated chemerin serum level in patients with breast cancer. **Materials and Methods:**In this cross-sectional study we enrolled 45 patients with breast cancer in Vali-asr hospital from June to December 2015 (range of age 18-60 years) and 40 healthy volunteers as a control group (range of age 22-56 years) were matched. Patients main inclusion criteria were having invasive breast cancer without any history of mastectomy surgery. In this experiment, we analyzed serum chemerin levels in both groups by ELISA method. Data was analyzed by using Spss 16 software and ANOVA test.**Results:**The patients serum chemerin average was 1536 ng/L±608 that in comparison with control group avarage 1919 ng/L ±544(p=0.04) was significantly lower . **Conclusion:** In conclusion, the results of this study showed significant reduced level of serum Chemerin in patients with breast cancer. Since, Chemerin plays important roles in inflammation, so lower chemerin levels results in tumor growth and progression.Key words: breast cancer, chemerin, Elisa

11186P

Expression of matrix metalloproteinase-13(MMP-13) in human gastric cancer at the presence of Helicobacter Pylori

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Introduction: Matrix metalloproteinases (MMPs) can degrade the essential extracellular matrix(ECM) components .MMPs are important regulators of tumor growth not only at the primary site but also in distant metastasis; hence the enzymes are considered as seriously targets for cancer therapy. MMP-13 is specially activated in gastric cancer and promotes the primary tumor toward an invasive phenotype. Helicobacter Pylori (H.pylori) stimulates gastric epithelial cells to produce MMP-13 in vitro. The purpose of this research is to reveal the relation between matrix metalloproteinase-13(MMP-13) expression and clinicopathological characteristics of gastric cancer at the presence of H.pylori infection in 50 patients.**Materials and methods:**The level of MMP13 expression was measured by quantitative PCR instrument and compared in two groups of normal and carcinomatous tissues. **Results:** The results showed 30% elevation of MMP13 expression in tumor tissues. There were no meaningful differences between the results of positive or negative H.pylori patients and gene expression. There was a relation between gene expression and tumor type (P value= 0.032). In addition, there was a significant relation between gene expression and stage in intestinal group (P value= 0.023). **Conclusion:** It t showed that in intestinal group, immune system plays an important role in reducing gene expression. The absence of Fas (a tumor necrosis factor receptor) expression in diffuse type gastric carcinoma cells may protect these cells from immune surveillance. Results also showed over expression (60%) in diffused group. These findings suggest that using MMP13 inhibitors in diffused group might contribute to the control of tumor growth. **Keywords:** MMP13, Gastric cancer, Helicobacter Pylori, Real-time PCR

11299P

Investigation of PD-1 genetic marker in ovary cancer in Iranian population

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Introduction: The programmed death-1 (PD-1) molecule has been found to be a critical component in immune regulation. Genetic association of PD-1 has been observed in certain autoimmune diseases and cancers. In this study, we aimed to investigate the polymorphisms of PD1.3 (+7146 G/A-rs11568821) and PD1.5 (+7785 C/T- rs2227981) in ovary cancer patients. **Materials and Methods:** One hundred patients with confirmed ovary cancer and one hundred age and sex matched healthy Iranian controls without history of autoimmune diseases or malignancie in first-degree relatives were enrolled. DNA was extracted using salting out method. Genotypes were determined using PCR-RFLP method. Data were analyzed by SPSS and Aarlequin software packages. **Results:** The frequency of GG, GA and AA genotypes at position +7146G/A in PD-1.3 gene, were 70(68.6%), 28(27.5%) and 4(3.9%) in patients and 89(88.1%), 11(10.9%) and 1(1%) in controls respectively. The frequency of G and A alleles were 168(82.3%), 36(17.7%) in patients and 189(93.5%), 13(6.5%) in controls. Statistical analysis indicated that the frequency of GG genotype and G allele were significantly higher in patients than controls, (P=0.003 for genotypes and P=0.0009 for alleles respectively). Distribution of the observed genotypes and alleles at PD1.5 locus, were not significantly different between patients and controls (P>0.05). Haplotype analysis did not show any significant differences between two groups. Conclusion: This study suggests that PD-1.3 (+7146 G/A) polymorphism may affect the ovary

cancer risk in Iranian population, due to significant association between PD-1.3 polymorphism and ovary cancer. **Key word:** Ovary cancer. Polymorphism PD-1

11310P

micro RNA let-7b and Lin28 gene expression relation survey in women breast cancer with 40-60 age via real-time pcr

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Introduction: Breast cancer is the cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nippleor a red scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath or yellow skin. Outcomes for breast cancer vary depending on the cancer type, extent of disease and person's age. Breast cancer most commonly develops in lining cells of milk ducts and lobules that supply the ducts with milk. Cancers developing from the ducts are known as ductal carcinomas, while those developing from lobules are known as lobular carcinomas. **Methods and materials:** We examined the expression of Lin28 and let-7b miRNA in 100 women breast cancer with 40-60 age. In this study, we investigated the association of Lin28 and let-7b miRNA with real-time pcr on gene expression level in breast cancer. **Results:** Result showed that the expression level of Lin28 (p=**0.032)** in these ages was upregulated. Upregulation of Lin28 increase the patient survival while it has no effect on let7b(p = 0.618) levels. **Conclusion:** Lin28 play its role in tumorigenesis by repressing biogenesis of microRNA let-7 and is considered as a potential therapeutic target for various human cancers. Lin28 is one of the most important RNA binding proteins for recognizing terminal loop of microRNA as a mechanism of expression control. Lin28 improve cancer via inactivating miRNA let-7.**Key woeds:** Lin28, let-7 miRNA, breast cancer, real-time pcr

11311P

Lin28, let7c miRNA and p21 gene co-expression pattern in T47D breast carcinoma cell line affected throw direct radiotherapy

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Introduction: A tumor-suppressor gene, let-7 microRNA (miRNA) family, is often inactivated in various human malignancies. Lin28 is a RNA-binding protein that has been well characterized for regulation of let-7 maturation in undifferentiated embryonic stem cells at post-transcriptional level. Oncogenic regulation of let-7 miRNAs has been demonstrated in several human malignancies but their correlation with Lin28 has not been studied in breast cancer. Resistance to radio therapy is a main difficulty for the effective treatment of cancers. Lin28 has been shown to contribute to breast tumorgenesis; though, the association between Lin28 and radioresistance remains unknown. **Methods and materials :** The carcinoma cell (T47D),were cultured and their miRNA expression profile were evaluated using Real time PCR. In this study, we investigated the association of Lin28 and let-7 miRNA with radiation effect on gene expression level in human breast cancer cell lines. **Results:** The results showed that the expression level of Lin28 was upregulated. Stable expression of Lin28 and treatment with radiation induced Lin28 expression, while down regulated p21 (p = 0.004).We further demonstrated that expression of let-7c (p = 0.006) was inversely correlated with those of Lin28 .**Conclusion**: Taken together, these results indicate that Lin28 might be one mechanism underlying radiation resistance, and Lin28 could be a potential target for overcoming radiation resistance in breast cancer.**Keywords:** Radiotherapy; miRNA; Breast Cancer

11331P

Up-regulated expression of miR-184 and in HER2-positive BT-474 breast cancer cell lines under treatment with Taxol

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Introduction: microRNAs (miRNAs) have been recently interested in different biological processes especially in tumorigenesis. These small non-coding RNAs are involved in multiple steps including generation, metastasis, immune-modulation and also in diagnosis, prognosis and molecular sub-typing. The aim of this study was to determine differential expression of miR-184 and miR-130a in breast cancer cell lines under treatment with Taxol chemotherapeutic agent. **Materials and methods:** Four breast cancer cell lines BT-474, SKBR-3, MDA-MB-231 and MCF-7 with different pattern of expression of HER-2, estrogen receptor (ER) and progesterone receptor (PR) were selected. IC50 of Taxol for cell lines was determined by MTT assay, and alterations in the expression of miR-184 and miR-130a after treatment with Taxol were determined by quantitative reverse transcriptase real-time PCR.**Results:** Mir-184 was significantly over-expressed in treated BT-474 (448 fold, p=0.0003), but its expression was down-regulated significantly in SKBR-3 (2.2, p=0.002), MDA-MB-231 (28.5 fold, p<0.0001) and MCF-7 (20.4 fold, p<0.0001). Expression of mir-130a was undeletable in both treated and untreated BT-474, SKBR-3, MDA-MB-231 and MCF-7 cell lines. **Conclusions:** Our findings show that higher expression level of HER-2 in comparison with low or no expression of this molecule could influence alterations in the expression level of mir-184 after treatment with Paclitaxel. Because of potential inhibitory effect of mir-184 on the proliferative nature of breast cancer cells, it could be postulated that Paclitaxel inhibit cell proliferation via over-expression of this miRNA. This effect might be one possible mechanism for better response of HER-positive breast cancer to therapy with Paclitaxel.

11333P

Association of rs1 polymorphism in OX40 gene with Basal cell carcinoma of skin

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Introduction: OX40 molecule plays an important role in stabilization and stimulation of T cell responses. Association of OX40 gene polymorphisms with different diseases has been found in previous studies. We investigated rs17568 G/A polymorphism of OX40 and its association with Basal cell carcinoma (BCC). **Methods and materials :** 152 patients with BCC, with an average age of 61.87±13.15 years who were referred for surgical treatment were investigated for rs17568 G/A polymorphisms in OX40 gene. The results were compared to 152 healthy peoples as control group who were age and sex matched with patients' group. Genotypes were identified using PCR-Restriction fragment length polymorphismmethod. **Results:** The frequencies of GG, GA and AA genotypes were respectively 63(41.44%), 61(40.13%) and 28(18.42%) in patients and 73(48.02%), 57(37.50%) and 22(14.47%) in controls. The frequencies of G and A alleles were 187(61.51%) and 117(38.48%) in patients and 203(66.78%) and 101(33.22%) in controls respectively. Despite of greater GG genotype and G allele frequency in patient and control groups we did not find any significant statistical association between rs17568 G/A polymorphism and risk of BCC disease (P: 0.45). We did not find significant differences in alleles frequencies between BCC

patient and control groups (P: 0.20). There was also no association between this polymorphism and the size of BCC tumors. **Conclusion:** Current study revealed that rs17568 G/A polymorphism does not play any role in susceptibility to basal cell carcinoma and its tumor size in the Iranian population. **Key words:** basal cell carcinoma, polymorphism, OX40, rs17568

11334P

Significant alterations in the expression of miR-224 and miR-1246 in breast cancer cell lines under treatment with Taxol

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Introduction: microRNAs (miRNAs) have been recently interested in different biological processes especially in tumorigenesis. These small non-coding RNAs are involved in multiple steps including generation, metastasis, immune-modulation and also in diagnosis, prognosis and treatment of cancer. Large number of miRNAs has been studied in breast cancer. In this study, differential expression of miR-224 and miR-1246 were studied in four selected breast cancer cell lines under treatment with Taxol. **Materials and Methods:** IC50 of Taxol for four BT-474, SKBR-3, MDA-MB-231 and MCF-7 breast cancer cell lines was determined by MTT assay. Expression pattern of miR-224 and miR-1246 before and after treatment with Taxol was determined using quantitative reverse transcriptase real-time PCR. **Results:** Expression of mir-224 was found in two SKBR-3 and MDA-MB-231 cell lines that significantly down-regulated after treatment with Taxol (2.1 fold, $p=0.001$, and 17.2 folds, $p<0.0001$, respectively). For mir-1246, significant up-regulation was found in HER2-overexpressing BT-474 (113 fold, $p<0.0001$) and SKBR-3 (1.4 fold, $p=0.02$), and significant down-regulation was seen in HER2-negative MCF-7 (45.5 fold, $p<0.0001$) and MDA-MB-231 (7.7 fold, $p<0.0001$). **Conclusion:** Expression of mir-1246 could be useful marker in molecular diagnosis of different sub-types of breast cancer, and an interesting marker for prediction of response to therapy with Taxol in HER2-positive and –negative breast cancers. On the other hand, differential expression of mir-224 could be applicable in only two SKBR-3 and MDA-MB-231 cell lines for response to therapy.

12331P

Up-regulated expression of miR-25 in Taxol-treated HER2-overexpressing breast cancer cell lines

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Introduction: MicroRNAs (miRNAs) are involved in different stages of tumorigenesis. Recently, miRNAs have been interested as useful markers for prognosis, diagnosis and response to therapy. Better response of HER2-expressing breast cancers to therapy with Taxol is well-documented, but exact molecular mechanism in not clear. In this study, we aimed to quantitatively investigate the differences in expression level of mir-25 and mir-93 in four breast cancer cell lines before and after treatment with Taxol. **Materials and methods:** Using MTT assay, IC50 of Taxol was determined for BT-474, SKBR-3, MDA-MB-231 and MCF-7 breast cancer cell lines. Total RNA was extracted from untreated and Taxol-treated cells and expression level of mir-25 and mir-93 was investigated by

quantitative reverse transcriptase real-time PCR.**Results**: Over-expression of mir-25 was seen in Taxol-treated HER2-positive BT-474 (48 fold, p<0.0001) and SKBR-3 (1.4 fold, p=0.23). Inversely, mir-25 expression was down-regulated in Taxol-treated HER2-negative MDA-MB-231 (50 fold, p<0.0001) and MCF-7 (52 fold, p<0.0001). Mir-93 was not expressed in both untreated and treated BT-474, SKBR-3, MDA-MB-231 and MCF-7 cell lines. **Conclusion:** It has been shown that mir-25 is associated with aggressiveness of breast cancer malignant cells. According to better response of HER2-positive breast cancers to taxol therapy, it might be predicted that expression of mir-25 down-regulated in HER-2 positive breast cancer cell lines. However, our results for expression level of mir-25 in HER2-positive and –negative breast cancer cell lines failed to support this hypothesis.

12338P

Soluble HER2 (sHER2) shows elevated level in sera of patients with high and low grades of meningioma

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Introduction: Human epidermal growth factor-2 receptor (HER2) is a transmembrane glycoprotein with tyrosine kinase activity. HER2-signaling pathway and its involvement in tumor proliferation, survival, and metastasis induction is elucidated and it can be a potential target for therapeutic intervention. HER2 is expressed in some human malignancies and it is overexpressed in 30% of meningiomas with epithelial differentiation. There are only a few studies on the relationship between HER2 expression and meningioma. Therefore, in this study we measured the levels of sHER2 as a biomarker in serum sample of patients with different grades of meningioma and its correlation with clinicopathologic parameters. **Materials and methods:** 68 meningioma patients and 20 age-sex matched healthy volunteers enrolled in this study. The soluble HER2 levels were measured by commercial sandwich ELISA assays according to the manufacturer's manual. Statistical analysis was performed using SPSS software. **Results:** When comparing sHER2 levels in the sera of patients with controls, sHER2 in sera of patients was significantly higher than controls (4.8 vs. 4.1 pg/ml; p value=0.013).**Conclusion:** Although more studies are needed to precisely determine the role of sHER2 in meningioma patients, it may be regarded as a possible intervention target for therapeutic purposes.

12339P

Investigation of glial fibrillary acidic protein (GFAP) serum levels in patients with meningioma

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Introduction: Glial fibrillary acidic protein (GFAP) is an intermediate filament expressed in glial cells that stabilizes and maintains the cytoskeleton of normal cells. GFAP is expressed by numerous cell types of the central nervous system (CNS) including astrocytes and ependymal cells. In the present study we measured the levels of GFAP in patients with different grades of meningioma and its correlation with clinico pathologic parameters. **Materials and methods:** Peripheral blood samples were obtained from 68 meningioma patients and

20 age-sex matched healthy volunteers. The GFAPlevels were determined bycommercial sandwich ELISA assays. S tatistical analysis was performed using SPSS software. **Results:** We found no difference among GFAP levels in the sera of patients with different meningioma grades and control group. Also, no relation was found between these two groups with different age and sex. **Conclusion:** Although the result of this study indicatedno significant difference in the case and control groups, to confirm this finding more studies with larger sample size are required.

Transplantation & Stem cells

Oral Presentations:

Stem cells

7596O

The effects of hyperthermia on immunomodulatory properties of mesenchymal stem cells (MSCs)

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Introduction: Hyperthermia could modulate inflammation and immune response. Considering the recriutment of Mesenchymal Stem Cells (MSCs) at inflamed tissuses and their immunomodulatory properties, the aim of this study was to examine the effects of hyperthermia on immunomodulatory properties of MSCs in Mixed Lymphocyte Reaction (MLR). **Materials and Methods:** Passages 4-6 of human umbilical cord vein mesenchymal stem cells were co-cultured with two-way MLR. In hyperthermia groups, the cells were incubated for 45 min at 41°C (Hyperthermia). CCK colorimetric assay was employed in order to examine the effects of MSCs on cell proliferation. Cell culture supernatant was used to measure IL-4 and TNF-α proteins, and cells were used to extract RNA and then to synthesise cDNA. RT-PCR was utilized to assess the mRNA expression of IL-10, IL-17A, IL-4, TNF-α, TGF-β1, FOX P3, IFN-γ, CXCL12 and β actin. **Results:** Co-culture with MSCs reduced prolifration of the lymphocytes at 37°C, whereas 41°C attenuated this effect. Hyperthermia increased expression of the mRNA level of IL-10, TGF-β1, and FOXP3 in the co-culture as compared with MLR, but it was ineffective on IL-17A and IFN-γ and also it reduced the expression of CXCL12. In co-culture, IL-4 mRNA and protein level increased at 37°C, while this effect was revoked at 41°C. No change was seen in the mRNA of TNF-α in hyperthermia and the TNF-α protein was reduced slightly in the co-culture supernatant. **Conclusion:** Hyperthermia increases lymphocyte proliferation in co-culture of MSCs with MLR and changes the cytokine profile, so it could be a modulator of immune response. **Keywords:** Mixed Lymphocyte Reaction (MLR), Immunomodulatory, Mesenchymal Stem Cells (MSCs), Hyperthermia

7702O

Regulation of telomere activity by Immune cell cytokines in hematopoietic cord blood cells

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Introduction: The telomere is a nucleoprotein structure in the end of eukaryotic chromosomes and its length is regulated by telomerase enzyme. In differentiated cells, the number of DNA hexamer repeats decreases and telomere length is reduced with each cell division. The aim of this study was to evaluate the influence of IL-2 (Interleukin), IL-7 and IL-15 on telomere length and hTERT (human Telomerase Reverse Transcriptase) gene expression in mononuclear and umbilical cord blood stem cells ($CD34^+$ cells) during development to lymphoid cells. **Materials and Methods:** Both MNCs (Mononuclear Cells) and $CD34^+$ hematopoietic precursor cells were isolated from umbilical cord blood and co-cultured for 21 days in the presence of different cytokines. Telomere length and hTERT expression level were evaluated in freshly isolated cells and after 7, 14 and 21 days of culture by real-time PCR. **Results:** Highest gene expression levels of hTERT and maximal telomere length were measured in MNCs at day14, when co-cultured with IL-7 and IL-15. There was also a significant correlation between telomere length and telomerase expression level in MNCs at 14^{th} day after being exposed to IL-7 and IL-15 cytokines ($r = 0.998$, $p = 0.04$). In contrast, IL-2 showed no distinct effect on telomere length and hTERT expression neither in MNCs nor $CD34^+$ cells. **Conclusion:** IL-7 and IL-15 increased telomere length and hTERT gene expression at 14^{th} day. It seems likely that cells in response to prolonged exposure of IL-7 maintain naïve phenotype; While IL-2 had no significant effect on telomere length and hTERT gene expression. **Keywords:** Telomere, Telomerase Enzyme, Differentiated Cells, T and B lymphocyte, CD34+ cells, Real-time PCR

10857O

Effect of Preconditioned Human Umbilical Cord Mesenchymal Stem Cells on Treatment of Pulmonary Fibrosis in Mice

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Introduction: Pulmonary fibrosis (PF) is a progressive lung disorder of unknown etiology. Mesenchymal stem cell (MSC) based therapy is a novel approach with high therapeutic potential for the treatment of lung diseases. H_2O_2 preconditioning is thought to enhance the therapeutic potency of engrafted MSCs. The aim of this study was to investigate the anti-inflammatory and anti-fibrotic effects of preconditioned MSCs in bleomycin-induced PF. **Materials and Methods:** PF was induced in Eight-week old male C57BL/6 mice by intratracheally administration of bleomycin (4U/kg) in 50 µL sterile PBS. Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) were isolated and treated with sub lethal concentration of H_2O_2. 1 week after the bleomycin injection, 2×10^5

preconditioned hUC-MSCs in 50 µL PBS were injected in the PF mice through intratracheally. On day 14 after stem cell transplantation, animals were sacrificed and lung was removed to assess MPO activity and pathological changes. **Results**: MPO activity was significantly increased in mice administrated with only bleomycin (PF group) compared to the control group. However, preconditioned hUC-MSCs transplantation resulted in significant reduction of MPO activity as compared to the PF group. Also, in the PF group, hematoxylin/eosin and Masson's trichrome staining showed that the pulmonary alveolus cavities were obviously decreased in size, the alveolar wall was thickened, and there was accumulation of inflammatory cells. However, preconditioned hUC-MSCs transplantation alleviates histological changes. **Conclusion**: Stem cell therapy using preconditioned hUC-MSCs reduced inflammatory and fibrotic effects in bleomycin-induced pulmonary fibrosis and could enhance the therapeutic potency for cell therapy. **Keywords:** Pulmonary fibrosis**,** hUC-MSCs**,** Myeloperoxidase**.**

11253O

LPS stimulation of adipose tissue-derived mesenchymal stem cells affectsIL-10 and TNF-α production of macrophages

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Introduction: Adipose tissue-derived mesenchymal stem cells have different molecular pattern recognition receptors and respond to their stimulation. It is important to determine that how these stimulations affect the immunomodulatory properties of mesenchymal stem cells. For this purpose we assess the immune-mudulatory effect of LPS treated MSCs on macrophage function in the cell to cell contact manner. **Materials and Methods:** adipose tissue derived mesenchymal stem cells were isolated from C57BL/6 mouse and characterized by flow-cytometry analysis of cell surface markers. MSCs were cultured and divided into two groups at passage two, first group treated with LPS for three days and the second one had no treatment. After treatment MSCs were co-cultured with C57BL/6 peritoneal macrophages. IL-10 and TNF-α production of the co-cultured cells in response to LPS were measured by ELISA method. **Results:** According to obtained results we found that the level of TNF-α production of macrophages was reduced after co-culture with LPS stimulated MSCs. However, PS stimulation of the co-cultured cells demonstrated a significant ($p \leq 0.05$) increase in TNF-α production of macrophages co-cultured with LPS stimulated MSCs. In addition, reduction in IL-10 production of macrophages was determined after co-culture with LPS stimulated MSCs. **Conclusion:** In this research, it was shown that MSCs stimulation with LPS will change their immune-mudulatory properties on macrophages by induction of more TNF-α production.

12385O

Isolation and expansion of CD44$^+$ cancer stem cells of tumor tissue from gastric adenocarcinoma patients

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Introduction: Gastric cancer is the fourth most common cancer worldwide, with a high rate of death and low 5-year survival rate. Recent studies suggest that cancer stem cells (CSCs) are responsible for tumor initiation, invasion, metastasis, and resistance to anticancer therapies. The isolation and identification of CSCs could help to develop novel therapeutic strategies specifically targeting CSCs. In this study, we enriched CSCs through spheroid colony formation by cultivating cells of tumor tissue from gastric adenocarcinoma patients in serum-free medium and isolated and identified them using cell surface marker CD44. **Materials and Methods:** Tumor tissues were dissociated by mechanical and enzymatic methods. For spheroid colony formation, the resulting cancer cells were cultivated in serum-free medium supplemented bFGF, EGF, and B27 in ultralow adhesion flask. For magnetic separation and surface marker analysis by flow cytometry, spheroid body-forming cells after enzymatic dissociation were selected with CD44 magnetic microbeads and detected with FITC-tagged anti CD44 antibody, respectively. **Results:** In serum-free medium, cells grew as non-adherent, three-dimensional spheroid clusters, called spheroid colony. After purifying $CD44^+$ cells, CD44 were positively expressed by the majority of tumor sphere cells by flow cytometry analysis. **Conclusion:** The purification and characterization of CSCs could lead to the identification of better targets for therapeutic interventions. As a functional approach, spheroid colony formation is particularly useful to enrich the potential CSC subpopulations and identified the existence of gastric cancer initiating cells in the $CD44^+$ population. However, further experiments such as a combination of two or multiple markers are required to identify and purify CSCs.

12414O

Immunomodulatory effect of human umbilical cord blood derived mesenchmal stem cells on activated T-lymphocyte

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Introduction: After identification and isolation of mesenchymal stem cells by Friedenstein in 1970, many studies have conducted about regenerative and immunomodulatory properties of mesenchymal stem cells and their application in treatment of different diseases. This study aimed to investigate the immunomodulatory effect of umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) on cytokine genes expression profile of stimulated T-lymphocytes populations. **Materials and Methods:** MSCs isolated from umbilical *cord blood samples* and cultured in DMEM supplemented with 10% FBS. Mesenchymal stem cells nature were identified by flow cytometry analysis and differentiation to the adipocyte and osteocyte lineage. The mesenchymal stem cells co-cultured with stimulated T-lymphocytes and the expression level of cytokine genes (IL-2, IL-4, IL-6, IL-10, IL-13, IFN-γ, TNF-α and TGF-β) assayed by Real Time PCR method. **Results:** Our findings confirmed the mesenchymal nature of isolated cells from umbilical cord blood source. The Evaluation of cytokines gene expression profile in co-culture system showed that mesenchymal stem cells can significantly decrease pro-inflammatory cytokines gene expression such as: IL-2, IL-6, IFN-γ and TNF-α in peripheral blood mononuclear cells and in contrast Induce the anti-inflammatory cytokines gene expression such as: IL-4, IL-10, IL-13 and TGF-β. **Conclusion:** Based on data collected in this study we concluded that mesenchymal stem cells derived from umbilical cord blood have modulatory effects on activated T-lymphocyte and more complementary studies are needed to clarify this function in clinical statues.

Transplantation

9735O

Evaluation of circulating miR-150 expression level in renal transplant recipients

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Introduction: microRNAs (miRNAs) are small noncoding RNAs and functional regulators of the immune system. It was reported to be involved in the set of diverse pathways associated with chronic allograft dysfunction (CAD), a foremost cause of renal graft loss. In this study, the researchers sought to evaluate the significance of circulating miR-150, a vital regulator in differentiation and activation of immune cells, in anticipating the renal graft function. **Materials and Methods**: The plasma miRNA levels of 53 renal transplant recipients including 27 with stable graft function (SGF) and 26 recipients with biopsy proven interstitial fibrosis and tubular atrophy (IFTA) were evaluated using real-time quantitative-PCR (q-PCR). **Results**: Increased expression levels of miR-150 (P< 0.001), were detected in IFTA group compared to SGF group. This was even higher in severe IFTA grade (P< 0.001) patients. Receivers operating characteristic (ROC) analysis showed that circulating miR-150 with 100% sensitivity and 80% specificity could discriminate IFTA from SGF group (area under the curve [AUC]= 0.963, 95% confidence interval of [CI]= 00.825 to 0.999, P< 0.001). **Conclusion**: Increased level of miR-150 may indicate active immune response and extra cell matrix accumulation and could discriminate all IFTA patients from normal individuals and, therefore, can be used for monitoring patients besides other validated miRNAs.

10795O

The role of Interleukin-35 gene expression in renal allograft survival

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Introduction: The main treatment for advanced renal failure patients is kidney transplant. Transplant rejection is a major constraint. Interleukin-35 (IL-35) is considered as a suppressive cytokine secreted by regulatory T-cells. Probably, higher expression of this molecule can be related to increase the allograft survival time. So, the aim of this study was to investigate the association of IL-35 gene expression with renal allograft survival time. **Materials & Methods:** This case-control clinical study included 40 patients dividing into two groups: 27 patients with long-term allograft survival (with no history of rejection in more than 5 years after transplantation) and 13 patients with early graft failure. Blood samples were taken from patients. Total RNA were extracted from Buffy coats and cDNA were

synthesized. IL-35 gene expression was measured by Real Time PCR and compared with study groups. **Result**: Statistical analysis showed that the expression of IL-35 gene was significantly higher in patients with long-term allograft survival compared to patients with early graft failure and control group (P <0.01). **Conclusion:** Increase the expression of IL-35 resulted in the survival of kidney transplant allografts.

10800O

The gene expression of FCRL molecules in Iranian kidney transplant patients

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Introduction: Fc receptor-like (FCRL) molecules belong to the immunoglobulin superfamily. Their genes located on chromosome 1q21-23 indicating the preferential expression of FCRL molecules by B cells and their potential to deliver activating or inhibitory signals. Recent study has demonstrated the FCRL1 expression in several B cell in renal transplant. **Materials and Methods:** Blood samples taken from EDTA-treated were collected from 30 patients in 1, 3, 7 days of post-transplantation. Total RNA was extracted from patient's Buffy coat and cDNA synthesis was carried out. Gene expression for FCRL1 was measured by Real Time PCR and compared with control group. **Result**: The results showed that the expression levels of FCRL1 was increased in patients group compared to the control group but not significant (P value> 0.05). **Conclusion**: The expression of FCRL1 can be used as a diagnostic marker in kidney transplant patients that requires further research in this area.

10828O

Effects of Mesenchymal stem cells on cytokine production in mixed lymphocyte reaction

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Introduction: It is shown that Mesenchymal stem cells (MSCs) can down-regulate immune cells activities by direct contact and/or secretion of soluble factors. Some recent studies have revealed the immuno-modulatory effects of MSCs on dendritic cells (DCs). However, the exact mechanisms involved in MSCs immuno-regulatory function has not well understood. In this study, first DCs were treated with MSCs and then the ability of MSCs treated DCs was studied to change cytokines secretion in mixed lymphocyte reaction (MLR). **Materials and Methods**: MSCs were isolated from the bone marrow of BALB/c mice. DCs were isolated from the splenocytes of BALB/c mice using a positive selection magnetic adsorption cell sorting (MACS). T-cells were isolated from lymph nodes of C57BL/6 mice using a negative selection MACS technique. The purity of isolated cells was assessed by flow cytometry. MSCs were co-cultured with DCs in 1:10 and 1:50 ratios. MSCs treated DCs were co-cultured with allogeneic T-cells in MLR. Levels of 13 cytokines were analyzed in MLR supernatants. **Results**: IL-2 and TNF-α levels were

lower in MLR supernatant included MSCs treated DCs (1:10 ratio) compared to untreated DCs. Surprisingly, IL-10 and IFN-γ levels were higher in MLR supernatant included MSCs treated DCs (1:10 and 1:50 ratios). **Conclusion**: MSCs inhibit immune responses by changing the secretion of Th1/Th2/Th17/T-reg cells related cytokines.

10881O

Expression of interferon regulatory factor 3 (IRF3) in HBV-infected liver transplant patients

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Introduction: Interferon regulatory factor 3 is one of the transcription factors which was induced by dsRNA and activated by bacterial lipopolysaccharide. It up-regulates IFN4α/β genes, interferon stimulated genes and some of the chemokines. In attention to HBV infection in studied patients, pre-transplantation, early activation of innate immune system of post transplantation, and change in expression level of IRF3 were important. **Materials and Methods**: In this project the expression level of IRF3 was evaluated in confirmed HBV infection patients who were undergoing liver transplantation in Namazi hospital, Shiraz, Iran between years: 2012-2014. According to the pathologic tests patients were divided into two groups: HBV Rejection (20 patients) and HBV Non –rejection (26 patients). The expression level of IRF3 in buffy coat of patients was evaluated in 1, 4, 7 days of post transplantation by Real time PCR using SYBR-Green PCR Kit. Data was analyzed by using spss version 18. **Results:** Comparison of IRF3 expression level between two patient groups showed that IRF3 expression level was significantly (p=0.049) up regulated in 1st day and none significantly down regulated in 4th and 7th day of post transplantation in HBV-Acute rejection group compared to HBV-Non rejection group. **Conclusion:** According to previous reports, up regulation of IRF3 in 1st day post transplantation may cause the ischemic reperfusion injury in HBV-Acute rejection of patients but down-regulation of this factor may result due to the presence and counteraction of HBV with IRF3 expression.

11243O

Up-regulation of miR-142-3p in renal transplant patients with chronic allograft dysfunction

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Introduction: Chronic allograft dysfunction (CAD) has remained the major cause of graft loss. miR-142-3p was implicated in biological processes and immune system. The aim of this study was to investigate the expression levels of miR-142-3p in biopsy and peripheral blood mononuclear cell (PBMC) samples of renal transplant patients following CAD with interstitial fibrosis and tubular atrophy (IFTA).It was investigated whether CAD with IFTA is associated with changes in miR-142-3p expression within biopsy and PBMC samples of renal transplant patients

following CAD with IFTA and whether its expression levels could be predict CAD with IFTA. **Materials and Methods:** The expression levels of miR-142-3p in biopsy and PBMC samples were surveyed from recipients with CAD with IFTA as well as subjects with normal biopsy results as controls. Levels of miR-142-3p were measured using TaqMan MicroRNA Assays. **Results:** In this study, expression levels of miR-142-3p was increased in biopsy and PBMC samples of the recipients with CAD with IFTA compared with recipients with normal biopsy results. We found a significant association between expression of miR-142-3p and CAD with IFTA. Moreover, CAD with IFTA of renal allografts could be predicted with a high level of precision by measurement of miR-142-3p levels within biopsy and PBMC samples. **Conclusion:** Measurement of miR-142-3p in PBMC samples could be used as a biomarker for non-invasive diagnosis of CAD with IFTA in renal transplant recipients. Also, as miR-142-3p was expressed in higher levels in biopsies, it might have a role in CAD with IFTA mechanism. **Keywords:** MicroRNA, Renal Transplantation, Acute Rejection

Poster Presentations:

6511P

Non-adherent bone marrow stem cells and adherent mesenchymal stem cell have the same potential in ameliorating experimental autoimmune encephalitis

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Introduction: Non adherent bone marrow derived cells have recently been described to give rise to multiple mesenchymal phenotypes. The aim of the current study was to evaluate the ability of non-adherent cell population of rat bone marrow in ameliorating the experimental autoimmune encephalitis (EAE). **Materials and Methods**: EAE was induced in Wistar rats by guinea pig spinal cord homogenates and complete Freund's adjuvant. Therapies were initiated at day 12 post immunization when the mice developed a disability score with 2×106 of routine adherent mesenchymal stem cells or non-adherent bone marrow cells. After day 33, the mice were sacrificed and the effects of cell therapy were investigated. **Results**: Clinical scores, leukocyte infiltration and lymphocyte proliferation were significantly diminished in a similar pattern in both treatment groups. Body weight increased significantly in the both treated groups compared to EAE rat without treatment. **Conclusions**: Non adherent bone marrow derived cells may be a simple and cost-effective way to cell-therapy of autoimmune disorder such as multiple sclerosis. **Keywords**: Non-Adherent Cell, Bone Marrow Cell, EAE

7516 P

Comparison of the effects of adherent and non-adherent mesenchymal stem cells on the Acetic Acid-Induced Ulcerative Colitis in wistar rat.

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Introduction: Mesenchymal stem cells (MSCs) have a good potential for inducing anti-inflammatory responses in inflamed tissues, hence they are used as therapy in auto-inflammatory disease. There are two population of cells in the bone marrow that can differentiate to MSCs in vitro. This study was conducted to compare the therapeutic potential of adherent and non-adherent MSCs in animal model of ulcerative colitis. **Materials and Methods**: Colitis induced by acetic acid in four groups of male Wistar rats; control group, adherent MSCs treated group, non-adherent treated group and control sham group. Adherent MSCs and non-adherent cells (2×106 cell) have been isolated and then injected into the peritoneum in different group separately. After 10 days, the rats were euthanized and evaluated for gross pathology, and production of inflammatory mediators in gut tissue. **Results**: Data showed that non-adherent MSCs have therapeutic potential compared to adherent MSCs via reducing the inflammatory mediators like nitric oxide and reactive oxygen substances and improving the gross pathology. **Conclusions**: As isolation of non-adherent MSCs is very rapid and simple, this approach may be a useful strategy to control colitis.

7532P

Co-Overexpression of HIF-1α and metallothionein 1A enhances functions, Resistance and cytokine production in human mesenchymal stem cells

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Introduction: The number of hematopoietic stem cells (HSCs) in an efficient stromal context is the most important factor determining the success of bone marrow transplantation (BMT), thus co-transplantation of reasonably functional mesenchymal stem cells (MSCs) can greatly improve the outcome of transplantations. Hypoxia inducible factor-1alpha (HIF-1α) and metallothionein 1 A (MT1 A) are the most important genes in the body and we taught this genes might change the efficiency of MSCs by affecting the production of some cytokines. To address this issue, human MSCs were manipulated to over-express HIF-1α and MTI A genes, in this study. **Materials and Methods**: Full-length cDNA of human HIF-1α and MT1 A were inserted into human bone marrow MSCs by pcDNA3.1 vector, and the effects of these co-over-expression on production of some hematopoietic growth factors was investigated. In co-culture of HIF-1α and MT1-A-over-expressing MSCs with HSCs, the effect of expression on HSCs was also evaluated. **Results**: co-Over-expression of HIF-1α and MT1 A in MSCs increased production of stem cell factor and glutathione in culture media. In co-culture of HIF-1α and MT1 A –overexpressing MSCs with HSCs, enhancement of colony formation and reduced differentiation of HSCs was observed. **Conclusion**: Co-Over-expression of HIF-1α and MT1 A in human MSCs can augment the production of some hematopoietic growth factors and also antioxidant agents, highlighting a rather effective role of MSCs in cell therapy specially Bone

marrow transplantation. **Keywords**: HIF-1α, MT1 A, Human Mesenchymal stem cells, hematopoietic stem cells, Stem cell factor, Glutathione, Co-Culture

7684P

Evaluation of gene expression changes of Fas, Fas L and Foxp3 after liver transplantation in autoimmune hepatitis patients with acute rejection compared to those without acute rejection

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Introduction: The liver is the largest of the abdominal viscera. It is involved in the synthesis and turnover of plasma proteins. Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease of unknown etiology. Liver transplantation is used as treatment of choice for the end-stage patients. Acute rejection is a well-known complication of allograft transplantation. Several mechanisms have role in graft rejection in liver transplantation such as changes in intra-graft cytokines, apoptosis induction by graft infiltrating lymphocyte. One of the most important apoptotic pathways is through the interaction of Fas and its ligand, FasL. Foxp3 + CD4+ CD25+ T-reg cells have been increasingly documented to suppress allograft. **Materials and Methods:** A total of 23 newly diagnosed AIH patients were enrolled in this study. Peripheral blood mononuclear cells (PBMCs) were isolated from the patients for total RNA extraction and cDNA synthesis. The expression of Fas, FasL and Foxp3 genes were assessed on days 1, 3, 5, and 7 days after transplantation by real-time PCR. Clinical and para-clinical information of the patients were extracted from the patients' medical files. **Results:** Real-time PCR showed various levels of Fas gene expression with no significant difference in different days in patients with acute rejection ($P = 0.52$). However, in patients without acute rejection a significant difference in Fas gene expression levels between day 1 and 3 after transplantation was observed ($P < 0.01$). Similarly, a significant difference was found in FasL gene expression between days 1 with 5 and 1 with 7 in patients without acute rejection ($P < 0.05$). A raise in FasL gene expression level was observed on days 5 and 7 compared to day 1 in this group of patients. **Conclusion:** In this study we found an increased Fas and FasL gene expression in patients without acute rejection and decreased in Foxp3 gene levels in both group of patients. These data indicated the active involvement of these molecules in immune response during liver transplantation and imply their importance particularly in relation to rejection the severity and renal dysfunction. **Keywords**: Liver allograft, acute rejection, Fas, Fas L and Foxp3,

9734P

Differential expression of circulating inflammation-associated miR-155 in renal transplant recipients

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Introduction: Chronic rejection is a major cause of renal allograft failure and is closely associated with the inflammation. Since miR-155 is related to the inflammatory response in multiple immune cell lineages, present study aimed to evaluate its plasma expression to determine its regulation in renal recipients and to determine its significance in anticipating the renal graft function. **Materials and Methods**: The plasma miRNA levels of 40 renal transplant recipients: 20 with stable graft function (SGF) and 20 recipients with poor graft function (PGF), 8 of them with biopsy proven chronic rejection, were evaluated using real-time quantitative-PCR (q-PCR). **Results:** The results showed that miR–155 was significantly (P= 0.005) up-regulated in plasma samples of recipients with PGF compared with normal graft recipients. This level was even higher in chronic rejection patients (P= 0.01). **Conclusion:** This study demonstrated that differential expression of miR-155 was observed in plasma samples of PGF patients that may contributed to active immune responses and inflammation-mediated glomerular endothelia injury. Therefore, miR-155 can be used for allograft monitoring and identification of risky chronic rejecting recipients.

9770P

Study of the relationship between genetic polymorphisms of TLR4 and CD14 with acute rejection in Renal transplantation

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Introduction: Toll-like receptors (TLRs) molecules are the important factors in transplant outcome.TLR4/CD14 (its Co-receptor) are cell surface markers that are associated with episode of acute rejection. Since the role of TLRs polymorphisms in renal transplantation is undeniable and the influence of CD14 polymorphism has not been elucidated in Iranian patients. So in the present study, the effect of TLR4 and CD14 functional polymorphisms were evaluated in acute rejection. **Materials and Methods**: Common Single nucleotide polymorphisms Asp299Gly (rs4986790), Thr399Ile(rs4986791) of TLR4 and -159 C/T (rs2569190) of CD14 genes were genotyped using Taq-man genotyping PCR from genomic DNA samples of 239 subjects in three groups (AR, SGF, HC). **Results**: There was a significant association between CD14 TT genotype in AR vs. SGF (P<0.0001) and HC (P<0.007). Moreover, recipients carrying CD14 TT genotype had a higher risk of acute rejection than those with CT or CC genotype. Interestingly, based on a logistic regression analysis CD14(TT), genotype of AR patients was still significant after including risk factor (CMV) (95% CI, 1.397-7.200; P=0.006). Also graft survival was analyzed according to studied polymorphisms and rejection-free survival was lower in recipients with TT CD14 genotype (log-rank test, P=0.000). **Conclusion**: The mentioned results indicating CD14 -159 C/T polymorphism, play crucial role in acute renal rejection. CD14 polymorphisms are correlated with renal graft surveillance. Therefore, 159C/T promoter polymorphism (rs2569190) of CD14 could be as a risk factor for predicting acute rejection in renal transplant patients. **Keywords**: Renal transplant, Acute rejection, TLR4, CD14.

8709P

Investigating the effects of bone marrow derived mesenchymal stem cells on USP7 expression

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Introduction: Mesenchymal stem cells (MSCs) capable to modulating the immune system. The cells of both the innate and adaptive immune system such as dendritic cells(DCs), naive and effector T cells, and natural killer cells have been altered to anti-inflammatory phenotype by MSC. Maturation, function and differentiation and of DC were affected by MSC. TolDCs induce FoxP3 Tregs from CD4 T cell. Treg cells are The central key of maintenance of self and environmental tolerance and homeostasis. USP7 have been detached ubiquitin molecules from FOXP3 protein so amount of FOXP3 protein keeping high. USP7 has been expressed in primary Treg cells and increase Foxp3 protein expression by decreasing Foxp3 polyubiquitination. **Materials and Methods:** MSCs were cultured and Freshly isolated Balb/c mice's DC were added to the MSC at different ratios. DCs were co-cultured harvested after 24 h. MLR were performed with T cells (C57BL/6) and cultured DC with MSC. T cell RNA was isolated after 48 h from MLR experiments and Quantitative RT-PCR experiments were performed for relative quantification of USP7 gene. **Result:** Gene expression of USP7 in MLR condition with MSC treated DC increased 3.5 fold in 1:2(DC:MSC) ratio co-culture,3.8 fold in 1:10 compare with control (MLR with not treated DC).also we observed significant correlation between FOXP3 and USP7(correlation coefficient=0.438, p-value =0.003). **Conclusion:** Our data reveal a molecular mechanism in which MSC can increase FOXP3 expression through USP7 and so detached ubiquitin molecules from FOXP3 protein. These data suggest that MSC generate Tol-DC that lead to induce steady FoxP3 Tregs.

10816

Effects of Mesenchymal stem cells on cytokine production in skin transplanted mice

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Introduction: Recently, mesenchymal stem cells (MSCs) have gained extensive notice as therapeutic cells for transplantation due to their immunoregulatory functions. However, precise mechanisms involve in MSCs

immunoregulatory activity is not well understood. In this study, in addition to investigate the ability of MSCs to increase the skin graft survival, we decided to clarify the effect of MSCs on secretion of various cytokines. **Materials and Methods**: Bone marrow MSCs (BM-MSCs) were isolated from BALB/c female mice. The purity of isolated cells was assessed by flow cytometry. Differentiation potential of isolated cells was assessed by culturing cells in differentiation media. Skin grafts were obtained from the back of C57BL/6 and transplanted to BALB/c mice. MSCs injected to transplanted Mice. Graft survival was checked daily. 13 cytokines related to Th2/Th17/Th1/T-reg cells were assayed on days 2, 5 and 12 after graft transplantation. **Results**: Injection of BM-MSCs improved skin graft survival time from 11 to 14 days. Interleukin-10 level in BM-MSCs treated mice was higher compared with untreated control group on days 2 and 5 after transplantation, while Interleukin-2, Interferon-γ and Interleukin-17 levels were lower in BM-MSCs treated mice. **Conclusion**: MSCs increase secretion of IL-10 as well as inhibition of IL-2, IL-17 and IFN-γ secretion thereby cause prolongation of skin allograft survival in mice model.

10835P

Effect of secretory factors from mesenchymal stem cell pulsed with Caffeine on the neutrophils functions

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Introduction: Caffeine is structurally similar to adenosine, and is capable of binding to adenosine receptors without activating them, thereby acting as a competitive inhibitor. Interestingly, adenosine receptor exists on the mesenchymal stem cells (MSCs).The aim of the present survey was to evaluate the effects of the conditioned medium of mesenchymal stem cells treated with caffeine on neutrophils. **Materials and Methods**: After isolation of mesenchymal stem cells from rats bone marrow, these cells were pulsed with different concentration of caffeine (0.1, 0.5 and 1 milimolar) at different times (24, 48 and 72 h). Then, MSCs cultured for 24 h without treatment and the supernatants of MSCs were collected and incubated with neutrophils for 4 h. Finally, the functions of neutrophils were evaluated. **Results**: Data indicated that the supernatants of MSCs pulsed with caffeine at low to moderate concentrations, preserved the phagocytic ability of neutrophils and established the MSCs ability to protect neutrophils from apoptosis. Moreover, the supernatants of MSCs decreased the production of potentially harmful Reactive oxygen substances more profound than MSCs without treatment. Nevertheless, high concentration of caffeine could interfere with some aspects of crosstalk between MSCs and neutrophils. **Conclusion**: It seems that caffeine has a dose-dependent effects on the effects of MSCs on the neutrophils. **Keywords**: Mesenchymal Stem Cells, Neutrophils, Caffeine.

10851P

Study of the gene expression level of Toll-like receptors 2 and 4 with kidney transplant rejection

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Introduction: Kidney transplantation is receiving the kidney from an alive or cadaver and transplanting it to a patient that has a nonfunctioning kidney. Transplant rejection is the main limitation in reaching a successful transplantation. Toll-like receptors (TLRs) are the receptors of innate immune system expressing on antigen presenting cells. Studies showed that TLR2 and TLR4 molecules have important roles on the outcome of kidney transplantation. The aim of this study was to investigate the gene expression level of these two molecules in rejected kidney transplanted patients with non-rejected ones. **Materials and Methods**: 101 non-rejected and 50 rejected kidney transplanted patients were enrolled in this study. Blood samples were taken from all patients, then RNA was extracted and cDNA were synthesized from all samples. Using SYBR Green Real-time PCR technique, the expression level of both genes were measured. **Results**: The results showed that the expression level of both TLR2 and TLR4 genes had significant increase in the rejected group compared to non-rejected group. **Conclusion**: Studies revealed that TLR2 and TLR4 molecules may have effect on the process of rejection in kidney transplanted patients.

10872P

Evaluation the effects of ROCK Y–27632 inhibitor, Ascorbic acid , Trehalose for increasing survival of human Wharton's jelly stem cells after cryopreservation.

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Introduction: Human Wharton jelly stem cells (hWJSCs) are used for cell based therapies in regenerative medicine. Expansion and storage of stem cells are an integral part of any stem cell bank and cell based therapy program. Efficient methods of cryopreservation that yield increased thaw-survival rates and continue to maintain stemness after thawing are thus important aspects of stem cell storage facility. **Materials and Methods**: HWJSCs Treated with Ascorbic acid (0.06, 0.125, 0.25, 0.5 mM) or Trehalose (35, 75, 125 mM) or Y-27632 (only 10 µM) 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) and apoptosis (Annexin V-FITC Assay) was evaluated. **Results**: Treated hWJSCs with Ascorbic acid or Trehalose or Y-27632 showed increased Thaw- survival, increased cell proliferation and no evidence of apoptosis. **Conclusion**: Using cryoprotectant substances are important for expansion of hWJSCs and would be useful for their storage in cord blood banks for regenerative medicine.

10874P

Differentiation of human mesenchymal stem cells wharton's jelly toward insulin producing cells in medium containing human platelet Lysate (HPL)

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Introduction: Type 1 diabetes is caused by the inability of the beta cells to produce enough insulin and regulate blood sugar. The differentiation of stem cells toward islet like cells seems to be a good option for treatment of patients. **Materials and Methods:** In this study, mesenchymal stem cells were isolated from human umbilical cord Wharton's jelly (HUMSCs) and differentiated toward insulin producing cells. The HUMSCs were harvested and differentiated in culture medium DMEM-F12, containing retinoic acid, exendin, nicotinic acid, epidermal growth factor, human platelet lysate (HPL), in 3 step protocol during 21 days. **Results:** The Islet like clusters were differentiated. The expression of insulin, glucagon and Glut were confirmed and the presence of insulin granules was detected by DTZ staining. The insulin secretion was confirmed by ELISA method. The usual differentiation medium contained fetal bovine serum (FBS) to provide growth factors. **Conclusion:** Using of HPL has great advantage that the medium is xeno-free and these protocols are safe for cell transplantation in humans.

10875P

Differentiation of Umbilical Cord Mesenchymal Stem Cell toward Insulin producing Cells using Mir-375 lentivirus.

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Introduction: Type1 diabetes is characterized by autoimmune destruction of pancreatic β cells, leading to reduced insulin secretion. Differentiation of mesenchymal stem cells (MSCs) into β-like cells offers new ways for diabetes treatment. The microRNAs (miRNAs) are key players in several stages of pancreatic differentiation. MSCs can be isolated from the human umbilical cord tissue and differentiate into insulin-producing cells. **Materials and Methods:** Human umbilical cord–derived stem cells (hUDSCs) were attained after birth, selected by plastic adhesion, and considered by flow cytometric analysis. For induction of differentiation, miR-375 was lentivirally overexpressed in hUDSCs. **Results:** In this study, we present a novel and cost-effective strategy in which over-expression of miR-375 promotes pancreatic differentiation in hUDSCs in the absence of any other growth factor. The expressions of PDX1and insulin were identified by quantitative RT-PCR ($P < 0.05$). **Conclusion:** In conclusion, Morphological assessment and expression analysis of islet cell marker demonstrated that hUDSCs are able to differentiate into insulin-producing cells by transduction with lentiviral vector miR-375.

10885P

The effect of human term and preterm umbilical cord vein derived from mesenchymal stem cells on lymphocyte proliferation

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Introduction: Mesenchymal stem cells (MSCs) have immuno-modulatory properties. Although MSCs can be isolated from various tissues, umbilical cord is an accessible source of them. Different gestational age can affect isolation rate and expansion of these cells in vitro. In this study the effect of human term and preterm umbilical cord vein MSCs on lymphocytes proliferation was investigated. **Materials and Methods:** MSCs were isolated from preterm (22-32 week) and term umbilical cords (38-40 week) by enzymatic method. Passage 2 and 5 of MSCs (7000 cells) were added into two-way MLR (co-culture). XTT colorimetric assay was used to assess lymphocytes proliferation on day 5th of MLR. **Results:** The isolated cells were plastic adherent and spindle-shaped. They were negative for CD45 and CD34 and positive for CD105 and CD73. They had osteogenic and chondrogenic differentiation potency. Term and preterm MSCs diminished lymphocytes proliferation in both passages. Passage 2 of term and preterm MSCs reduced lymphocyte proliferation 37.9% and 14.5% in co-culture, respectively, and also co-culture of passage 5 of term and preterm MSCs decreased proliferation 40.7% and 26.2%, respectively. **Conclusion:** The results indicated that term MSCs can suppress lymphocyte proliferation more efficiently than preterm MSCs. Also, the older passages of these cells reduced proliferation to a greater extent as compared to early passages which could result to the higher purity of MSCs.

10912P

The comparison of migratory ability of term and preterm human umbilical cord vein derived mesenchymal stem cells

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Introduction: Mesenchymal stem cells (MSCs) can migrate to the injured and inflamed tissues in response to chemokines and growth factors and can modulate immune responses there. Therefore these cells could be a noteworthy candidate for cell therapy. The migratory ability of MSCs toward chemotactic mediatorsis a determinant factor in the outcome of cell therapy. Here we examined the migration ability of term and preterm human umbilical cord vein MSCs in vitro. **Materials and Methods**: MSCs were isolated from term (38-40 weeks) and preterm (22-32 weeks) umbilical cord veins by enzymatic method, and then cultured up to passage 4. The migratory ability of MSCs were assessed by transwell chemotaxis assay. To this aim, MSCs were resuspended in the serum free culture media and added to the upper side of the chambers. In the lower side, culture media containing 10% and 30% FBS was used as a source of chemo-attractant and positive control respectively. **Results**: It was verified that the both of term and preterm cells were negative for CD45 and CD34, and positive for CD105 and CD73. Isolated cells had capability of differentiation to osteogenic and chondrogenic lineages. It was found that migrated MSCs in term group were 49% while in preterm group were66% (P= 0.03). **Conclusion**: According to our study, preterm MSCs have higher migratory capability as compared to term MSCs and this characteristic could be useful in cell therapy.

11052P

TNF-α modulates the immunosuppressive effects of mice Adipose-derived MSCs on Dendritic cells and T cells

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Introduction: Mesenchymal stem cells are a progenitor cells that have capabilities to differentiate to different cell types. Also, MSCs possess immune suppressive effects on DCs differentiation and T cells activation through wide range of soluble factors and receptors. The properties of MSCs change through activation of cytokines particularly IFN-γ and TNF-α. **Materials and Methods:** The DCs phenotypes and functions including the expression of co-stimulatory and co-inhibitory molecules and capabilities of DCs to induce allogeneic activation of CFSE-labeled splenocytes as well as cytokines production when they differentiate in presence of MSCs, TNF-α activated MSCs, IFN-γ activated MSCs and IFN-γ & TNF-α activated MSCs were analyzed. Treg population and T cells polarization were investigated using flow cytometry and real-time PCR respectively. **Results**: Here, it was shown that IFN-γ slightly enhances immunosuppressive effects of MSCs on immune system through induction of tolerogenic DCs with elevated expression of IDO and increasing Treg population. Conversely, TNF-α decreases immunomodulation properties of MSCs on immune cells through the enhancement of co-stimulatory molecules such as ICOSL and HLA-DR, reduction of PDL-1 and PDL-2 expression and decrease of TGF-β and IL-10 in DCs as well as inhibition of T cells polarization into T_H2 and Treg. **Conclusion**: Taken together, these data showed a crucial effects of microenvironments on MSCs behaviors indicating that functions of MSCs differentially were altered in presence of different cytokines. **Keywords**: Mesenchymal stem cells; Dendritic cells; T cells; TNF-α; IFN-γ; IDO

11090P

The effect of pro-inflammatory cytokines on immunophenotype, differentiation capacity and immunomodulatory functions of human mesenchymal stem cells

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Introduction: Mesenchymal stem cells (MSCs) are nowadays considered as a new approach for cells therapy of inflammatory disorders such as autoimmune diseases. Due to the local pro-inflammatory cytokine milieu, the immuno-phenotype, multi-lineage differentiation capacity and immuno-modulatory functions of MSCs could be altered by inflamed-micro-environments. This study was conducted to investigate the effects of pro-inflammatory cytokines on MSCs properties. **Materials and Methods**: In this study, human bone-marrow-derived MSCs (BM-MSC) and adipose-tissue-derived MSCs (AD-MSC) were cultured with or without Interleukin-1β, IL-6 and IL-23 as pro-inflammatory cytokines that are essential for polarization of IL-17-producing Th (Th17) cells. The surface markers, co-stimulatory molecules and their differentiation capacity were measured in cytokine-untreated and cytokine-treated MSCs. MSCs-mediated immuno-modulation was analyzed by their regulatory effects on mixed lymphocyte reaction (MLR) and the level of IL-10 and TGF-β production. **Results:** Pro-inflammatory cytokines showed no effect on MSCs morphology, immunophenotype and co-stimulatory molecules except up-regulation of CD45. Adipogenic and osteogenic differentiation capacity increased in CD45+ MSCs. Moreover, cytokine-treated MSCs preserved the suppressive ability of allogeneic T cell proliferation and produced higher level of TGF-β. **Conclusion:** It was concluded that pro-inflammatory cytokines up-regulated the efficacy of MSCs in cell-based

therapy of degenerative and inflammatory disorders. **Keywords:** Mesenchymal stem cell; CD45+ MSC; Pro-inflammatory cytokine; Immunomodulatory function; TGF-β; Th17 cell;

11112P

The Hepatocyte Growth Factor (HGF) can act as immune modulator by increasing IL10, TGFβ and IFNγ cytokines in Mesenchymal Stem Cells: in vitro study

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Introduction: The Hepatocyte Growth Factor (HGF) has important role as a modulator of immune system. This factor producemainly by Bone Marrow Mesenchymal Stem Cells (BM-MSCs.) The purpose of this study was the evaluation of the effect of HGF on human mesenchymal stem cells to produce the gene of IL10, TGFβ and IFNγ cytokines and suppression of the proliferation of Peripheral blood mononuclear cells **(**PBMCs.). **Materials and Methods:** In this study, the BM-MSCs were Isolated, cultured, characterized and then treated with HGF at dose of 20 ng/ml in different times. IL10, TGFβ and IFN-γ gene were measured by qRT-PCR. Also, the ability of the HGF in suppression of the proliferation of PBMNC cells were evaluated by using MLR. Results were analyzed by SPSS software and T-test. **Results**: The results showed significant production of IL10, TGFβ and IFN-γ upon HGF treatment in RNA level ($P<0.05$). Also a significant decrease of PBMCs proliferation co-cultured with HGF treated-MSCs were seen ($P<0.05$). **Conclusion**: The findings showed that using HGF could decrease proliferation of HSCs by altering expression of key cytokines in the process of modulating the immune system. **Keywords**: Hepatocyte Growth Factor, Mesenchymal Stem Cells, Immune Modulating.

11125P

Mesenchymal stem cell educated macrophages increases TNF-α/IL-10 ratio in response to Leishmania major infection

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Introduction: Mesenchymal stem cells (MSCs) are sensors and switchers of inflammation that exist in all tissues and migrate to inflammation site. MSCs can refunction macrophages in bidirectional interaction to M1 or M2 phenotype dependent to the inflammatory or anti-inflammatory conditions. Macrophages are the main host of leishmania that prevent leishmaniasis development if fight correctly or may help disease exacerbation through reduced nitric oxide and increased IL-10 production. This study has designed to assess MSCs effect on macrophage cytokine responses to L.major. **Materials and Methods**: Macrophages and MSCs are isolated from susceptible BALB/c mice. MSCs surface marker and differentiation potential was confirmed. Macrophages were educated with MSCs for three days in the transwell system. MSCs were removed and macrophages were treated with L.major at 1:10 ratio. TNF-α and IL-10 production were assessed by ELISA after 72h incubation. **Results:** The results showed a significant ($p\leq0.05$) shift to increase in TNF-α/IL-10 ratio in MSCs educated macrophages in response to L.major,

in spite of suppression in both TNF-α and IL-10 production of MSCs co-cultured macrophages compared to control. **Conclusion**: This results introduced a novel aspect of immuno-modulatory effect of mesenchymal stem cells on macrophages. Reduction in IL-10 production, make MSCs a candidate for cell therapy in infection.

11165P

The effect of adipose tissue-derived mesenchymal stem cells Conditioned media on phagocytostic activity and nitric oxide production by peritoneal macrophages

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Introduction: Adipose tissue-derived mesenchymal stem cells (AD-MSCs) can migrate to inflamed site and exerts its immune-modulatory effects on tissue resident immune cells like macrophages. To investigate the effect of MSCs soluble factor on macrophage function, we assess phagocytosis potential and nitric oxide production of macrophages after treatment with MSCs conditioned media. **Materials and Methods:** MSCs were isolated from adipose tissue of C57BL/6 mice. MSCs surface markers were analysed using flow-cytometry method. 72 hours conditioned media (CM) of MSCs at passage 2, were collected. C57BL/6 peritoneal macrophages were harvested and treated for 72h with MSCs conditioned media. After treatment, macrophages were treated with yeast particle at 1:10 ratio and phagocytosis percent was measured by microscopic examination. Nitric oxide production was measured in the supernatants of treated macrophages by Griess method. **Results:** The results indicated a significant increase in nitric oxide production of macrophages after treatment with MSCs conditioned media. Phagocytosis percent of yeast particles were reduced significantly in CM treated macrophages compared to control. **Conclusion:** These results confirmed the immune-modulatory effect of MSCs soluble factors. Increase in nitric oxide production beside the decrease in phagocytosis percent, suggest an inflammatory role for MSCs conditioned media.

12506P

Investigation of natural killer cell activity in patients with acute and chronic renal transplant rejection

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Introduction: Kidney transplantation is the best treatment of end-stage kidney failure. Despite an increasing awareness of innate immunity, the roles of natural killer cells in transplant rejection have not been clearly defined. NK cells are a subset of lymphocytes that play a central role in the innate immune response. **Materials and Methods:** In a case-control study, subjects in patients with 11 acute and 11 chronic kidney transplant rejection and control samples in people having kidney transplant with optimal activity and healthy individuals, each group consisting 22 people respectively, were selected. The marker expression of CD107a, following stimulation with K562 and the amount of these cells in these groups were measured by flow cytometry techniques. In order to

analyze the data distribution comparison used through Kruskal-Wallis test and in order to their two by two comparison used through Mann-Whitney test. **Results:** Based on the current study results, the amount of CD107a in acute kidney transplant rejection showed significant increase in comparison to stable graft group and the P-value was 0.04. However, the percentage of NK cells in patients suffering acute and chronic transplant rejection compared with control groups was decreased. **Conclusion:** Our results showed the percentage of NK cells in patients with acute and chronic kidney transplant rejection was decreased; nevertheless, the expression of CD107a was increased. This finding demonstrated that the amount of cells circulating in peripheral blood of patients suffering rejection decrease but the cytotoxicity of these cells will increase. **Key words:** Kidney transplant rejection, Natural killer cells, CD107a, Cytotoxicity.

12507P

Assessment secreting intracellular IFN-γ of natural killer cells in patients with kidney transplant rejection

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Introduction: Kidney transplantation is a recognised treatment for patients with end stage kidney disease. Despite an increasing awareness of innate immunity, the roles of natural killer cells in transplant rejection have not been clearly defined. **Materials and Methods:** In a case- control study, subjects in patients with 11 acute and 11 chronic kidney transplant rejection and control samples in people having kidney transplant with optimal activity and healthy individuals, each group consisting 22 people respectively, were selected. The secreting intracellular cytokine of natural killer cells, IFN-γ, and the amount of these cells in these groups were measured by flow cytometry techniques. In order to analyze the data distribution comparison used through Kruskal-Wallis test and in order to their two by two comparison used through Mann-Whitney test. **Results:** Based on the current study results, the intracellular secretion of IFN-γ in acute and chronic kidney transplant rejection, was increased significantly compared with stable graft group (P-value=0.022 and P-value=0.003 respectively). However, the percentage of NK cells in patients suffering acute and chronic transplant rejection compared with control groups was decreased. **Conclusion:** Our results showed the percentage of NK cells in patients with acute and chronic kidney transplant rejection was decreased; nevertheless, secretion of IFN-γ was increased. This finding demonstrated that the amount of cells circulating in peripheral blood of patients suffering rejection decrease but the cytotoxicity of these cells will increase. **Keywords:** Kidney transplant rejection, Natural killer cells, IFN-γ, Cytotoxicity.

Vaccine

Oral Presentations:

6504O

Immunogenicity study of artificial and immunogenic epitopes from Newcastle virus F gene in an animal model

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INTRODUCTION : Newcastle disease virus (NDV) is an infectious agent of a large variety of birds, including chickens, which poses a real threat to the poultry industry. NDV is embedded by spikes consisting of glycosylated HN and F proteins. While the mean death time after vNDV infection is 2–6 days, therefore the presence of preexisting antibodies prior to infection is the most critical way for protection from this disease. Antibodies produced against the fusion (F) trans-membrane surface glycoproteins are able to neutralize NDV and subsequently the infection by blocking viral attachment and inhibition of viral fusion with the host cell membrane. **MATERIALS AND METHODS:** In this research, bioinformatics studies were implemented to define the most immunogenic epitopes of F protein. Based on these analyses the synthetic F gene was cloned and expressed in heterologous system (*Escherichia coli*), using the appropriate vector (pET32a). Subsequently, the purified recombinant protein was *dialyzed* against phosphate buffer (PBS) and was injected to the mice subcutaneously (3 times) and the raise of specific antibodies was assayed in immune sera. **RESULTS:** The results showed that immunization of mice with this recombinant protein could elicit significant serum IgG antibody titers. Our data show that the recombinant F protein could be recognized by the mice sera immunized with commercial vaccine. **DISCUSSION:** The reactivity of vaccine strain with sera from F protein immunized mice suggested that the F protein is able to present similar epitopes with vaccine strain and hopefully could stimulate the immune system against the infectious viruses in the environment. **Keywords:** Newcastle disease virus, Fusion protein epitopes, Recombinant protein, Immunogenicity, IgG

7708O

A potent multivalent vaccine for modulation of immune system in atherosclerosis: An in silico approach

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Introduction: Atherosclerosis is classically defined as an immune-mediated disease characterized by accumulation of LDL-c over intima in medium and large sized arteries. Recent studies have demonstrated that both innate and adaptive immune responses are involved in atherosclerosis. In addition, experimental and human models have recognized many autoantigens in pathophysiology of this disease. Oxidized low-density lipoproteins (ox-LDL), β2 glycoprotein I (β-2-GPI) and heat shock protein 60 (HSP60) are the best-studied of them which can represent promising approach to design worthwhile vaccines for modulation of atherosclerosis. **Materials and methods:** In silico approaches are the best tools for design and evaluation of the vaccines before initiating the experimental study. In this study, we identified immunogenic epitopes of HSP60, ApoB-100 and β-2-GPI as major antigens to construct a chimeric protein through bioinformatics tools. Additionally, we have evaluated physic-chemical properties, structures, stability, MHC binding properties, humoral and cellular immune responses, and allergenicity of this chimeric protein by means of bioinformatics tools and servers. **Results:** Validation results indicated that 89.1% residues locate in favorite or additional allowed region of Ramachandran Plot. Also, based on Ramachandran plot analysis this protein could be classified as a stable fusion protein. In addition, the epitopes in the chimeric protein had a strong potential to induce both the B -cell and T-cell mediated immune responses. **Conclusion:** Our results supported that this chimeric vaccine could be effectively utilized as a multivalent vaccine for prevention and modulation of atherosclerosis. **Keywords:** Atherosclerosis, multivalent vaccine, HSP60, ApoB-100, β-2-Glycoprotein I

9731O

Improved anti-Treg vaccination targeting FoxP3 could induce both cellular and humoral responses in mice

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Introduction: Apparently, regulatory T cells (Treg) play a critical role in attenuating immune responses against tumor cells in tumor microenvironment. Therefore, instructing immune response against Treg expressing Foxp3 transcription factor is a hopeful approach to decrease Treg frequency. In current study, we used a fusion construct expressing Fc portion of IgG and Foxp3 (previously constructed by our group) to effectively instruct immune response against Treg. **Materials and methods:** DNA construct containing Fc portion of IgG fused to Foxp3 and its recombinant fusion protein were injected as DNA and protein vaccines respectively into C57BL/6 mice versus

control groups of vaccination. After 2 weeks, mice were bled and Specific IgG againt FoxP3 was measured in serum. Frequency of Treg in spleen, cytokine assay, CFSE proliferation assay and cytotoxicity assay were performed. **Results:** Flow cytometry 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. Increase of anti-FoxP3 specific IgG was also significant in the same group. IFN-γ secretion, In vivo and In vitro cytotoxicity assay and CFSE Proliferation assay against FoxP3 protein confirmed the efficiency of the vaccination. **Conclusions:** We conclude that targeting Treg expressing FOXP3 by our novel fusion construct cause more efficient immune responses against Tregs and decrease its content. This novel approach can be considered for efficient elimination of Treg in tumor bearing mice besides other means of immunotherapy. **Key words:** Regulatory T cell; FOXP3; Fragment c (Fc)

9757O

Whole recombinant yeast vaccine expressing leishmania major (L. major) antigen LmSTI1 protects mice against cutaneous leishmaniasis

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Introduction: The lack of a proper adjuvant is one of the obstacles in vaccine development against leishmaniasis. Whole recombinant yeast vaccine exhibiting intrinsic adjuvant properties of yeast cell walls is considered as a novel approach to induce protective immunity in cancer and infectious diseases. Herein, we describe the immunological responses of whole recombinant yeast expressing leishmania antigen LmSTI as a candidate vaccine against cutaneous Leishmaniasis in BALB/c mice. **Materials and Methods:** Four- to 6-week-old Female BALB/c mice were immunized in three doses with three weeks intervals. Groups 1 to 4 received 1YU(1x105) heat-killed yeast expressing LmSTI1 (K-YLM), 1YUheat-killed yeast (K-Y), 20 µg recombinant LmSTI1 formulated in montanide ISA-720 (L-720) and PBS, respectively, followed by challenging with L. major promastigotes. The course of the disease was monitored until 8 weeks after challenge. **Results:** Results showed that the mice immunized by K-YLM are able to produce the strongTh1immune responses, as well as high levels of IFN-y and IL-17A with no significant differences of IL-4 and IL-10 before and after challenge. In addition, the level of IgG and IgG2a antibodies was high in both L-720 and K-YLM groups before and after challenge. DTH responses elicited a significant increase ($P<0.05$) in vaccinated groups along with decreased lesion development and lived parasites in mouse lymph node even 8 weeks after leishmania infection, indicating long-term protection in the murine model. **Conclusion:** In this study, we introduced whole yeast cell expressing LmSTI1 for cutaneous leishmaniasis, highlighting its potential capacity as a candidate vaccine against leishmaniasis.

9758O

Listeriolysin O Plasmid as Genetic Adjuvant for Hepatitis C virus DNA vaccine

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Introduction: *Hepatitis C Virus* (HCV) is a major cause of liver disease in the world. To develop HCV vaccine, Induction of potent T cell response against immunogenic and conserved regions such as NS3 (non-structural protein 3) is considered. To enhanced DNA vaccine potency, The Listeriolysin O (LLO) of *Listeria Monocytogenes* with CD4 and CD8 epitopes is an alternative approach to modulate DNA vaccine. Herein, we cloned NS3, LLO in pcDNA3.1 plasmid and evaluated T cell immune response of NS3 DNA vaccine with LLO adjuvant in BALB/c mice. **Materials and Methods:** cDNA corresponding to partial immunogenic length of NS3 (NS3p, aa 1191-1380) and 442 amino acids of N-terminal LLO fragment were cloned separately into the pcDNA3.1 plasmid. The recombinant plasmids (pNS3, pLLO) were transfected to HEK293T cell line and protein expression was confirmed by Western blotting. The mice were immunized three doses with co-delivery two plasmids or separately and pcDNA3.1 (as control groups) in 3-week intervals and their immune responses were evaluated using cytotoxic T lymphocyte (CTL) activity by lactate dehydrogenase releasing method and IFN-g assay by ELISPOT. **Results:** The experiments showed plasmids encoding recombinant NS3 and LLO were constructed and confirmed by restriction enzyme analysis with expected sizes and sequencing. Proper expressions of the recombinant proteins were confirmed using western blotting. The immunization results indicated that the co-delivery pNS3 and pLLO immunized group was significantly enhanced T cell response especially Th1 responses compared to control groups ($p<0.05$). **Conclusion:** This results demonstrated that pLLO can be a potent adjuvant for enhancing NS3 DNA vaccine-induced immunity.

9800O

A live nonpathogenic *Leishmania tarentolae* vaccine containing sand fly salivary antigen PpSP15 and CpG motifs induces the generation of Th17 and Th1 cells against *Leishmania major* infection in BALB/c mice

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Introduction: Cutaneous leishmaniasis is a zoonotic, vector-borne disease causing a major health problem in several countries. No vaccine is available and there are limitations associated with the current therapeutic regimens. A cellular immune response to PpSP15, a protein from the sand fly Phlebotomus papatasi, was sufficient to control *Leishmania major* infection in mice. In an effort to retain the immunological benefits, while avoiding the side effects of leishmanization, we immunized mice with nonpathogenic recombinant *L. tarentolae* secreting PpSP15 along with immunostimulatory oligo deoxynucleotides (CpG ODN). **Materials and Methods:** We generated a recombinant *L. tarentolae*-PpSP15 strain delivered in the presence of CpG ODN and evaluated its immunogenicity and protective immunity against *L. major* infection in BALB/c mice. In parallel, different vaccination modalities using PpSP15 as the target antigen were compared. Humoral (IgG1, IgG2a) and cellular (IL-6, IL-5, IL-17, IFN-γ, TNF-α) immune responses were evaluated before and at three and eight weeks after challenge. Footpad swelling and parasite load were assessed at eight and eleven weeks post-challenge. **Results:** In this study, the level of humoral and cytokine expression and parasite load in different modalities showed the immunogencity of prepared recombinant *L. tarentolae*-PpSP15. Apart from the specific immunogenicity of PpSP15, CpG has an important role in inducing Th1 and Th17 cells in combination with a non-pathogenic live *L. tarentolae*. **Conclusion:** Our results show that

vaccination with *L. tarentolae*-PpSP15 in combination with CpG as a prime-boost modality confers strong protection against *L. major* infection that was superior to other vaccination modalities used in this study.

10959O

Oral administration of Lactococcus lactis displaying Acinetobacter baumannii phospholipase D induces a protective immune response in murine model

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Introduction: Acinetobacter baumannii is an opportunistic pathogen which can cause human life-threatening infections due to increasing rate of antibiotic resistance A. baumannii strains. phospholipase D (PLD) is as an important virulence factor associated with A. baumannii development in the host. It was proven that immune responses elicited by antigens expressed on L. lactis surface are stronger than those expressed intracellularly. The present study was designed to evaluate immunogenicity of phospholipase D displayed on Lactococcus lactis as a vaccine candidate against A. baumannii. **Material and Methods:** In order to produce PLD on L. lactis surface, a chimeric gene was designed. The synthetic gene was then subcloned into a lactococcal vector for the expression of gene under the control of nisin a inducible promoter. Mice vaccine group received four cycles of orogastric immunizations at 2-week intervals. Each cycle consisted of gavaging 2 × 109 CFU for 3 consecutive days of induced recombinant L. lactis suspended in 100 µL sterile PBS. Control group received L. lactis:vector in the same manner. The serum IgG immune responses were analyzed and the mice were challenged with lethal doses of A. baumannii. **Results:** Immunized mice group exhibited IgG rise and 100% survival rate at 108 and 109 lethal doses of A. baumannii. Those receiving 1010 and 1011 bacteria were protected 80% and 40% respectively. All the control groups died within 24 hours of challenge. **Conclusion:** Recombinant L. lactis displaying PLD was potentially effective in triggering a high antibody response and protecting mice against A. baumannii. **Key Words:** Acinetobacter baumannii; Phospholipase D; Lactococcus lactis; Immunity; Display

11019O

Construction of Recombinant Saccharomyces boulardii as a vaccine delivery vehicle via oral system

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Introduction: Saccharomyces boulardii (S. boulardii) is the best known probiotic yeast. Recombinant technology has led the way to monumental advances in the development of useful molecules, including the development of safe probiotics. The development of novel approaches using recombinant technology and probiotics that allow accurate targeting of therapeutics to the mucosa is an interesting area of research. Because ovalbumin is a model protein for immune stimulating, therefore ovalbumin was used as an antigen and transformed into S. boulardii. **Method:** For construction of plasmids we used pYES-2 backbone including 2 micron and Ura3 gene that is suitable for Ura3-auxotrophic S. boulardii strain, constructed in our laboratory, after that we cloned ovalbumin gene along with mating factor in front of GAL-1 promoter in pYES-2 plasmid. In addition to Gal-1 promoter, we used two more constructs that contained a continuous promoter PGK-1 and TEF-1 respectively.**Results:** After cloning of ovalbumin gene into pYES-2, all three constructed plasmids confirmed by digestion and sequencing, after that we expressed all the constructs in BY-4742 strain and then expression confirmed by western blot. **Conclusion:** Recombinant S. boulardii can be an appropriate choice to deliver a new antigen and therapeutic proteins via oral system.

11151O

Comparison of antibody response to different combinations of recombinant and native *Bordetella Pertussis* antigens in BALB/c mouse

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Introduction: Bordetella Pertussis (BP) is the etiologic agent of whooping cough which still causes a significant mortality in children. Efficacy improvement of the current acellular pertussis vaccines is a health priority. In this study, the mouse antibody response to recombinant fragments of filamentous hemagglutinin (FHA) and Pertactin (PRN) and native pertussis-toxin (PT) has been investigated following immunization with different formulations of the antigens. **Materials and Methods:** Four and two overlapping recombinant fragments of FHA and PRN, respectively, were expressed in *E-coli* and purified by affinity chromatography, while PT was purified from bacterial suspension of BP. The purity and concentration of antigens were determined by SDS-PAGE and BCA assays, respectively. BALB/c mice were immunized twice with different combinations of antigens and received a respiratory challenge with wild type BP. Serum sample, bronchoalveolar lavage (BAL) and splenocytes of mice were collected for measurement of antibody against recombinant fragments and native antigen, using an antigen-based ELISA. Lung clearance was also investigated by CFU-assay. **Results:** Dominant antibody response against two fragments, PRN2 and FHA3, spanning amino acids 341-699 and 1877-2250 of the mature PRN and FHA molecules, respectively, was observed following vaccination with recombinant antigens as well as acellular standard vaccine. CFU-assay results showed bacterial growth in culture of the BALs of unvaccinated challenged mice, but no bacterial colony was observed in vaccinated mice. **Conclusion:** Our results suggest that PRN2 and FHA3 are the immunodominant fragments of PRN and FHA molecules. In addition, all formulations were able to block the bacterial colonization similar to the standard acellular vaccine. **Keywords:** Bordetella pertussis, acellular vaccine, Pertactin, filamentous hemagglutinin, Pertussis toxin

11291O

Immunization with recombinant Crimean-Congo Hemorrhagic fever virus Gn protein expressed in insect cells results in induction of T helper 1 (Th1) response and induction of IL-10

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Introduction: Crimean Congo hemorrhagic fever (CCHF) is one of the deadly hemorrhagic fevers that are endemic in Asia, Africa, Eastern Europe, and the Middle East. It is a tick-borne zoonotic viral disease caused by CCHF virus of genus Nairovirus (family Bunyaviridae). Iran is an endemic area and Sistan-Baluchestan Province is considered as hot spot for this disease. Currently there is no vaccine against CCHFV. The recombinant technologies have opened new strategy for developing a vaccine against this highly pathogenic virus. In this report, we investigated the immune response by the recombinant Gn from CCHFV in adjuvant. **Material and Methods:** CCHFV Gn protein expressed by baculovirus expression systemwas inoculated subcutaneously in mice groups with Freund's adjuvant. Then spleen and lymph nodes of immunizedmice were removed and single cell suspensionculture was sprepared and stimulated with recombinant Gn. IL-10 levels were evaluated using the ELISA technique. **Results:** Spleen and lymph nodes cells from immunized micewith recombinant Gn produced high level of IL-10, which was significantly higher than that in the control groups, suggesting the induction of a T helper 1 (Th1) response. **Conclusion:** Based on these results, it can be concluded that baculovirus could be considered as a good platform in production of recombinant Gn antigen and would be of value in the development of subunit vaccine studies against CCHFV.
Keywords: Crimean Congo hemorrhagic fever; Vaccination; IL-10; Baculovirus.

Poster Presentations:

4492P

The study of serum antibody level in patients with Acinetobacter baumanni infection and comparing with healthy people and intensive care unit personnel in Laleh and Masih Daneshvari hospitals in Tehran, to determine an immunogenic proteins for vaccine candidate

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Introduction: Nosocomial infections with bacterial origin are considered as one of the most dangerous threats to global health. Among the most important causes of bacterial infections, Acinetobacter baumanni is the main concern. The purpose of this study wasto determine immunogenic proteins for vaccine candidate. **Methods:** In this study, 70 serum samples from Daneshvari and Laleh hospital in three groups of patients (20 samples) with positive sputum culture of Acinetobacter, ICU nurses (20 samples) and normal controls (30 samples) have been collected. PCR was performed based on the presence of OXA-51 gene for bacterial isolates. Dot blot test for screening has been done and the presence of antibodies against Acinetobacter baumanni were measured by ELISA. Pooled serum samples containing high titers of antibody has been prepared and IgG antibody was isolated by affinity chromatography. The MACS technique was done to separate the bacteria with high virulence factors. The high virulent bacteria with positive serum was studied by Western Blot. To evaluate the results of Western blot bands, MS Spectrometry was done. The packed bands were sent to PTY LTD Proteomics Company in Australia for MALDI TOFF. Results were analyzed using the MASCOT software. **Results** :Western blotting showed 5 bands in the areas 60, 35, 20, 15, 10kd of marker. Evaluation of the software Mascot MOLDI TOF spectroscopy indicates the presence of OMP-A protein of Acinetobacter baumannii in 35kd band, carbapenem-associated resistance protein precursor in 25kd band and stress induced bacterial acidophilic repeat motif in 60kd band. **Conclusion:** By the pursuit of superficial bacterial antigenic index (Omp-A) and bioinformatics methods the outer membrane protein can be evaluated as a candidate for Research on animal models used in vaccine production. **Keywords:** Nosocomial infection, Acinetobacter baumanni, Vaccination.

7566P

A Model of Cost Effectiveness of HPV Vaccination in Iran

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Introduction: The human papilloma virus (HPV) is one of the most common sexually transmitted viruses. Chronic infection with certain subtype of the HPV is the primary cause of cervical cancer and its precancerous lesions. Despite of screening programs for cervical cancers, it remains the second most common cause of cancer-related death among women worldwide. Vaccines are essential tools for preventing the diseases. The aim of this study was to describe a model to estimate the cost –effectiveness of HPV vaccination in Iran. **Materials and methods:** We develop an incidence based model based on available data in Iran on the epidemiology of HPV related health outcomes. Incremental cost –effectiveness ratio (ICER) calculation and one way sensitivity compares the cohort of 15-years old girls with and without vaccination. Similar to US study model approach we compare the baseline screening strategy with the second strategy including routine Gardasil (HPV) vaccination. We measured the cost per QALY ratio as the ICER difference between two strategies. **Results:** WHO has recommended that a health care technology is cost effective if the ICER is less than triplet of GDP (Gross Domestic per Capita).comparing the cost – effectiveness threshed of Iran to other country shows that this vaccine is not cost – effective and according to the sensitivity analysis was limiting the indication of vaccination. **Conclusion:** Gardasil vaccine is not recommended in Iran based on case parameters values. **Key Words:** model, HPV, Vaccine

7707P

Production of new recombinant fusion protein as a potential vaccine for targeting angiogenesis

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Introduction: Angiogenesis is very important in cancer growth and metastasis. Basic fibroblast growth factor (bFGF) as one of the most important angiogenesis factors is a good target for cancer vaccine. Due to low immunogenicity, it cannot stimulate an effective immune response. Theoretically, Pseudomonas toxin as a potent immunogenic carrier when fused to low immunogenic antigens significantly increased immunogenicity of it. In this study, we tried to perform the molecular cloning and expression of bFGF conjugated with immunodominant domain of pseudomonas toxin. **Materials and Methods:** *It was synthesized* and expressed using recombinant DNA technology in the bacterial expression system. Expression of recombinant protein was verified using SDS-PAGE and western blot technology. Finally, it was purified using Ni NTA column. **Results:** The 37 kDa band in SDS-PAGE and western blot was aligned completely to designed sequence. Purified recombinant protein also showed as a clear single band near to 37 kDa. **Discussion:** In this study, new recombinant fusion protein as a new potential vaccine was produced.

9782P

The Effects of Recombinant Leishmania Major Lipophosphoglycan 3 (LPG3) on Activation and Cytokine Secretion of Human T Lymphocytes

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Introduction: Leishmania major, the main causative agent of Cutaneous Leishmaniasis (CL), remains as a serious public health concern in many tropical and subtropical regions. Some Leishmania antigens have been introduced to serve in vaccination which creates long-lasting protection against leishmaniasis. Lipophosphoglican 3 (LPG3) is a member of heat shock protein 90 (HSP-90) family and plays a role in assembly and synthesis of lipophosphoglycan (LPG) that implicates in parasite virulence. The aim of this study was to investigate the ability of recombinant LPG3 (rLPG3) to induce human T lymphocyte activation in vitro. **Materials and Methods:** Peripheral blood mononuclear cells were obtained from heparinized blood samples of 10 normal healthy volunteers. Naïve T-cells were then isolated by MACS technology and their purity was assessed by flow cytometry. T-cells were co-cultured with different concentrations of rLPG3. After 48 hours incubation, the activity of treated T-cells was investigated by assessment of cytokine secretion and CD69 surface expression. **Results:** The purity of isolated T lymphocytes was over than 90%. rLPG3 showed stimulatory effect on IFN-γ secretion by treated T lymphocytes ($P<0.01$). Moreover, rLPG3 induced CD69 activation marker on the surface of human T lymphocytes ($P<0.01$). **Conclusions:** Recombinant LPG3 could potentially activate T lymphocytes and consider as a powerful adjuvant in vaccination strategies against leishmania infection.

9817P

GM-CSF, a potent adjuvant for polarization to Th-17 pattern: an experience on HIV-1 vaccine model

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Introduction: Disturbance in function of humoral and cellular immune system components such as T cells and B cells is considered as an indicator of HIV disease . So that, several studies demonstrate that use of an effective adjuvant for induction of two arms of adaptive immune system is really essential.one of the best adoption is Granulocyte-macrophage colony-stimulating factor (GM-CSF); because GM-CSF cytokine is able to induce immune cellular and humoral system responses through increasing of IFN-γ, IL-4 cytokines and Antibody isotypes. Here, the effect of GM-CSF as an adjuvant on the polarization into TH17 was assessed. **Materials and methods**: Experimental mice were immunized with HIV-$1_{tat/pol/gag/env}$ DNA vaccine with GM-CSF and Boosted with recombinant vaccine. Lymphocyte proliferation was evaluated using Brdu method, and CTL activity, IL-4, IFN-γ, IL-17 cytokines, Total antibody and IgG1 and IgG2a isotypes were assessed with ELISA. Results were analyzed using graph pad prism software. **Result:** Immunization of candidate vaccine using GM-CSF as an adjuvant increased lymphocyte proliferation, IFN-gamma and IL-17 cytokines. Also total antibody and both IgG1 and IgG2a were increased when vaccine injected with GM-CSF. **Conclusion**: The results of this study indicate that GM-CSF cytokine is able to Induce humoral and cellular immune responses and also polarization to Th17 pattern responses.

10781P

Expression of a fusion protein of two high immunogenic insilico fragments of *clostridium novyi* alpha toxin and *clostridium perfrin*gens type D epsilon toxin

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Introduction : *Clostridium perfringens* epsilon toxin and *Clostridium novyi* alpha toxin are two important and potent toxins cause gas gangrene in humans and animals. According to the importance of these two toxins, vaccination against their secreting agents seems crucial in Livestock industry. Multi-epitope vaccination technology is a good alternative for toxoid vaccines and is more effective. The aim of this study is to insilico design a fusion protein of two high immunogenic fragments from two mentioned toxins and expression of fusion protein in E.coli BL21 D3 bacterial host. **Materials and methods:** The Primary sequences of alpha and epsilon toxins were retrieved from NCBI and uniprotKB database. The secondary structure (α-helix and β-sheet) of fusion protein was predicted by PSIPRED Homology modeling of fusion protein was obtained by I-TASSER. B cell epitopes predicted by using

IEDB server, Kolaskar and Tongaonkar antigenicity method and SEPPA tool. The purified PCR products of two separate genes were cloned with T/A cloning kit. Recombinant plasmids were enzymatically digested and ligated together with an appropriate linker as spacer. Recombinant fusion protein was expressed in pET28 (a+) into BL21 DE3. Immunological methods were used to confirm presence of recombinant fusion protein. **Results:** the fusion protein was analyzed immunologically and the result shows that it can compare with anti serum of the natural toxins. This product, after more experimental works can be used as a novel subunit fusion protein for production of polyclonal antibodies against both alpha and epsilon toxins. In addition, this fusion protein with multifunctional antibody production capability can be used in diagnostic tests and as a candidate for vaccine application.

10884P

Novel formulation of FMD vaccine shows superiority versus traditional Iranian FMD vaccine

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Introduction : FMD is one of the most important viral infectious disease among animals, specially ruminants cloven hoofed, that have harmful effect of trade in livestock and livestock products. One way to prevent this disease is vaccination in cattle. In this study, a novel formulation of FMD virus was prepared in Montanide ISA 201VG and its immunogenicity was compared with traditional FMD vaccine formulated in alum adjuvant. **Materials and Methods:**Killed virus was formulated in alum and also Montanid ISA 201VG adjuvants, and was injected twice with two weeks interval to experimental Balb/c mice with matched control groups. Ten days after last vaccination, TNF-a and IFN-gamma cytokines and total antibodies were assessed with ELISA method. **Results:** The results show that, both formulations of vaccine induced humoral immune responses but vaccine formulated in Montanide ISA 201VG was more potent in the release of INF-γ cytokine and induction of Th-1 immune response and also TNF-a production. **Conclusion:** It is seemed that Montanide ISA 201VG formulated vaccine is a potent and better vaccine in the induction of Th1 immune responses and may be a good candidate for replacement of alum based FMD vaccine in Iran.

10892P

Th1/Th17 pattern of oil-based formulation of HPV16 E7d Vaccine

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Introduction: HPV is known as a main factor of cervical cancer which is the second most common cancer worldwide among women that sometimes leads to death. So prevention of such disease seems to be necessary. In

order to experience a good vaccination and receive an appropriate result, cellular immunity and inflammatory responses must be stimulated. To do so, Th1 and Th17 must be considered as vaccine's targets. Thereby, we formulated the candidate vaccine in oil-based adjuvant to receive more potent immune responses versus alum-based one. **Materials and Methods:** The candidate vaccine was formulated in oil-based adjuvant Montanide and alum adjuvant and administrated to C57BL/6 mice three times with 2 weeks interval. Ten days after last vaccination, spleen cell culture supernatants were collected and IFN-γ and IL-17 cytokine levels were assayed by ELISA method. **Results:**The results showed that the oil-based adjuvant-formulated vaccine leads to increase of IFN-γ and IL-17 cytokine levels versus alum formulated one. **Conclusion:** The study indicated that formulation of candidate vaccine with oil-based adjuvant leads to induction of cellular and inflammatory responses.

10896P

FMD formulation in Montanide ISA-61 VG : Induction of Th1/Th2 immune platform

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Introduction: Foot-and-mouth disease virus is a member of Aphthovirus genus and the Picornaviridae family. The virus causes disease in cloven hoofed animals that are highly contagious and cause economic loss to society. Currently available FMD vaccines are mainly based on inactivated viral antigens formulated in alum. The aim of this study was to evaluate the immunogenicity of FMD vaccine formulated in Montanide ISA-61 VG with alum based one. **Materials and Methods:** FMD virus is produced in BHK-21 cell cultures. In-activated virus is used in formulation of vaccine in alum and Montanide ISA-61 VG . Experimental mice were immunized subcutaneusly with vaccine formulations two times, with two week interval. Ten days after last immunization, IL-4 and IFN-γ cytokines, total antibodies and IgG subtypes were determined with ELISA method. **Results:** Results show that immunization with both formulations of vaccines induced Th1/Th2 immune platform in the experimental groups. **Conclusion:** Based on this study results, Montanide ISA-61 VG can be used as an adjuvant for FMD virus. However more studies like challenge in animal model are needed to present the new formulation as a candidate vaccine.

10924P

R26, a novel oil-based adjuvant, comparison of adjuvant activity of R26 with Montanide ISA 206 and Montanide ISA 266

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Introduction: Cervical cancer is the second most common cancer worldwide among women and HPV is the most important factor in cervical cancer infection that sometimes causes fatality. Since HPV-16 E7 protein is expressed in all stages of cervical cancer and is found on cancercell surfaces abundantly, this protein would be an appropriate candidate in designing therapeutic vaccines. In this study, adjuvanticity effects of Montanide ISA 206 and 266 were assessed versus R26 adjuvant on HPV-16-E7d vaccine model. **Materials and Methods:** In this study, after preparing Montanide ISA 206, 266 and R26-formulated candidate vaccine, the vaccine was administrated three times with two weeks interval with 20 µg E7d protein. Then, two weeks after last vaccination, lymphocytes proliferative responses with Brdu method and total antibody production were evaluated with ELISA. **Results:** The results showed that the candidate vaccine formulated with Montanide 206, 266 and R26 stimulated lymphocytes proliferative responses, also total antibody production appropriately. **Conclusion:** The study demonstrated that HPV-16E7d formulated with these adjuvants is able to induce cellular and humoral immune responses and formulation in R26 adjuvant induced comparable response to Montanide ISA 206 and 266 adjuvants.

11099P

Immunotherapy of Atherosclerosis: A Novel and Robust Approach for Lipid Lowering

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Atherosclerosis remains a most significant global public health problem and there is still a clinical need for a novel therapeutic strategy to decrease its incidence. Alternatives to pharmacological treatments for atherosclerosis are highly desirable in terms of cost and compliance. Atherosclerosis, the primary and main cause of cardio vascular diseases, is a progressive and multifactorial process characterized by lipid abnormity. A decrease in high-density lipoprotein cholesterol (HDL-C) or an increase in low-density lipoprotein cholesterol (LDL-C) plays an important role in atherosclerosis development. Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that mediates transfer of cholesteryl esters from HDL to LDL, and thereby increases the concentration of LDL-C known as bad cholesterol. Hence, High activity of CETP develops atherosclerosis formation and inhibition of excessive CETP activity to the normal level is important to prevent atherosclerosis. Several lines of in vivo studies and clinical trials have been shown that inhibition of CETP through active (vaccine) or inactive (antibody) immunotherapy can decrease LDL-C and increase HDL-C concentrations leading to amelioration of the atherosclerosis lesions. Clinical studies, including phase III clinical trials, are now underway to describe the effect of CETP inhibition by immunotherapy on cardiovascular disease, and the safety and efficacy profile of theses approach. The present systematic review is conducted to introduce theses novel therapeutic approach and its benefits and effectiveness in patient with high cholesterol at risk for cardiovascular disease.

10999P

In silico analysis of a multi-epitope chimeric protein as a subunit vaccine for Brucellosis

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Introduction: It has been established that using a subunit vaccine targeting multiple antigens could be considered as a perfect approach for prevention and treatment of brucellosis. In last decade more studies revealed the most effective immunogenic antigens of brucella including Omp28, bp26, BCSP31, SOD and L7-L12. **Method:** *In silico* design is an essential tool for vaccine evaluation prior to experimental studies. Hence, immunogenic epitopes of Omp28, bp26, BCSP31, SOD and L7-L12 through bioinformatics tool were determined. Then, a chimeric DNA was synthesized from dominant B and T cell epitopes of this immunogenic antigens. The chimeric DNA structure, its mRNA, and deduced protein were analyzed by related bioinformatics software. Afterward, the three dimensional (3D) structure was predicted by Modeler software. At last, validation of the predicted protein was evaluated by Ramachandran plotstatistics and the B cell epitpes on the surface of the predicted model were mapped. **Result:** The predicted 3D structure of the chimeric protein showed that most of the dominant epitopes were folded individually. Also, validation experiment showed that most residues of chimeric protein are located in favorable regions of the ramachandran plot. Finally, this predicted protein was able to induce cell and humoral immune responses. **Conclusion:** *In silico* analysis confirmed that this chimeric protein can be effectively expressed and utilized as a vaccine against brucellosis. **Keywords:** Brucellosis; In *silico;* multi-epitope

11040 P

In vitro evaluation of differentiation and maturation of human dendritic cells transfected with synthetic mRNA that encoding core antigen of hepatitis C virus .

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Introduction: Hepatitis C virus is a blood borne disease estimated to infect more than 350 million people globally and is a leading cause of liver cirrhosis, transplantation and hepatocellular carcinoma. Current gold-standard therapy often fails, has significant side effects in many cases and is expensive. The fact that a significant proportion of infected people spontaneously control HCV infection in the setting of an appropriate immune response suggests that a vaccine for HCV is a realistic goal but no vaccine is currently available. The present study was designed to investigate the possibility of a new vaccine against the hepatitis C virus based on mRNA encoding of Core antigens of hepatitis C virus. **Material and methods:** The nucleotide sequence of mRNA encoding core antigen of HCV virus by the Bioinformatics program was designed and in PGE plasmid vector was prepared .Then in vitro transcription reactions are used to synthesize mRNA from this recombinant DNA template. Nanoparticles encapsulated this mRNA synthesized and delivered to Monocytes isolated from human Buffy coat and the

activation and differentiation of monocyte to dendritic cells was examined . **Result and conclusion:** In conclusion, Our preliminary results showed that immature DC transfected with synthetic mRNA , developed a characteristic of mature DC morphology and up-regulate the surface expression of the major histocompatibility complex type II molecule human leucocyte antigen-DR, the co-stimulatory molecules CD80, CD86 and CD40 and the differentiation marker CD83. **Key words:** Hepatitis C virus, mRNA, Core

11070P

In-silico Prokaryotic expression vector contains Neuraminidase immunogenic epitopes of Influenza virus in order to inducing optimized immune system

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Introduction: Development of an universal Neuraminidase peptide–based Influenza vaccine has considerable value. In spite of experimental procedures, computational analysis is a convenient way to screen and select B-cell and T-cell antigenic epitopes. The aim of this study was to design a multi-epitope universal vaccine for major neuraminidase proteins, NA1 of Influenza A H1N1, using bioinformatic models. **Material & method:** we predicted the most probable immunogenic epitopes of NA1 from H1N1, based on BCPREDS defaulted models, while solvent accessibility of structure was extrapolated. The 3D molecular model of N1 protein was constructed by Swiss Model server, whereas multiple-sequence alignments were provided from NA proteins for epitopes prediction. After that, N-Glycosylation sites excluded from estimated epitope regions, then by other bioinformatics analysis, selected epitopes fused in tandem, reverse translated and codon optimized to the relevant sequence.The final protein parameters like antigenicity analyzed by Protean program. Finally, DNA sequences containing relevant epitopes of NA protein modified for introducing into expressing vectors. **Result & discussion:** In early epitope screening, near 140 epitopes selected that 20 epitopes with higher degree of solvent accessibility and higher degree of homology refined from 26 common H1N1 sero types. These epitope sequences fused together in tandem repeats. Analyzed plots showed that major parts of new protein constructs have hydrophilic properties; thus harboring antigenic potency. Finally this sequence ligated into pET28a bacterial expression vector. The new recombinant protein harbouring 20 B-cell epitope seems to be a suitable antigen based on computational methods as a universal vaccine candidate for H1N1.

11076 P

Evaluation of Preventive Effect of polyclonal antibody against ExotoxinA-Flagellin Pseudomonas aeruginosa infections in mice

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Introduction: Antibiotic resistance and the need for long-term treatments especially for chronic infections necessitate the development of a vaccine against *Pseudomonas aeruginosa* infection. **Materials and Methods:** A 1-year-old New Zealand White rabbit was immunized by Exotoxin A- Flagellin in combination with Freund's adjuvant. immunized rabbit serum was collected and precipitated by %50 ammonium sulphate. purified antibody was evaluated by injection of it to 5 groups of mice and they were challenged with a 2X LD50 of *P. aeruginosa* for protection assay. **Results:** Passive immunization of mice with exotoxin A-flagellin antibody showed significant protection against intra-peritoneal challenge with (2X LD50) *P. aeruginosa.* **Conclusion:** Results of this study suggested that recombinant ExoA- Flagellin is a preventive protein which can be used as a new vaccine candidate against *P. aeruginosa*.

11116P

In-silico Prokaryotic expression vector contains Heamagglutinin immunogenic epitopes of Influenza virus in order to inducing optimized immune system

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Introduction: Development of universal Heamagglutinin Influenza peptide–based vaccine with more serotypes coverage has considerable value, computational analysis is a convenient way to screen and select B-cell antigenic epitopes. The aim of the present study was to design a multi-epitope universal vaccine for major neuraminidase proteinsHA1 Influenza A H1N1, using bioinformatics models. **Material &Method:** We predicted the immunogenic epitopes of HA1 influenza H1N1, based on BCPREDS defaulted models, while solvent accessibility of structure were extrapolated. The 3D molecular was constructed by Swiss Model server, N-Glycosylation sites excluded from estimated epitope regions, then by other bioinformatics analysis, selected epitopes fused in the tandem, reverse translated and codon optimized to the relevant sequence. The final protein parameters like antigenicity analyzed by protean program. Finally, DNA sequences containing relevant epitopes of HA1 proteins modified for introducing into expressing vectors. **Result:** In epitope screening, near 115 epitopes selected That among them 20 epitopes with higher degree of solvent accessibility and also higher degree of homology refined from 18 common H1N1sero types. These epitope sequences fused together in tandem repeats. Evaluation of new recombinant protein sequences indicated a molcular wieght of 40.3 kDawith 360 amino acids beside positive charges. Analyzed plots showed that major parts of new protein constructs have hydrophilic properties thus harbor antigenic potency. Finally this sequence ligated into pET28a bacterial expression vector. **Conclusion:** The new recombinant proteins harbouring 18 t CELL&B-cell epitope seems to be a suitable antigen based on computational methods as a universal vaccine candidate for H1N1. **Key words:** In silicon analysis; Heamagglutinin ;influenza ; Universal Vaccine

11157P

Proapolipoprotein A-1 derived from Fibroblasts as an auto-antigen in human

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Introduction: Fibroblasts, as the architect of connective tissue in different organs including the heart, are the most abundant cells of this member. According to our previous research, analysis of mass spectrometry showed that fibroblasts secrete proapolipoprotein A-1 under heat shock circumstances. In this study, the presence of antibodies against this protein was evaluated in human plasma. **Material and methods:** Fibroblasts were isolated from newborn foreskin and were cultured in 25cm^2 flasks until reach to 90% confluence. In order to heat shock stress, the flasks were incubated in 43°C for 2h. Then the cell culture medium was removed and fresh serum-free DMEM was added. Flasks were incubated in 37°C for 6h then supernatant was collected. The supernatants were concentrated by Amicon ultra centrifugal filter and were coated in ELISA plate. ELISA test was performed by five serum samples of atherosclerotic patient, who were confirmed by cardio angiography. Then the optical density of samples was measured with ELISA reader in 450nm. **Results:** The results of optical density which is measured by Elisa reader showed that 3 out of 5 patients have a remarkable level of IgG antibodies against proapolipoprotein A-1. **Conclusion:** Preliminary data from this study suggest that the secreted proapolipoprotein A-1 derived from fibroblasts under heat shock conditions can be an appropriate candidate for vaccine designing and atherosclerosis prevention.

11162P

An In silico analysis of 2014-2015 Iranian Human Papilloma Viruses L1 Protein: immunogenicity, antigenicity, and epitope mapping

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Introduction: Papillomaviruses are small tumor viruses with a genome of approximately 8000 base pairs. They are found in many different types and infect a wide range of animals and several primates including humans. HPV genome encodes six characterized proteins as E6 oncoprotein, E7 oncoprotein, E1 Replication protein, E2 Regulatory protein, L1 major capsid protein and L2 minor capsid protein. The aim of this research was a comparison analysis among recent Iranian L1 protein sequences and 3 major vaccine sequences and determining efficiency of these vaccines for Iran population. **Materials and Methods:** All sequences HPV L1 protein from Iranian isolates in 2014 and 2015 were selected and obtained from NCBI data bank. CLC sequence viewer and CLUSTAL X softwares were used to translate, alignment and construct phylogenetic trees. To predict B-cell and T-cell epitopes, we were employed ABCpred, BcePred Bepipred, and immuneepitope programs. Modification sites such as phosphorylation, glycosylation, and disulfide bonds determined by using GlycoEP, NetNGlyc, N-glycosylation, NetPhosK, NetPhos, and DISPHOS softwares. Secondary and tertiary structure and structure validation of all sequences analyzed by Qmean, (PS)^2, Phyre2server, and I-TASSER. **Results:** Many conserve regions were determined in all sequences. We found three major mutations in aa 176, 353, 474. Mentioned mutations showed the huge effect on B-

cell and T-cell epitopes and physico-chemical properties of L1 protein. Five disulfide bonds were determined in L1 protein; also several N-link and O-link glycosylation sites. **Conclusions:** our results showed some differences between approved vaccines and Iranian patients' sequences. So it is essential to consider these differences to design an effective vaccine for Iran population against HPV infection.

11196P

Immunization Evaluation of HIV-1 polyepitopic DNA candidate vaccine in mice model

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One of the priorities of global health and the challenges of today's world is the AIDS with 2 million deaths annually and 7,000 new infections daily (the fourth leading cause of death in the world). Because of genetic variety of HIV, lack of appropriate animal models and related natural protective immunity, the efforts to produce an effective vaccine against this virus failed. It is recognized that the scope and breadth of the immune response induction is very important to increase effectiveness of candidate HIV vaccines. Therefore, with regard to mammalian polyclonal immune response, putting together some immunogenic epitopes (from proteins which have important roles in the viral cell cycles and pathogenesis) into a single vaccine may induce strong immune responses against virus and so it may decrease the number of vaccine administrations. In this study, pcDNA3.1-*tat*/*pol*/*gag*/*env* eukaryotic expression vector was used as a candidate DNA vaccine and its immunogenicity was evaluated. BALB/c mice were immunized three times with 2 weeks intervals, subcutaneously, with candidate vaccine. Two weeks after the last immunization, the sera levels of IgG_1 (as a symbol of humoral immunity) and IgG_{2a} specific antibodies, proliferative responses, CTL Cytotoxicity and production of cytokine IFN-γ (as symbols of cellular immunity) were evaluated by ELISA (Enzyme- Linked Immunosorbent Assay) method. Totally, the results showed that the candidate DNA vaccine with subcutaneously injection has not been able to induce efficient cellular and humoral immune responses compared to the control groups. **Keywords**: HIV, DNA vaccine, cellular Immunity, humoral Immunity

11197P

Efficiency Evaluation of a Candidate Vaccine Based on HIV-1 *tat*- DNA in Mice Model

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HIV infection has created a major global health challenge. and extensive efforts have been made to prepare an effective anti HIV vaccine. However, all efforts have remained inconclusive to date. Various candidate HIV-1 vaccines have been developed including, recombinant protein DNA vaccines, subunit vaccines, live vectored

recombinant vaccines and various prime-boost combinations. Since the 1990s, most researchers devoted to use Tat (trans-activating proteins) as an immunogen of HIV vaccines. The HIV-1 *tat* gene codes a 14-16 kDa protein. It is an essential activator of HIV-1 transcription and is expressed during the early stages of viral infection. Recent phase II clinical investigations demonstrated that therapeutic immunization with Tat induced durable and safe immune responses. In this study, pcDNA3-*tat* eukaryotic expression vector was used as a candidate DNA vaccine and its immunogenicity was evaluated. BALB/c mice were immunized three times with 2 weeks intervals, subcutaneously, with candidate vaccine. Two weeks after last immunization, IFN-γ cytokines and IgG2a antibody levels were measured with direct and indirect ELISA (Enzyme-Linked Immunosorbent Assay) methods, respectively. Results showed that immunization with HIV-1 tat led to a significant increase in IFN-γcytokine production and IgG2a (symbols of cellular immunity) in comparison with the control groups (*p-value*<0.0001). Taken together the Tat DNA vaccine, because of conserved sequence and inducing high level of antibody has potentials as a suitable candidate vaccine against the HIV-1 virus. **Keywords:** HIV, Recombinant DNAVaccines, DNA Vaccines, ProteinTat, Cellular Immunity.

11202P

HBSAg formulated with Naloxone versus conventional HBSAg vaccine and Comparison to Fenderix

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Introduction: Hepatitis B virus is a major cause of liver infection and it considered the tenth leading cause of death in the world.The consequences of infection with this virus are cirrhosis of the liver or liver cancer.Due to the low efficiency of the alum adjuvant in inducing the appropriate immune response and presence of non-responder individuals to the vaccine it is necessary to design an effective vaccine.Naloxone is a morphine like receptor antagonist.In this study the effect of the Naloxone was compared with Fenderix vaccine. **Methods:** In this study hepatitis B vaccine was injected three times to female balb/c mice. One group recieved mixture of Naloxone with HBS vaccine and the other group recieved routine vaccines and a group recieved Fendrix vaccine from GSKcompany's and PBS was injected into control group. Finally,the formulated vaccine with Naloxone compared with the Fenderix vaccine by measuring levels of total anti-HBSAg,IFNɣ and IL4 by ELISA and splenic lymphocyte proliferative response against HBSAg was evaluated by BRDU method.Statistical analysis of data was performed by using SPSS software. **Results:** The level of total anti-HBSAg antibody and lymphocyte proliferation in Naloxone formulatd vaccine was comparable with Fenderix but IL4 and IFNɣ level significantly increased in vaccine formulated with Naloxone. **Conclusion:** Naloxone induced the appropriate immune response. The humoral responses was comparable with Fenderix.The cytokine response was higher than Fenderix vaccine and it can shift immune response to Th1 pattern.The findings suggest that Naloxone has an effective role in optimizing hepatitis B vaccine as an adjuvant. **Key words:** Adjuvant, HepatitisB vaccine, Naloxone

11214P

Evaluation of Immunogenic properties of recombinant fusion protein 4xM2e-HA influenza A virus expressed in MDCK cell line

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Introduction: 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. we triedto produce a universal vaccine 4M2e-HA that can be produced in large scale in reasonable time **Methods:** In this study a recombinant 4xM2e-HA gene of influenza A virus was designed and expressed in MDCK cell which could be secreted from 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. , also antibody against fusion protein could neutralize both heterologous and homologues influenza virus **Conclusion:** These findings suggest that4xM2e- rHA expression in MDCK cell may provide a new approach for developing a novel universal 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 4xM2e- rHA 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.

11226P

Evaluating the effectiveness of Montanide adjuvant to improve immunogenicity of hepatitis B vaccine

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Introduction: HBV infection is a life-threatening infection of liver that can lead to chronic liver disease. The common treatment in the control of viral replication and liver damage inmany patients has not been successful. Immunotherapy achievements have shown promising effects in the treatment ofchronic hepatitis B. Montanide ISA 720is a relatively new adjuvant which has metabolized natural oils and pure compound which is named Mannide. This adjuvant acts as a depot to slow release of antigens and to protect it from proteolytic enzymes and improved antigen presentation toT cells. Ability to stimulate both humoralandcellularresponse is a Montanaide advantage, taking into account the characteristics of the Montanide adjuvantis confirmed by research,we decided to evaluate the

effect of this formulations of hepatitis B vaccine with Montanide ISA 720that has not been tested to date. **Methods:** Inthisexperimental studyhepatitis B vaccinewas injectedtwo times to female balb/c mice . One group received formulated vaccinewithMontanaide ISA 720 adjuvant and a group received vaccine with alum adjuvant andthe other group received PBS as acontrol.AntiHBS Agproliferative responseof spleen lymphocytesevaluated byBRDUmethodandthe overall level of IFNɣ, IL4and antibodyagainstHBS Ag byELISA. Statisticalanalysis of data was performed using SPSS software. **Results:** Montanide significantly increased theoverall level ofantibodyagainstHBS Ag, IFNɣ and IL4 cytokinescomparedto the control group, lymphocyte proliferationlevels increased in comparison with control group as well. **Conclusion:** The results show Montanide ISA 720 as a oil based adjuvant can extremely promote level ofcellular and humoral immuneresponsesversushepatitis B vaccine. **Key words:** Adjuvant, HepatitisB vaccine, Montanide ISA 720

12372P

Development of Lieshmania major based vaccine against prostate cancer

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Introduction: Prostate cancer is a high mortality cause in American men. One out of every three men who are diagnosed with cancer each year has prostate cancer. Recently the development of cancer vaccines has offered the promise of a safer and more effective way for alternative to conventional cancer therapy. One of the best candidates is prostate specific antigen (PSA) is kalikerin serine protease (KLK3) which is secreted by prostatic epithelial cells. It is widely used as a serum marker for prostate cancer. High level of PSA is produced in malignant tissue. PSA is one of important target antigens against prostate cancer. The gentamicin-attenuated Leishmania major has been established in the present of gentamicin. It has been shown that the attenuated line is safe in 1800 volunteers. Leishmania has been shown with some benefit in comparing with viral or bacterial vectors. **Method and Materials:** The sequence of PSA gene containing 711 nucleotides has been designed. The gene inserted into Nco1 and Kpn1 site of an inducible vector. For transfection the linearized the attenuated line of L. major–PSA electroporated. The recombinant plasmid prepared in large scale with high purity, linearized and electroporated in to parasite. The transformants selected by plating solid medium containing bleomycin. **Conclusion:** Integration of PSA gene in the genomic DNA of the recombinant cells confirmed by PCR analysis and Western-blotting

12374P

Modification of immune response versus HPV-16 E7d vaccine via utilization of Naloxone/Montanide mixture as adjuvant

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Introduction: Adjuvants are defined as pharmacological and immunological components that are able to enhance antigen-specific immune responses which are used in combination with Antigen in order to stimulate immune responses . Studies show that Naloxone can shift immune responses toward Th1 patterns. In the present study the potency of Naloxone/Montanide mixture as an adjuvant is studied for E7d vaccine model. **Materials and Methods:** Recombinant pET28a/E7d plasmid was transformed to E.coli BL21 (DE3) and under induction with 1 mM IPTG the protein was expressed. After expression and purification of recombinant protein with Ni-NTA column, the protein was filtered and concentration was detected using Bradford method .Experimental C57BL/6 mice were vaccinated three times at two week intervals with 10 µg of E7d protein that formulated with Montanide, Naloxone or mixture of both adjuvants with Naloxone, Montanide and PBS control groups. Two weeks after last immunization, sera of experimental mice were collected and spleen cell suspension was prepared for immunoassay. Total antibody, IL-4 and IFN- cytokines were assessed with ELISA method and lymphocyte proliferation was evaluated with BRDU method. **Results:** Results indicated that the mixture of NLX-Montanide significantly increased the vaccine immunogenicity and IFN-gamma response. **Conclusion:** Naloxone/combination mixture increased IL-17 and lymphocyte proliferation versus each adjuvantation. Also this mixture as an adjuvant increased IgG2a versus other groups**.**

12380P

Cloning, expression and protein purification of ORF2 HEV virus in prokaryote system

Divbandi M

Introduction: Hepatitis E virus (HEV) is one of the major causes of hepatic failure worldwide and has a high mortality among pregnant women. Recently chronic HEV infections were reported. Therefore, it is necessary to develop an effective vaccine against this virus. Although a recombinant hepatitis E vaccine has been licensed in China, there are no other approved vaccines available in other countries. The aim of this study is to clone and express the truncated open reading frame 2 (ORF2) proteins- a hepatitis E virus capsid protein- in E. coli, which might be a potential HEV vaccine candidate in future. **Material and Methods:** The truncated orf2 gene which encodes 368-606 amino acid of the ORF2 protein (this part of the protein is named HEV239), synthesized in pUC18 and sub cloned into PET26b expression vector. The construct was confirmed by PCR and digestion. The production of HEV239 protein was confirmed by SDS-PAGE and Western blotting. The target protein was purified by native method for future purposes. **Results:** The highest amount of protein production in Rosetta was obtained by adding IPTG. expression and purification of HEV239 protein was confirmed by SDS-PAGE and western blotting. The size of the protein was 26 kDa. The highest amount of HEV239 in elution buffer was obtained at pH 4.5(and 2molar urea). The yield of the purified protein was about 400 µg/dl of culture media. **Conclusion:** In this study, HEV239, was expressed in E. coli and purified successfully for future purposes such as designing vaccine against HEV infection.

12391P

Assessment of features and examination of structural and functional role of the amino acids that make neurotoxin botulinum (BoNT) type E

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Introduction: botulinum neurotoxin can cause an often fatal food poisoning, botulism. The most effective means of defending against the toxin is by inducing a protective immune response through vaccination. Assessment of different features of proteins and identification of structural and functional amino acids can be useful in selection of suitable area for vaccine production. **Material and Methods:** ProtParam software, http://www.expasy.org/tools/protparam.html, computes various physico-chemical parameters, theoretical isoelectric point (pI), molecular weight, total number of residues, half-life and instability index of protein. Protein sequence was analyzed by using conseq software, http://conseq.tau.ac.il/. Software parameters were adjusted as follows: PSI-BLAST with five repetitions, Uniprot databases, e-Value equal to 0.01 and Maximum likelyhood (ML) as the method for calculating the degree of amino acid residue protection. **Result:** Neurotoxin botulinum E protein sequence contains 1589 amino acid. Its molecular weight is 130,522.6 Da and isoelectric point (pI) was calculated 5.16. The biocomputed half-life in mammalian reticulocyte, yeast and E. coli was 4.4h, 20h and 10 h, respectively. Instability index was calculated 26.99. Thus Expasy's ProtParam classifies this protein as stable. In the output of Conseq software program, surface and non-surface residues are determined, and their conservation scores are calculated. According to this principle that the conserved surface residue probably has a functional role and non-surface residues have a structural role, the role of residues will be determined. **Conclusion:** By obtaining the aforesaid information and considering that an appropriate vaccine containing exposed areas of the protein structure, this region is recommended as a good candidate for vaccine production.

Veterinary Immunology

Oral Presentations:

10988O

Examination of Urease immunogenicity against Brucella abortus and Brucella melitensis: influence of nanoparticulation versus traditional immunization

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Introduction: *Brucella* (B) species are causative agents of brucellosis, a worldwide zoonotic illness causing abortion in domestic animals and Malta fever in humans. In this study, the potential of Urease was evaluated with Freund's adjuvant (Urease-IFA) and N-trimethyl chitosan (TMC/Urease) nanoparticles against brucellosis. **Materials and Methods:** The *urease* gene was expressed in *E. coli* BL21 (DE3). After purification, the recombinant Urease was loaded onto TMC nanoparticles by ionic gelation with tripolyphosphate. Particle size and loading efficiency of the nanoparticles were determined. Urease-IFA was administered intra-peritoneally while TMC/Urease nanoparticles were administered orally and intra-peritoneally. Additionally, vaccinated mice were challenged with virulent *B. melitensis* and *B. abortus*. **Results:** Oral administration of TMC/Urease elicited low titers of specific IgG, while i.p. immunization with Urease-IFA and TMC/Urease induced high specific IgG production. According to cytokine assay and antibody isotypes, intra-peritoneal (i.p.) immunization by Urease-IFA and TMC/Urease nanoparticles induced a mixed Th1-Th2 immune response, whereas oral administration of TMC/Urease nanoparticles induced a mixed Th1-Th17 immune response. In lymphocyte proliferation assay, splenocytes from i.p. vaccinated mice showed a strong recall proliferative response. Additionally, the i.p. vaccination with TMC/Urease nanoparticles exhibited a high degree of protection. **Conclusion:** All together, our results indicate that TMC nanoparticles are a potent delivery system for i.p. administered *brucella* antigens.

Poster Presentations:

7537P

Study effects of dietary 1% inulin as prebiotic on some immunological parameters of young Ross chicks

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The growing rate of broiler breeding has led to an increasing demand on the use of new chemical materials. Among these chemicals include probiotics of which inulin type fructones have been under further study. Intestinal microbes play an important role in normal nutrition, physiologic, immunity and protection of the host animal. In this study, 100 Ross-308 chicks were selected. Then the chicks were divided into two groups of 50 broilers. Chickens accumulation was adjusted to 6 chickens per square meter. Water and sugar solution of 5 % was applied for feeding on the first day. The environment was exposed to 24-hour lighting during the experiment. From the second day, the control group was fed with basal diet and the treatment group was fed with basal diet plus 1 % inulin. After 19 days, feeding was cut in 19th day and blood sampling was taken on the 20st day from 15 chicks of control and test groups. The method of WBC count was improved hemocytometer, anti-SRBC was hem agglutination and IgM and IgG were determined by the method of Cheema et al (2003). There was no significant differences on anti-SRBC on $p<0.05$ level. Heterophils and Lymphocytes showed no significant differences but there was significant increases in total WBC between inulin group to control ($p<0.05$). in IgG and IgM rates were no significant differences. In conclusion inulin host protection mechanisms are different from host immune system. **Key words**: Inulin, Ross, WBC, Anti body, Immune

7546P

Evaluation of some of the factors associated with immune system following effect of magnetic field in rats

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Introduction: This study was designed to investigate the effects of electromagnetic fields (EMF) uniformly on the immune system of 12 Norwegian male Wistar rats used as experimental model. **Materials and Methods:** These rats were divided into three control, treatment one and treatment two groups. The first treatment group was subjected to electromagnetic field for four hours and fifteen days. The second group received this treatment for eight hours and fifteen days. At the end of the fifteenth day, blood samples from rats were taken and were prepared to be analyzed. Then, blood cells were counted and after conducting biochemical tests, the data were analyzed using SPSS computer application, one-way ANOVA and Tukey range tests. **Results:** The results revealed that the level of WBC increased in the first experimental group compared to control group; yet, it decreased in the second experimental group. The

level of total protein was relatively decreased compared to control group; however, level of albumin was decreased significantly and at the same time Gamma globulin was increased. Eventually, the increase in the number of lymphocytes and decrease in the number of neutrophils and monocytes are the most significant effects of electromagnetic field on immune system. **Keywords:** Immune system; Magnetic field; Rats.

7554P

Study distribution of immune cells of uterus 10 days after pregnancy of rats by immuneohistochemical method

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Introduction: Pregnancy in mammals is major example of natural immunologic tolerance that despite father antigen, fetus won't attack by mother immune system. Uterus immunity has important role in pregnancy success. **Materials and Methods:** In this study, the distribution of immune cells in uterus tissue of pregnant rats in 10 days after pregnancy were studied by immuneohistochemical method. For staining used monoclonal antibody, Alkaline Phosphatase and HRP enzyme and color were studied with markers. There were significant differences on population and dispersion of CD86+, CD11b+, CD11C+ and MCH-II+ cells. Recent study showed the role of uterus mucous membrane immunity in order to prevent damages against fetus of pregnant rats. **Conclusion:** The presence of immune cells in certain areas resulted to avoidance of damage response of mother immune system against fetus.**Key words:** pregnancy, rat, immune cells, uterus

7611P

Investigating the CIAV infection rate on Tabriz broiler farms in 2015 by serum analyzing and PCR

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Introduction: Chicken infectious anemia virus in 2 to 4 week old chicks cause infectious anemia. According to clinical and sub clinical effects of CIAV on immune system, infections of this virus cause sensitivity of host to other pathogens. The aim of this study was investigating the rate of infected broilers in Tabriz 2016. **Materials and Methods:** For this, with sampling of 19 broiler farms and ELISA test with PCR method were used. **Results:** 31 percent of broiler farms in 1/100 dilution of ELISA test were positive and in PCR study 5 samples of 6 samples were positive. **Conclusion:** Results of this research showed high rate of CIAV infection in Tabriz. **Key words:** CIAV, ELISA, PCR, Broiler

8703P

Evaluation ofsome of the factorsassociated withimmune systemfollowingUV radiation in rats

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Introduction: This study was designed to investigate the effects of UV radiation on the immune system of 12 Norwegian male Wistar rats used as experimental model. **Materials and Methods:** These rats were divided into three control, treatment one and treatment two groups. The first treatment group was subjected to UV radiation for four hours and fifteen days. The second group received this treatment for eight hours and fifteen days. At the end of the fifteenth day, blood samples were taken from rats and were prepared to be analyzed. Then, blood cells were counted and after conducting biochemical tests, the data were analyzed using SPSS computer application, one-way ANOVA and Tukey range tests. **Results:** The results revealed that the level of WBC increased significantly in the both treatment groups compared to control group. Also, the level of total protein was relatively increased in both treatment groups compared to control group. **Conclusion:** Uv radiation can change functional immune cell
Keywords: Immune system; UV ; Rats.

10802P

The relation between the severity of Babesia ovis infection on sialic acid level andinnate and acquired immunity in naturally infected sheep

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Introduction: *Babesia ovis* is one of the most important tick-borne diseases of sheep. This study was designed to evaluate the effect of severity of *B.ovis* parasitemia on the sialic acids levels and potential of immune responses in naturally infected sheep.**Material and Methods:** Infected animals comprised of 38 Iranian fat-tailed sheep, about 1-3 years old, naturally infected with *B. ovis*, were divided into four groups with respect to parasitemia rates (low: 0.1-0.3%, moderate: 0.4-0.9%, high: 1-2.5% and very high: >2.5%). As a control group, ten clinically healthy sheep reared under the same management and environmental conditions were also sampled. The concentrations of total sialic acid, lipid-bound sialic acid and protein-bound sialic acid were measured in groups. For evaluation of immune response, peripheral blood mononuclear cells (PBMCs) were isolated from blood of animals by Ficoll-Hypaque gradient. Finally, the respiratory burst of phagocytes and the proliferation of lymphocytes were evaluated in PBMC population. **Results:** Sialic acid concentrations showed significant increase in infected goats compared to controls. Parasitemia rate was positively correlated with sialic acid concentrations. The data of NBT reduction assay and MTT proliferation method showed that the respiratory burst of phagocytes and the proliferation of lymphocytes were significantly decreased in infected sheep. The parasitemia rate was also positively correlated with decrease in NBT and MTT assays compared to control group. **Conclusion:** This survey documented that the degree of *Babesia ovis* parasitemia was correlated significantly with the serum sialic acid concentrations or reduction in innate and acquired arm of immune system. **Keywords:** *Babesia ovis*, Sheep, Parasitemia, Sialic acid, Acquired immunity, Innate immunity.

Late Abstracts

Poster Presentations:

12423P

Investigation of CTLA-4-318C/T gene polymorphism in cases with type 1 diabetes of Azerbaijan, Northwest Iran

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Introduction: Type 1 diabetes (T1D) is a T-cell mediated autoimmune multifactorial disease. CTLA-4 encodes an immunoregulatory glycoproteinsurface receptor that is synthesized by activated T lymphocytes. This study analyzed the association of CTLA-4-318C/T gene polymorphism with susceptibility of T1D.**Material and methods:** One hundred and fifty-three T1D patients and 189healthy controls entered this study. CTLA-4-318C/T genotyping was performed by tetra-primer amplification refractory mutation system–polymerase chain reaction (T-ARMS–PCR) analysis. **Results:** The allelic andgenotypic frequencies of −318C/T gene polymorphism were similar in patients and controls. **Conclusion:** The current study demonstrates that CTLA-4-318C/T polymorphism was not linked with a higher genetic risk for T1D.

12424P

Association of CTLA-4-318C/T gene polymorphism with clinical course of type 1 diabetes

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Introduction: Type 1 diabetes (T1D) is a T-cell mediated autoimmune multifactorial disease. CTLA-4 encodes an immunoregulatory glycoproteinsurface receptor that is synthesized by activated T lymphocytes. This study analyzed the association of CTLA-4-318C/T gene polymorphism with clinical course and laboratory findings of T1D. **Material and methods:** One hundred and fifty-three T1D patients entered this study. CTLA-4-318C/T genotyping was performed by tetra-primer amplification refractory mutation system–polymerase chain reaction (T-ARMS–

PCR) analysis. **Results:** Younger age, earlier age at onset, higher HbA1c levels, higher frequency of Glutamic acid decarboxylaseantibodies (GADA) and Insulinoma Associated-2 Autoantibodies (IA-2A) were observed in T1D patient carriers of CT genotype. **Conclusion:** The current study demonstrates that the presence of a CT genotype was associated with ayounger age of onset, poor control of HbA1c level and positive anti-GAD or IA-2 serum autoantibodies in Iranian Azeri population.

12425P

Gene Expression of CXCL8 in peripheral blood mononuclear cells of Iranian patients with Chronic Myeloid Leukemia

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Introduction: Chronic myeloid leukemia (CML) is a clonal disorder of hematopoietic stem cells. CXCL8 (also known as IL-8) is a CXC chemokine that in addition to its role in regulating inflammatory responses has particular relevance to tumorigenesis and angiogenesis. CXCL8 is a mitogen for endothelial cells and stimulates both endothelial proliferation and capillary tube formation**.** The aim of this study is to evaluate gene expression of CXCL8 in peripheral blood mononuclear cells of Iranian patients with CML. **Material and methods:** We investigated the mRNA expression of CXCL8 in peripheral blood mononuclear cells of thirty-three patients with CML and 66 healthy controls by Quantitative Real time PCR. **Results:** In spite of decrement of CXCL8 mRNA expression in peripheral blood mononuclear cells of CML patients compared with controls, there were marginally significant differences in expression of CXCL8 mRNA in PBMCs of CML patients and healthy controls (p= 0.06).**Conclusion:** Our data show that CML is associated with a decreased expression of CXCL8 mRNA. CXCL8 may be important in CML pathogenesis.

12426P

Investigation of CXCR2 gene polymorphisms in cases with Leprosy Iranian population

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Introduction: Leprosy, caused by Mycobacterium lepra, is a human chronic infectious disease causing damaging inflammatory lesions in the skin and peripheral nerves. Several types of study support a role for host genetics in likely to be involved in directing the cellular infiltration in the various forms of Leprosy lesions. CXC group, which contains IL-8, predominantly attracts neutrophils, but also monocytes and T lymphocytes, which express the IL-8 receptors CXCR1 and CXCR2. CXCR1 is activated only in response to binding of IL-8 and granulocyte chemotactic protein-2. Alternatively, CXCR2 is activated by multiple CXC chemokines. In the present study, for the first time in the world, we examined polymorphism in the CXCR2 +1208 T/C and +785 C/T 3ˈuntranslated region with

respect to Leprosy in a population-based case-control study in Iran. This polymorphisms have the ability to alter the processing, stability and translation of mRNA. **Material and Methods:** One hundred ten Leprosy patients and 165 healthy and ethnic-sex-age matched controls were included in this study. CXCR2 +1208T/C and +785C/T gene polymorphisms were genotyped via allele specific PCR (ARMS-PCR) method. **Results:** The allelic andgenotypic frequencies of +1208T/C and +785C/T gene polymorphisms were similar in patients and controls **Conclusion:** The current study demonstrates that CXCR2 +1208T/C and +785C/T polymorphisms were not linked with a higher genetic risk for Leprosy.

12427P

Up regulation of CXCR1 and CXCR2 mRNA expression in peripheral blood mononuclear cells of patients with Chronic Myeloid Leukemia

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Introduction: CXCL8 (also known as IL-8) is a CXC chemokine that in addition to its role in regulating inflammatory responses has particular relevance to tumorigenesis and angiogenesis. The biological effects of IL-8 are mediated by binding to two-cell surface G protein–coupled receptors, termed CXCR1 and CXCR2. CXCR1 and CXCR2 are expressed on the cell surface of most of the myeloid lineage, such as mature PMN and monocytes. CXCR1 is activated only in response to binding of IL-8 and granulocyte chemotactic protein-2. Alternatively, CXCR2 is activated by multiple CXC chemokines. The aim of this study is to evaluate gene expression of CXCR1 and CXCR2 in peripheral blood mononuclear cells of patients with Chronic Myeloid Leukemia (CML). **Material and Methods:** We investigated the mRNA expression of CXCR1 and CXCR2 in peripheral blood mononuclear cells of thirty-three patients with CML and 66 healthy controls by Quantitative Real time PCR. **Results:** Expression of CXCR1 and CXCR2 mRNA was significantly higher in CML patients than in controls (p=0.001and p=0.01 respectively). **Conclusion:** Our data show that CML is associated with an increased expression of CXCR1 and CXCR2 mRNA. Up-regulation of CXCR1 and CXCR2 may be important in CML pathogenesis.

12428P

The effect of IL-4 and integrin αVβ5 on Monocytes proliferation in giant cell granuloma patients

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Introduction: It is now well established that IL-4 and integrins have a central role in the development of monocytes to multinucleated giant cells (MGCs). The aim of this study was to Evaluation of the synergistic effect of IL-4 and integrin αVβ5 on Monocytes proliferation in the peripheral blood samples of patients with giant cell granuloma. **Material and methods:** Monocytes were isolated from peripheral blood samples of patients with giant cell granuloma and healthy controls using human Monocyte Isolation Kit II. Isolated monocytes were then cultured in the absence or presence of IL-4 and integrinβ5 (10 and 20 ng/mL), and following MTT assay was performed to

determine proliferation. **Results:** Both IL-4 and integrin β5 induce proliferation of monocytes in a dose-dependent manner. Proliferation was significantly enhanced by increasing the IL-4 dose from 10 to 20 ng/mL. When IL-4 and integrin β5 were combined, they had synergistic effects at low doses. **Conclusion:** In this study, we showed an elevation Proliferation when stimulated by IL-4 and β5 integrin. It is strongly indicated that IL-4 and β5 acts as an important mediators during macrophage to macrophage fusion and development of giant cells. **Keywords:** IL-4, integrin β5, Apoptosis, giant cell granuloma

12429P

Adaptive response induced by long term low doses of ionizing radiation exposure in workers of radiopharmaceutical facility

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Introduction: Long term low doses of ionizing radiation can induce DNA damages and adaptive responses. Due to the importance of these responses the aim of this study was to evaluate the adaptive cytogenetic responses in the workers of radiopharmaceutical facilities that are occupationally exposed to external radiation and internal contamination. **Methods:** The Micronucleus (MN) assay was performed for evaluation of DNA damages and irradiation of blood samples (30 tests and 30 controls) with 2Gy gamma rays were used for evaluation of adaptive responses. **Results:** The results showed the MN frequency and other genotoxic damages of nucleoplasmic bridges and nuclear buds were significantly higher and nuclear division indexes were lower in the workers in comparison with the controls. After 2 Gy gamma irradiation DNA damages were lower in the test group in comparison with controls. **Conclusion:** The higher incidence of DNA damages in the test group (at zero doses) and lower damages after 2 Gy irradiation shows the adaptive response induced by long term exposure to low doses of ionizing radiation in the test group. The adaptive responses are indicator of individual radiation sensitivity due to the genetic background and may cause resistance to radiotherapy programs, therefore should be considered in cancer treatments. **Keywords:** Micronucleus assay, Radio-adaptive response, Occupational exposure, Radiopharmaceutical

12511P

Evaluation of the IgE-immunoreactivity of *Amaranthus retroflexus* polcalcin with inhibition assays

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Introduction: *Amaranthus retroflexus* (pigweed) pollen is one of the main sources of pollen allergy in tropical and subtropical regions. In this study we aimed to evaluate the allergenicity of the recombinant polcalcin from *A. retroflexus* pollen. **Material and Methods:** Recombinant polcalcin was cloned in pET21b and expressed in *E. coli BL21* system. The purification of expressed polcalcin was conducted by Ni-IDA affinity chromatography. Following extraction of total proteins from *A. retroflexus* pollens we determined the IgE-binding capacity of the produced proteins by inhibition assay using sensitive patients' sera. Firstly, crud extract proteins were transferred on PVDF sheets and probed with polcalcin (10µg/ml) or BSA (background control) pre-incubated patients' sera overnight at 4°C on a rocker. The evaluation of immunoreactivity of the absorbed sera was followed by routine ELISA or blotting methods. **Results:** Polcalcin was successfully expressed in *E.coli* BL21 and was purified by affinity chromatography. Inhibition assay results demonstrated that same IgE epitopes are present in recombinant protein and could reduce IgE-reactivity of polcain in the crud extract. **Conclusion:** Inhibition assays demonstrated that the allergic subjects' sera reacted with recombinant polcalcin similar to natural polcalcin in crude extract of *A. retroflexus.* We believe that this protein could be used as an allergen in diagnostic and immunotherapy procedures. **Keyword:** Allergy, Amaranthus *retroflexus*, polcalcin, Recombinant allergen, inhibition assays

12512P

Expression of walnut major allergen in the Escherichia coli and determination of its IgE-binding capacity

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Introduction: Walnut is one of popular allergenic nuts; however, there is scarce data about its allergens. In the present study we expressed and purified a recombinant form of walnut major allergen (2S Albumin) and determined its IgE-binding capability. **Material and Methods:** cDNA was synthesized from walnut total RNA. Then 2S albumin-coding sequence was amplified by conventional PCR using specific primers with overhanging sequences to *BamH1* and *Xho1* restriction sites and ligated to pET21b. The construct was amplified in competent *E. coli* TOP10 cells and the recombinant allergen was expressed in *E. coli* BL21 cells. The recombinant 2S Albumin purified by Ni-IDA affinity chromatography system. Finally, its IgE-reactivity was compared with crude extract by ELISA and Western blotting with walnut allergic patients' sera. **Results:** The successful expression and purification of 2S albumin in bacterial cells was confirmed by SDS-PAGE. SDS-PAGE revealed a single 18 kDa protein band in the purified fraction which showed considerable IgE-reactivity in ELISA and Western blotting. DNA sequencing revealed high similarity of the insert with other 2S Albumin coding sequences. **Conclusion:** Since the recombinant 2S albumin showed comparable IgE-reactivity with total extract, we concluded that the produced protein could be used as an allergen in diagnostic procedures. **Keyword:** Allergy, Jugland regia, 2S Albumin, Recombinant allergen

12513P

Relationship of Regulatory T Cells and Soluble Human Leukocyte Antigen-G (sHLA-G) in Multiple Sclerosis pateints

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Introduction: Regulatory T cells (Treg) are considered as pivotal players in MS pathogenesis. Also soluble human leukocyte antigen (HLA)-G has recently been suggested as immunomodulatory factors in multiple sclerosis (MS). We aimed to investigate the relationship of HLA-G molecules and Treg cells in Relapsing-Remitting Multiple Sclerosis (RRMS) patients and compare it to healthy controls. **Materials & Methods:** Patients with RRMS (n=200) and healthy subjects (n=200) were studied. Then sHLA-G levels (sHLA-G1 and sHLA-G5) were measured using ELISA method. Treg (CD4+CD25+ Foxp3+) cells in patients who had sHLA-G>10 U/ml were characterized by using flow cytometry. **Results**: Our data showed that there was no significant differences between RRMS patients and healthy controls in sHLA-G concentration ($p>0.05$). Treg cell frequencies were higher in the patients who had sHLA-G >10 U/ml compared to healthy subjects ($p<0.05$). **Conclusion:** Collectively, there was significant correlation between sHLA-G and frequency of Treg cells in treated RRMS patients and healthy individuals. It seems that high level sHLA-G has been instrumental in raising frequency of Treg cells in treated patients and could be associated with remission of MS disease . **Keywords:** Multiple sclerosis, Regulatory T cells, sHLA-G

12514P

Investigation of Apoptosis Effect of Dichloromethane fractions of*Scrophularia oxysepala* extract in WEHI-164 cells

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Introduction: Breast cancer is a prevalent malignancy among women, especially in developing countries. There have been mentioned potentials as anticancer agents for some plants. Hence, herbals may play essential role in prevention and treatment of cancers. This paper tended to judge cytotoxic effects of Dichloromethane fractions of *Scrophularia.oxysepala extract* on WEHI-164 breast cancer cell line. **Materials & Methods:** In present study Dichloromethane fractions were examined using MTT test (3-(4, 5-dimetylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide) and Trypan-blue assays were used to analyze cytotoxic activity of *S. oxysepalaextract* in WEHI-164 breast cancer cell line. Apoptosis induction was determined using TUNEL (terminal deoxytransferase (TdT)-mediated dUTP nick- end labeling) test and DNA fragmentation analysis. Relative mRNA expression was quantified by quantitative real-time PCR. **Results:** Results revealed an effectively inhibited cell growth and viability due to treatment with dichloromethane fractions. Cell death assay and DNA fragmentation analysis using TUNEL test indicated induction of apoptosis in WEHI-164 cell line by dichloromethane fractions of *S.oxysepalaextract* through decreasing Bcl-2 and increasing caspase-3, which highlights the possibility of mRNA over expression in WEHI-164 breast cancer. **Conclusion:** Findings suggest that fractions of *S.oxysepala*extract may cause apoptosis in breast cancer cells. The findings indicate that such fractions contain potentially anti-cancer components that inhibit proliferation of cancer cells by causing DNA damage. Results suggest that up-regulation of caspase-3 can effectively trigger apoptosis while down regulation of bcl2 can inhibit the proliferation of WEHI-164 breast cancer cells.

12515P

Effect of HMGA2 siRNA and Doxorubicin loaded TMC Nano-particles on metastasis of metastatic breast cancer cell line (MDA-MB-231) and their synergic evaluation

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Introduction: Disruption of the normal regulation of cell-cycle progression and section stand at the heart of the events lead to cancer. Complex networks of regulatory factors, tumor microenvironment, and stress signals, such as those resulting from damaged DNA, dictate cancer cells proliferate or die. Recently, High-mobility group protein 2 (HMGA2), a non-histone nuclear-binding protein and its down-regulators; vimentin, MMP-9 and E-cadherin are shown to contribute to tumor progression and metastasis. **Material and methods:** Thus in this study we checked simultaneous delivery of HMGA-2 siRNA and the anticancer drug doxorubicin to enhance the anticancer treatment effects. For this purpose, real time PCR (expression of HMGA2 and down-regulators). **Results:** Our results showed that dual delivery of Dox and HMGA-2 siRNA by TMC (trimethyl chitosan) significantly silenced HMGA-2, vimentin, and MMP9 mRNAs, but led to overexpression of E-cadherin mRNA. **Conclusion:** In conclusion, we demonstrated that Dox and HMGA-2 were successfully encapsulated in TMC NPs and had a great potential for HMGA-2 and its downstream signaling molecules silencing. Moreover, the HMGA-2 mRNA gene silencing in MDA-MB-231 was closely related to the drug sensitivity to Dox and led to a significant cytotoxic effect.

12516P

Identification of Acute Promyelocytic leukemia (APL): Variation in Surface CD Markers

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Introduction: Acute Promyelocytic Leukemia (APL) is one of the highly aggressive disease. Diagnosis of APL traditionally relies on the morphologic identification of the leukemic cells, followed by flow cytometry. it's more rapid to diagnosis than molecular cytogenetic detection of the T (15;17) and other rare variant translocations(2). **Material and method:** Immunophenotyping was performed in 15 case, in the age 8 - 82 years' old, of Acute Promyelocytic Leukemia (APL), morphologic identification, characterized surface markers such as CD34, HLA-DR, CD13 and CD33 by flow cytometry. **Results:** Among the cases of AML in this study, 15 were of the APL classification by morphologic identification. all of the 15 APL cases, CD33 demonstrated expression in all of the cases except one of them. Expression of CD13 and CD34 were observed in 9 (60%) and 12(80%) of the 15 cases, respectively. HLA-DR was expressed weakly or dim in 5 (33.33%) of the 15 cases.in this study just one of the case the triad of CD33+/CD34–/HLA-DR–. only 28.57% of cases express CD14 or CD64. **Conclusion:** identification of APL need flow cytometry, cytogenetic and morphologic characterization. In all cases we examined we have different surface marker and it seems we need a panel of surface marker to identified APL and also morphologic character can help identified confirmedly. Use a panel in side of the morphologic character is a helpful way to identified APL from other kind of AML and cytogenetic rearrangement helpful for response to therapies.

12518P

Evaluation of Epigenetic changes on p53 gene in MCF-7 cell line treated with extract of *Scrophularia amplexicaulis*

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Introduction: 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. herbal medicines are attracted a great deal of interest in this field. **Materials and Methods:** In this research, MCF7 breast cancer cells grown in RPMI medium were exposed to different concentrations of *S.amplexicaulis* 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:** S.amp has cytotoxic effects on P53 gene in MCF7 cells. MSP showed extract of S.amp cause significantly changes in methylation status on P53 gene. Real-time PCR has showed increase expression on P53 gene in MCF-7 cells, after treatment by S.amp**.** **Conclusion:** Extract of *S.amp* led to apoptosis in MCF7 cell line. There was detectable methylation change in P53 promoter region. According to our findings, the higher expression of P53 gene and apoptosis by this extract was caused by reverted methylation of the promoter region on P53 gene.

12519P

Immunotherapy of Ovarian Cancer

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Ovarian cancer is the most lethal malignancy among gynecological cancers. The lethality of ovarian cancer is due to diagnosis at advanced stages and metastasis to other parts of the body. Therefore, conventional therapies such as surgery, chemotherapy, and radiotherapy usually are not able to control the advanced stages of the disease. So, new therapy strategies are required to improve the results of this malignancy. Given to emphasis on preventing metastasis to abdominal cavity, as well as easy access to the peritoneal cavity in order to delivery of genes, ovarian cancer is an ideal option for gene therapy. Regarding the role of immune system in the pathogenesis of cancer and following the demonstration of the immunogenicity of ovarian tumors, several methods based on immunotherapy have been developed. This article reviews the rationale for immunotherapy and the main approaches under investigation in treatment of ovarian cancer, with an overview on human umbilical cord mesenchymal stem cells. **Keywords:** Ovarian cancer, Gene therapy, Immunotherapy, Stem cells

12520P

Knockdown of SNAIL1 via siRNA impedes migration of EJ138 bladder cancer cells

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Introduction: Epithelial–mesenchymal transition (EMT), a key process in the tumor metastatic cascade, is characterized by the loss of cell–cell junctions and cell polarity as well as the acquisition of migratory and invasive properties. SNAIL1 transcription factor induces epithelial-mesenchymal transition (EMT) in which the epithelial cells downregulate cell-cell adhesion genes such as E-cadherin and upregulate mesenchymal genes such as vimentin, leading to increased invasion and migration. **Materials and Methods:** To investigate the role of SNAIL1 in EMT, we utilized the specific siRNA to knockdown SNAIL1 expression in human bladder cancer cell line with typical EMT features. Then we performed realtime and the wound Healing assay to evaluate the migration of EJ138 cells before and after of knockdown. **Results:** The Quantitative RT-PCR analysis showed that down-regulation of SNAIL1 gene enhanced the expression of E-cadherin and reduced the expression of vimentin. Our results indicated that SNAIL1 suppression in EJ138 cells prominently impeded cell migration. **Conclusion**: Finally, the migration of cancer cells conferred by SNAIL1 provides a selective advantage to malignant cells to separate from the primary tumor and migrate to distant territories. By regarding to the roll of SNAIL1 in EMT and migration, it can be considered as a potent therapy for bladder cancer.

12521P

Synthesis of a dendritic polyester possesses 12 acrylate end groups for resin-based dental (nano-)composites

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Introduction: Dental caries and dentin hypersensitivity are the most common clinical diseases in oral health worldwide. When caries break in more than half of tooth enamel, dentist removes the decayed materials and replaces it with appropriate materials. In this respect, resin-based composite fillings have stimulated great interest as materials of choice for replacing amalgam as a restorative material for posterior restorations. However, the low degree of conversion, and undesirable consequences of residual monomer into the human body such as allergic reactions and sensitization in patients are the main drawbacks of these materials. **Materials and Methods:** In this study, a dendritic polyester possesses 12 acrylate end groups was synthesized. The synthesized dendrimer was subsequently photo-copolymerized with functionalized SiO2 nanoparticle to produce a dental nanocomposite. **Results:** It is expected that the synthesized dendrimer shows suitable polymer mechanical properties, reduce shrinkage or shrinkage stress, and high degree of conversion, mainly due to its high number of functional end groups. **Conclusion:** Due to high degree of conversion, and high cross-link density, as a result of the large number of reactive functional end groups we envision that the synthesized dental nanocomposite has lower allergic reactions and sensitization in comparison with conventional resin-based dental (nano-)composites.

12522P

A bioinformatics analysis to predict potential Micro-RNAs inhibiting processes of angiogenesis byMMPs in cancer

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Introduction: Increased size of the tumor results in hypoxic and Acidic environment that leads to increased production of several types of growth factors which are necessary to formation of blood vessels, followed by metastasis. Matrix metaloproteinases (MMPs provide space for tumor growth and also release growth and angiogenesis factors and induce angiogenesis in tumor by breaking down the extracellular matrix. Today it has been proved that micro-RNAs could regulate gene expression by pairing with an mRNA molecule in different ways. The purpose of this research is to identify Bioinformatics micro-RNAs that may have maximum inhibition of MMPs angiogenesis potential in tumors. **Material and Methods**: Using the results of previous studies, MMP genes involved in tumor angiogenesis (MMP1-2-3-8-9-10-11-13), angiogenesis factors such as VEGF, FGF and IL8, as well as genes involved in chemotaxis, such as MPC1, CSF1 and PDGF, that are activated by MMP activity, was detected. Then bioinformatics research was carried out to identify micro-RNAs that are connected to these genes, using mirwalk and Microrna.org. **Results:** Based on the findings mir-1302, mir-516a, mir-512, mir-511, mir-516b and mir-548 had the highest number of binding sites. **Conclusion:** So these micro RNAs are options for cell culture and laboratory examination in order to prove our results and to find new ways to prevent the development of cancer by inhibiting angiogenesis. **Keywords**: angiogenesis inhibition, bioinformatics, micro RNA, MMPs

12523P

Evaluation of Nrf2 expression level by IHC and real time PCR in colorectal cancer

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Introduction: Nuclear factor erythroid 2-related factor 2(Nrf2) is a transcription factor that binds antioxidant response element(ARE) DNA sequences and stimulates transcription of detoxifying genes. Nrf2 preserves not only normal cells but also cancer cells from cellular stress, and increases survival of both normal and cancer cells. In this study we aimed to evaluate Nrf2 mRNA and protein levels in biopsy specimens of patients with and without colorectal cancer. **Material and Methods**: Sixty cases of colorectal cancer patients and 60 controls were enrolled. The Nrf2 expression was evaluated by real time PCR and immunohistochemistry in case and control samples.

Between-group- differences were performed by chi-square, Fisher's exact, or Mann-Whitney U test. The correlation between Nfr2 expression and clinicopathological features was analyzed by Kaplan-Meier test, univariate and multivariate analysis. **Results:** We demonstrated that Nrf2 has different expression among tumor and normal tissues. In addition to, Nrf2 expression was found in gastric cancer cell nucleus, with significant correlation with clinicopathological features, including tumor size, lymph node metastasis, and histological analysis (all $P<0.05$). **Conclusion:** Our data show that the elevated expression of Nrf2 may be related to colorectal carcinogenesis and may play important role in promoting tumor progression. **Keywords:** Nrf2, IHC, Real time PCR, colorectal cancer

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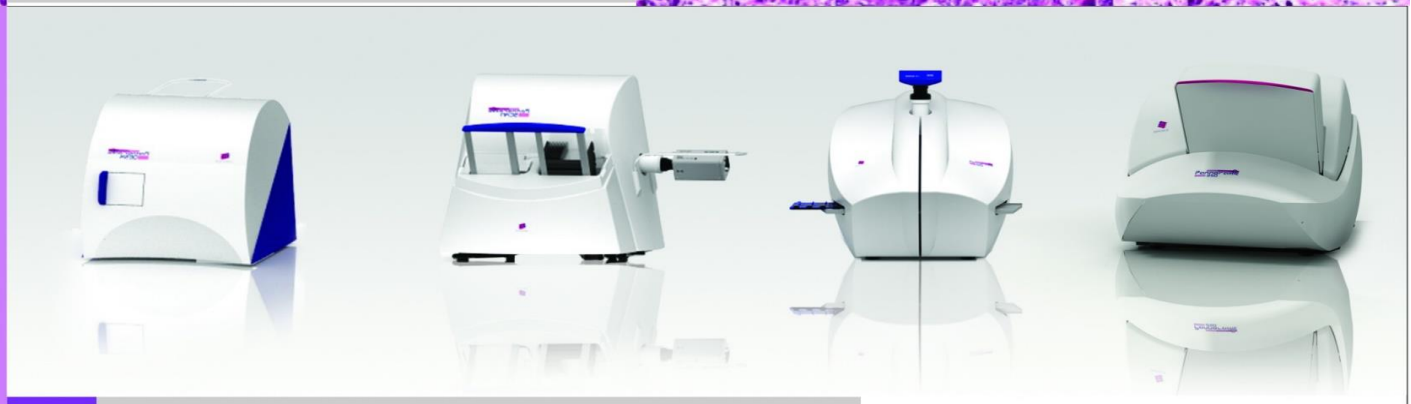

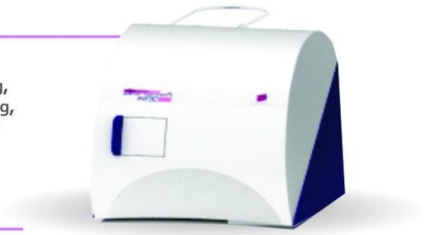

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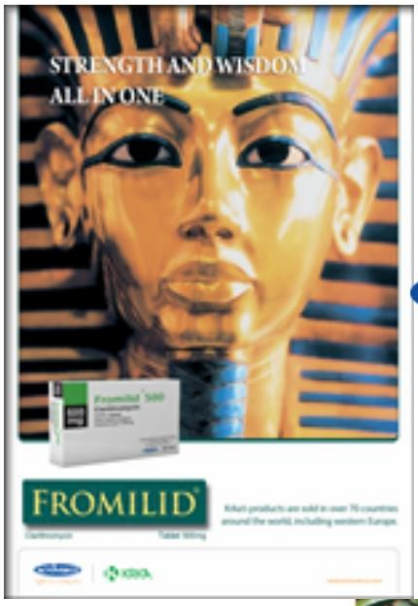

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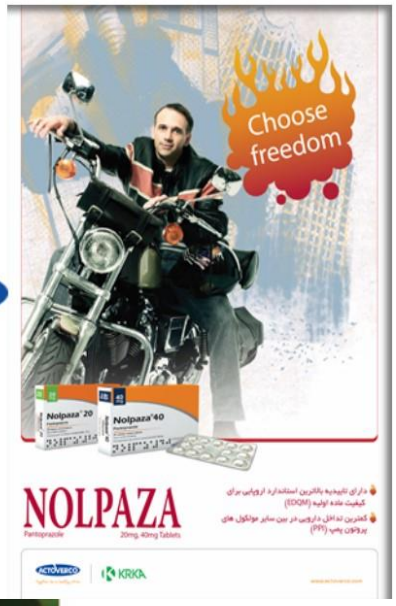

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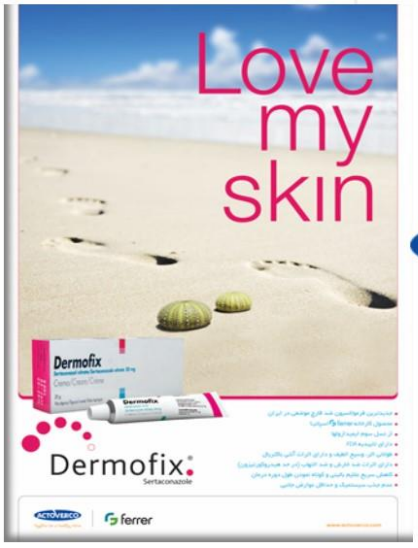

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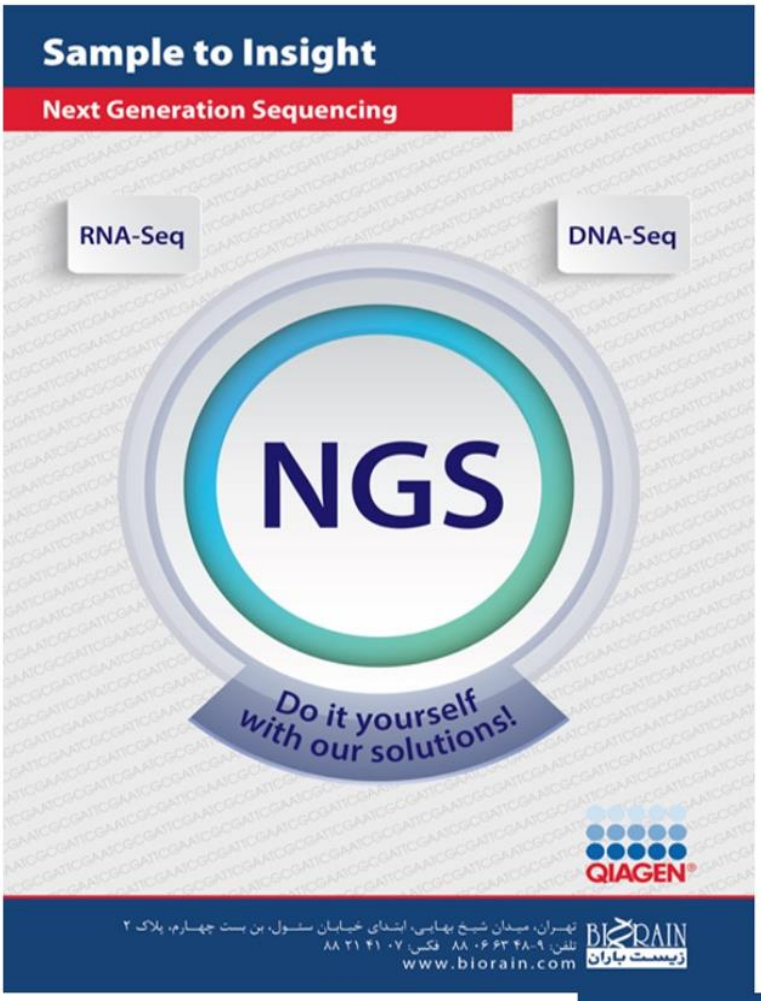
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