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ABSTRACT BOOK

11th INTERNATIONAL CONGRESS OF IMMUNOLOGY AND ALLERGY

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In the Name of God

11th International Congress of Immunology and Allergy

Tehran-Iran

"Immunology from Bench to Bedside"

26-29 April 2012

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Welcome Message

11th International Congress of Immunology and Allergy of Iran

It is our great pleasure to welcome you to the 11th International Congress of Immunology and Allergy of Iran (ICIA), which is being held in Tehran, 26-29th April 2012. Founded in 1991, Iranian Society for Immunology and Allergy (ISIA) has become the largest association of immunologists in Iran. ISIA's activities have been dedicated to advancing research and education in the field of immunology in Iran, and in addition to its numerous meetings, workshops and immunology courses, it has been publishing the "Iranian Journal of Immunology", one of the first immunology-focused journals in the middle-east region. Having successfully organized and completed 10 International Congresses over the last two decades, ISIA is now honored to hold its 11th biannual International Congress in 2012. The mission of this congress is not only to contribute to the ever-growing standards of basic and clinical research in immunology, but to provide a platform for further communication between basic researchers and clinical scientists. While the translation of basic knowledge to clinical applications have always been of interest to the scientific community, the wealth of molecular data derived from high-throughput technologies in the first decade of 21st century is incomparable to anything that had been resulted from classical biological research in the decades before that. This has put biomedical scientists, including immunologists, in a particular position, whereby integration of data resulted from system-level studies might revolutionize our understanding of the structure and function of biological systems, as well as their deviation from the normal behavior; i.e. disease. It is not an exaggeration to say that, for the first time in the history of biology, biologists are in a unique position to convert a "reductionistic" picture of biological systems into a "holistic" view, something that will have profound implications for the way we see disease, its mechanisms and its treatment. While the current congress is covering a spectrum of subjects from basic and molecular to clinical immunology, to get closer to the realization of this long-awaited dream of biology, we would like to use this opportunity to also foster collaboration and research in the areas of systems immunology, computational immunology and other related interdisciplinary areas.

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

Mohammed Vojgani, MD, Ph.D
President of ISIA and 11th ICIA

Tooba Ghazanfari, Ph.D
Scientific Secretary of 11th ICIA

University of Nebraska Medical Center

Director, Laboratory of Transplantation Immunology
Professor, Department of Pathology and Microbiology
COLLEGE OF MEDICINE

Dear Friends and Colleagues,

It is a great pleasure to welcome you to the Eleventh International Congress of Immunology and Allergy. I am confident that you will find this to be an exciting and informative congress and am disappointed to be unable to join you this week. I encourage you to communicate with one another regarding your research and clinical observations, as well as, your ideas and goals. Through these discussions, you will establish new collaborations from which to forge critical, new scientific and clinical directions and accomplishments. This establishment of international scientific collaborations will accelerate progress in the identification, development, and establishment of novel and effective biomarkers and prophylactic and therapeutic strategies. I wish you every success in this effort!

The field of immunology is in the midst of a paradigmatic shift moving our knowledge and focuses from the bench to the bedside and back again in pursuit of regulatory and therapeutic insight into disease-associated immunopathologies. Successes have been many over the past 25 years with the discovery, development, and delivery of new therapeutics and technologies. The omics have extended into immunology research and the clinic including new drugs, prognostic markers, and therapeutic strategies, such as cellular and genetic therapeutics. However, a brief review of national or international morbidity and mortality statistics underscores a critical need to identify and prioritize targets for investigation, as well as, to develop and establish goals addressing those issues limiting our progress. As Editor-in-Chief of *International Immunopharmacology*, I have interacted with many of the participants and the leadership of this Congress including the Scientific Secretary, Dr. Tooba Ghazanfari, Professor at Shahed University, as well as, Dr. Zuhair Hassan, Professor at Tarbiar Modarres University. As such, I am confident of the success, both short and long term, of this meeting.

The theme of this international congress is critical to the future development of immunology and its interface with the immunopathology of allergy, as well as, other diseases you will discuss at this symposium. I would like to again express a warm-hearted welcome to all gathered here and wish you every success in your interactions and an improved understanding of this complex and exciting field.

Sincerely yours,

James E. Talmadge, Ph.D.
Professor Department of Pathology and Microbiology

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- Gene Specific Silencing in Immunology Research
- Gene Targeting in Leishmania via Transfection
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- How to Choose a Research Topic and Write a Proposal
- How to Prepare a Scientific Presentation
- ImmunoHistochemistry (IHC)
- Immunoinformatics
- Immunotherapy Targeting Cancer Stem Cells
- Microarray Applications in Medicine
- Models of angiogenesis
- Multi-color Flow cytometry (FACS Canto, FACS Aria, LSRII & FACS Calibur)
- Primer Design
- Production of Mouse, Chimeric and Human Monoclonal Antibodies
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- Real-time PCR
- Reference Manager Software
- The Matrix Method of Literature Review
- Two-Dimensional Gel electrophoresis and Western Blot

Contents:

The Authors are responsible for the contents of their abstracts.

ALLERGY

Oral Presentation

1. Effect of Treatment with Intranasal Corticosteroids and Antihistamines on Cytokine Profiles of Patients with Allergic Rhinitis

Farrokhi Sh[1,2*], Mousavi T[2], Arshi S[3], Varasteh A[4], Javahertarash N[3], Falak N[4], Rezaei N[5], SalekmoghadamA.R[2]
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Background: Patients with allergic rhinitis (AR) show increased production of the Th2-related cytokines. This study was performed to determine cytokine profiles secreted by PBMCs of patients with AR sensitized to Chenopodium album (Ch.a) pollens before and after treatment with intranasal corticosteroids (INCs) and antihistamines. Materials and Methods: PBMCs of twenty patients with AR, before and after 45 days therapy and from 20 healthy subjects were tested for normal baselines levels stimulated with natural or recombinant Ch.a in 96 h. The levels of Th2 (IL-4 and IL-13), Th1 (IFN-γ) and immunoregulatory (IL-10) cytokines were measured, using ELISA. Results: The production of IL-4 by the patients' cells stimulated with both Ch.a and rCh.a was significantly higher than normal levels before therapy (p=0.04 and p=0.02, respectively). After therapy, a significant decrease in production of IL-4 and a significant increase in production of IL-10 were found in PBMCs stimulated with natural Ch.a, in comparison to the results before treatment (2.7 *vs.* 14.6 pg/ml, p=0.03 for IL-4; 26.7 *vs.* 13.3 pg/ml, p=0.04 for IL-10). Similarly, these results were seen in the production of IL-4 and IL-10 in exposure to rCh.a allergen after therapy in comparison with before (2.2 *vs.* 15.5 pg/ml, p=0.01 for IL-4; 33 *vs.* 14.1 pg/ml, p=0.03 for IL-10). Conclusion: This study suggests INCs and antihistamines have the capacity to inhibit the production of IL-4 and shift Th2/Th1 responses, probably due to increase the level of immunoregulatory IL-10. Therefore, it could be concluded that therapy with INCs and antihistamines has pharmacologic and immunologic therapeutic effects on AR patients.
Key words: Allergic rhinitis, Cytokines, PBMCs, Intranasal corticosteroids, Antihistamines, ELISA

2. Anti-inflammatory Effect of Intravenous Immunoglobulin (IVIg) in a Murine Model of Allergic Airway Disease: Induction of Foxp3^{+} Regulatory T-cells in an Antigen-Specific Manner

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Background: IVIg has been utilized to treat severe steroid-dependent asthma, but the mechanism of action remains unclear. IVIg inhibits bronchial reactivity in a murine model of allergic airways disease likely via induction of TReg. We assessed the antigen specificity as well as mechanism of TReg induction by IVIg. Materials and Methodes: CD4^{+}Foxp3$^{(GFP-)}$T-cells were purified from lymphoid organs of the TCR-OVA-specific-Foxp3^{GFP} (dTg) mice and adoptively transferred to WT syngenic C57BL/6 animals. Recipient mice were sensitized and challenged either with OVA or Ragweed. Challenged mice received IVIg or HSA as a control protein. Induction of CD4^{+}Foxp3$^{(GFP+)}$Treg was determined by flow cytometry in cells from lungs and lymphoid organs. A suppression assay was performed using CFSE-labled OVA-specific CD4^{+}T-cells plus enriched Foxp3^{GFP+}TRegs from adoptively transferred mice. The proliferative response of OVA-specific T-cells to OVA stimulation was measured by flow cytometry. Splenic dendritic cells were pretreated with IVIg, OVA or OVA-IVIg and co-cultured with CD4^{+}T-cells from dTg mice. The induction of Treg was assessed by flow cytometry after 5-days. Results: Induction of OVA-specific Foxp3^{GFP+}TReg in lung, dLNs and spleen of OVA-IVIg-OVA mice was 4-5 fold higher compared to control groups. In Ragweed-sensitized mice adoptively transferred with TCR-OVA-specific-Foxp3^{GFP+} T-cells, IVIg induced endogenous TReg but not OVA-specific Foxp3^{GFP+}TReg. Foxp3^{GFP+}TReg from OVA-IVIg-OVA mice inhibited proliferation of OVA-specific T-cells 8-fold more efficiently than Foxp3^{GFP+}TReg from other groups. Finally, OVA-IVIg pretreated DCs induced Foxp3^{GFP+}TReg*in-vitro* significantly higher than DCs pretreated with OVA or IVIg alone. Conclusions: IVIg induces Treg in an antigen-specific fashion. The induction of Treg appears to be mediated through conditioning of DCs.
Keywords: Anti-inflammatory Effect, IVIg, Foxp3^{+} Regulatory T-cells

3. ELISPOT for Evaluation of the CellularOrigin of TNF-α , IFN-γ , IL-13 and IL-4 Cytokines in Breast Milk and their Relationship with Atopic Dermatitis in Breastfed Infants

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Background: Breastfeeding is considered as the best method of feeding in infants. Atopic dermatitis is one of the most common chronic inflammatory skin diseases in infants. It is a matter of debate that breastfeeding prevents exacerbation of atopic diseases, especially in the infants with positive family history.This study was aimed to determine the cellular origin of TNF-α, IFN-γ, IL-13 and IL-4 cytokines in breast milk and their relationship with atopic dermatitis in breastfed infants. Materials and Methods: The current work is a case-control study. Twenty-six infants with atopic dermatitis were selected from mothers who had breastfed their infants as long as 1-24 months. They were admitted in asthma and allergy clinic in Afzalipour hospital; Twenty-six infants without atopic dermatitis were selected from mothers who had healthy infants without any other allergic disease. A data collectionform was filled. The number and type of cytokine-producing cells (including Epithelial cells, Fat cells, Lymphocyte or other Mononuclear cells) were determined using hemocytometer and stained smear respectively. The size and the color intensity of cytokine producing spots indicative of cells were determined using enzyme-linked immunospot (ELISpot) assay in milk samples.The number ofeach cytokine-producing cell was individually tested for determination of means, differences between 2 groups by Mann-Whitney U-test.Data were analyzed by using SPSS.Results:There were no significant differences in the numbers of cytokine-producing cells between 2 groups. The percentage of TNF-α, IFN-γ, IL-13 and IL-4producing cells (predominantly Mononuclear cells), size and color intensity of spots made by cells were determined. Conclusion: Our results indicate the majority of cytokine-producing cells are Mononuclear cells compatible with the presence of more number of these cell types in milk sample.
Keywords: TNF-α, IFN-γ, IL-13, IL-4, Atopic Dermatitis, Breastfed Infants

4. Evaluation of Allergy and Eosinophilia Level in Peripheral Blood of Patients with Cardiovascular Disease in Ilam

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Background: The cardiovascular diseases are one the most common causes of the deaths occurred in developing countries. Recently many have been performed to assess the relationship between the higher eosinophilia and allergy levels with the incidence, progress and severity of the cardiovascular diseases but the exact correlation between these two still remains to be understood. The current study was designed to measure the levels of allergy and the eosinophilia amongst the patients with cardiovascular diseases in Ilam. Materials and Methods: This case control study was carried out employing 59 cardiovascular patients randomly selected among those admitted to the Mostafa Khomeini Hospital of Ilam at 2010

as the case and 55 healthy individuals without any history of allergy and parasitic infections as the control group. A questionnaire was completed by each participant. 7 ml blood was taken from each participant for the CBC measurements and sera were extracted from the rest for IgE using ELISA. Data were analyzed using Epi-Info program in statically software. Results: There was a significant relationship for the variables such as the family history of cardiovascular disease (P<0.001), diabetes (P<0.003), hyperlipidemia (P<0.0001), high blood pressure (P<0.0001) and physical activity (P<0.0001) between the case and the control groups. The mean IgE titer in case group was 95.3 ± 71 and 62.44 ± 49 in control group. The mean eosinophila level in peripheral blood was 3.95 ± 1.057 in case and 1.53± 0.57 in control group. The differences between the IgE and eosinophilia levels in the case and the control groups was statistically significant (P<0.0001). Conclusion: It can be concluded that the higher amounts of IgE and the eosinophilia can be considered in incidence, severity and the progress of different types of the cardiovascular diseases so that the cardiologists can rely on the role of these variables in diagnosis of cardiovascular diseases.
Keywords: Allergy, eosinophilia, cardiovascular disease, antibody

5. Effects of Cyclic Nucleotide Phosphodiesterases (PDE) Inhibitors on Human Skin Mast Cells (HSMC), Human Lung Mast Cells (HLMC), and Basophils

Eskandari N, Bastan R, Peachell P T

Background: The Royal Hallamshire Hospital, Academic Unit of Asthma & Allergy, Dept, Of Immunity & Infectious Diseases, Sheffield University, UK Mast cell and basophil are thought to be central to inflammation that has an allergic basis as allergens activate these cells in an IgE-dependent manner to generate mediators such as histamine, eicosanoids and cytokines. PDE is known to exist as multiple molecular forms of enzyme that metabolize the second messengers . Studies of our own have shown that, of a variety of isoform-selective drugs, the PDE4-selective inhibitors, such as rolipram, attenuate the IgE-mediated release of histamine from human basophils but not from human lung mast cells (HLMC). The main aim of the present study was to characterize the type and role of PDEs regulating human skin mast cells by using selective and non-selective PDE inhibitors. Materials and Methods: Cells were pre-treated for 15 min with these agents and then challenged with an optimal releasing concentration of anti IgE (1:300) for a further 25 min for the release of histamine. Results: The data show that all the selective PDE inhibitor compounds (10-5 M) were ineffective whereas the non-selective PDE inhibitor, theophylline (10-3 M), inhibited histamine release from HSMC (74±4% inhibition; p<0.05). None of the selective PDE inhibitors had any effect on histamine release from HLMC whereas, in basophils, compounds with activity at PDE 4 (rolipram, denbufylline, Ro-2017, Org 30029) were effective inhibitors of histamine release. Conclusion: The data suggest that unlike most inflammatory cells, PDE-selective inhibitors are ineffective stabilizers of HSMC activity which is similar to HLMC.
Keywords: PDE, HSMC, HLMC, Basophils

Poster Discussion Presentation

6. Evaluation of proteins allergenicity and toxicity using by bioinformatics tools

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Although, by the end of the year 2010, more than 130 million ha were under the cultivation of transgenic plants, one of the main concerns about transgenic plants is risks derived from them. For risk analysis of GM crops laws, regulations and guidelines have been established to assure the safety of GM crops in each country. Evaluation of proteins expressed in transgenic crops, using bioinformatics tools, is one of those offered approaches. In this study using bioinformatics tools and current guidelines proposed by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and three methods amino acid 80, 6 or 8 and full lengh for allergenicity prediction based on protein sequence, we have investigated the allergenic effects and structural similarity of ectopically expressed Cry1Ab,Cry1Ac, CryIII, Chitinase, PRI and gluganase proteins with existing allergens. Thus, allergene proteins were digested *in silico* using Peptide Cutter andPeptideMas softewer. Evalutions of epitye potential of digested fragment were carried out by soaftwer.
Keywords: Allergin, Biosafety, Bioinformatic, Database

7. Basophil Activation Test (BAT) In the Diagnosis of Insect Venom Allergy in Mastocytosis Patients

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Background: Mastocytosis is a heterogenous disorder characterized by clonal mast cell proliferation that has different variants. Insect venom allergy(IVA) is more prevalent in mastocytosis patients compared to the general population and the reactions are more severe and life threatening. The diagnosis of IVA usually relies on a positive history of systemic reaction to a sting, positive skin test and/or specific IgE. However, in mastocytosis patients, specific IgE levels are usually low or even absent. Basophils, as the mediators of allergic response, have attracted attention in recent years and diagnostic tests based upon their degranulation are of special interest. The flowcytometric assays evaluating surface markers of basophils, named basophil activation tests (BATs), are rapid, in vitro tests for the diagnosis of different types of allergies. In this study we aimed to evaluate BAT as a diagnostic test in mastocytosis patients with IVA. Materials and Methods: Seventeen mastocytosis patients with IVA based on their history and skin tests and 6 mastocytosis patients without any history of IVA, were included. BAT was performed by assessing CD63 expression on basophils upon stimulation with 5 log concentrations of wasp venom ranging from 0.5-5000ng/ml. Roc curves were used in order to calculate sensitivity and specificity of BAT. Results: BAT could significantly discriminate between mastocytosis patients with and without IVA in wasp venom concentrations of 50 and 5000ng/ml (p,0.005). BAT was 87% sensitive and 100% specific in 500ng/ml of wasp venom concentration. The cut off value was discovered to be 1.11%. Conclusion: BAT was discovered to be specific and acceptably sensitivity for the diagnosis of IVA in mastocytosis patients. It can be valuable in mastocytosis patients with a history of insect sting and undetectable IgE. In conclusion, BAT is a novel diagnostic test for IVA in mastocytosis patients.
Keywords: BAT, Insect Venom Allergy, Mastocytosis Patients

8. A Study of Avicennia Marina Pollens Allergenicity in Two Regions of Bushehr Province in Iran

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Background: Pollens can play an important role in allergic diseases and many studies have revealed that allergenicity increases in industrial cities. In Bushehr province *Avicennia marina (A.marina)* has grown in two regions Assaluyeh and Bordekhoon. Assaluyeh is an industrial city with petrochemical factories but Bordekhoon is devoid of petrochemical pollutants. The aim of this study was to compare the allergenicity of *A.marina* pollens in these two parts of Iran. Materials and Methods: Pollens from those two regions were collected and examined by scanning electron microscopy. Pollens extracts were prepared in PBS and Pollen proteins pattern was detected by SDS-PAGE. As an experimental model, Balb/c mice divided in four groups (n=10). First and second groups as control groups received PBS and Aluminum hydroxide (Alum) respectively. Third group was treated by Bordekhoon pollen extract plus Alum, and forth group was treated by Assaluyeh pollen extract plus Alum. All injections were performed intraperitoneally for four times. Intradermal skin tests with pollen extracts of Bordkhoon and Assaluyeh was performed and wheal diameter was measured after 30 minutes. Eosinophily was detected in blood smears and total serum IgE level was measured

in each group. Immunoblot assay was also performed with mice serum samples and conjugated anti IgE (Koma Co., Seoul, Korea) to detect allergen bands. Results*:* Present study revealed that *A. marina* pollen surfaces of Assaluyeh and Bordekhoon were different. Pollen proteins profiles of two regions showed some different bands. Intradermal Skin tests revealed that wheal diameter significantly increased in mice treated with pollen extracts of two regions in comparison with control groups (pv=0). Blood smears of groups treated by Bordekhoon and Assaluyeh pollen extracts showed eosinophily in comparison with control groups. Statistical analysis showed that eosinophily in blood of mice treated by Assaluyeh pollen extract was significantly higher than the group treated by Bordekhoon pollen extract (pv=0.011). Total serum IgE level in mice treated by pollen extracts of two regions increased in comparison with control groups, but it was significantly higher in group treated by Assaluyeh pollen extract (pv=0.003). The immunoblot analysis revealed two bands in each region that reacted with the IgE antibodies induced in mice treated by pollen extracts of two regions.Conclusion: Results of this study showed that *A.marina* pollens allergenicity increased in Assaluyeh in comparison with Bordekhoon, probably by air pollution. But for more exactly investigations it needs to be studied further to detect that these changes were result of air pollution or not.

Keywords: Avicennia Marina Pollens, Allergenicity Bushehr

Poster Presentation

9. Different Isoforms of Cuc m 3 and Their Expression Patterns in Different Parts of Cucumis melo

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Background: Allergy to melon (Cucumis melo) is one of the most common food allergies in Iran. Since allergic patients show different reactions to various parts of Cucumis melo, this study aimed to compare the characteristics of different isoforms of Cuc m 3, one of allergens of Cucumis melo, as well as their expression levels in different parts of Cucumis melo, in order to understand the molecular mechanisms of their expression. Materials and Methods: Sequences obtained from our pervious study (accession numbers EU556704 and EU679402) showed that in addition to mRNA fragment which coded a 16.96 kd protein, there was one more mRNA sequence that coded a smaller PR-1 (Cuc m 3) protein. As there is an increased expression level of PR proteins after exposure to pathogens, we used eight groups of melons which were under different dosage of poison as well as one group without pesticide poison spray. To assess PR-1 expression in the presence and absence of plant pathogen, we designed one forward and two reverse primers specific for larger and smaller mRNA fragments, respectively; and examined the presence of these mRNA fragments in pulp and peel of melon. Using a semi-quantitative RT-PCR, we compared the expression level of both small and large mRNA fragments from Cuc m 3 to that of tubulin-β. Results: RT-PCR confirmed the presence of two different size mRNA fragment of Cuc m 3 obtained from our previous study. Interestingly, both coding region and 5' end of smaller isoform were different from those of larger isoform. RT-PCR also showed that expression level of larger isoform was higher in melon groups under low dose or no poison than those which were under high dose of poison; however, the expression level of smaller isoform had no correlation with the dosage of poison and was higher than larger form Furthermore, in all groups of melon, the expression level of both larger and smaller isoforms of Cuc m 3 were higher in melon peel compared to its pulp.
Conclusion: Our results suggest that melon constantly express a small isoform of Cuc m 3 as a member of PR-1 protein family which is more abundant in the peel of fruit compared to its pulp. After exposure to pathogens including poison, it expresses a larger isoform of Cuc m 3 (another member of PR-1 protein family). Further investigation is needed to determine the allergencity of smaller isoform of Cuc m 3.

Keywords: Allergy, Cucumis melo, Cuc m 3 isoforms

10. The Comparison of TH1 and TH2 Cytokines Gene Expression in Allergic and Non-Allergic Patients with Nasal Polyps by PCR

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Background: Several studies are in the process to determine probable role of immune system in Etiopathogenesis of nasal polyposis. In order to elucidate the probable participation of Th1, Th2 lymphocytes in induction and progression of nasal polyposis in two groups of allergic and non-allergic patients this study was designed. Materials and Methods: Seventy five patients with nasal polyposis with mean age of 38 years (18-81 years) were studied. They were examined for total serum IgE, specific serum IgE and skin test to differentiate them as allergic and non-allergic patients. To determinate the possible correlation of allergic reaction of upper respiratory tract and nasal polyposis, cytokine gene expression was evaluated on extracted RNA by RT-PCR. Results: Frequency of IFN-γ and IL-4 gene expressions were more in allergic patients in comparison to non-allergic individuals (IFN-γ: 39.5% Vs. 14.2%, P= 0.3; IL-4: 44.7% Vs. 18.9%, P=0.02, respectively). IL-10 and IL-12 (P35 & P40 fractions) genes were not significantly different in these two groups. 10, IL-12 (p35,p40) genes do not differ significantly in two groups. Conclusion: This research suggests that an imbalance of Th1 and Th2 cells plays an important role in pathophysiology of nasal polyp. Thus although nasal polyposis is a multifactorial diseases with several different etiological factors, chronic persistent inflammation is undoubtedly a major factor irrespective of the etiology.

Keywords: Nasal polyposis, Th1, Th2 cells, Cytokines, RT-PCR

11. Genetic Association Study of TGF-β Gene Polymorphisms with Allergic Rhinitis

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Background: Allergic rhinitis (AR) is the most common chronic inflammatory disease of nasal airways induced by IgE–mediated type I hypersensitivity. That is caused by the interaction of multiple genetic and environmental factors. Transforming Growth Factor Beta-1 (TGF-β1) is a multifunctional cytokine witch play a key role in regulation of immune responses to maintain peripheral tolerance against antigens, including auto antigens and foreign allergens. The aim of present study is to investigate whether genetic polymorphisms at codons 10 (+869 T>C), 25 (+915 G>C) of TGF-β gene predispose to AR in Iranian population. Also, the relationship between this SNPs and serum IgE &IgA levels and peripheral blood eosinophil countsin this population was evaluated. Materials and Methods: In a case–control study, 155 AR patients and 163 allergy-free controls were recruited according to age, gender and living area. Two single nucleotide polymorphisms (SNPs), +869 T>C, +915 G>C, were investigated by using PCR sequence-specific primer (PCR-SSP) technique.Serum IgE & IgA were measured by ELISA and Nephlometric method respectively and peripheral blood eosinophil counts based on the eosinophil numbers per total cell numbers. Statistical significance was defined as $p<0.05$ (using SPSS 18). Results: The finding showed that there were not statistically significant differences between cases and control groups ($p>0.05$). Additionally, there was no significant relationship between these SNPs and activity disease indexes. Conclusion: Although genetic polymorphisms at codons 10 and 25 of TGF-β gene may change functionally or quantitatively TGF-β cytokine; this study showed that polymorphisms in this regions cannot be associated with susceptibility and pathogenesis of AR in Iranian population.

Keywords: TGF-β, Gene polymorphisms, Allergic Rhinitis

12. Prevalence of Aeroallergens in Allergic Disease in Ahvaz

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Background: Allergic diseases are extremely common disorders worldwide. Recognition of various environmental allergens is of great importance. The purpose of this study was to determine sensitivity to common allergens by skin prick test. Materials and methods: In this cross sectional study, 410 Volunteers with the sign and symptoms of allergic diseases who referred to Ahvaz Gahad-e-daneshgahi polyclinic during 2010-2011 were investigated. All patients were subjected skin prick test with 24 common allergenic extracts. Data were analyzed by SPSS-18 software using Chi square test. Results: There were 410 allergic patients of whom 213 (52%) were males and 197 (48%) were females, with a mean ±SD age of 31.7±14.4 years.In 259patients (63.2%) hada history of allergiesinthe family. Three hundred sixteen patients (77.1%) were skin test positive to at least one aeroallergen and ninety four patients (22.9%) were negative for skin-prick test (SPT). Among those with positive test responses, 27 (6.6%) subjects had positive SPT to indoor allergens alone, 195 (47.6%) subjects to outdoor allergens alone, and 94(22.9%) subjects reacted to both of them. Among indoor and outdoor allergens, mites (44.9%) and weeds (78.3%) were the most common aeroallergens, respectively. Salsola kali with frequency of 72.2% was the most prevalence individual allergen. In this study the frequency of positive skin tests in men was higher than women (78.2% *vs.* 76.1%) but this difference was not significant (p=0.348).The mean total IgE serum levels were determined as 153.9 IU/ml. Conclusion: The study showed outdoorallergens especially weeds are the main allergensinthe region that can cause allergies. Considering the abundance of weeds in this area, recognition of common allergens and warning patients to avoid them could be an effective way to control the disease progress.
Keywords: Allergy, Skin test, Allergen, Prevalence

13. Investigation the Prevalence of Respiratory Airborne Antigens Sensitivity in Patients Suffering with Allergic Rhinitis in Kerman

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Background: Allergic rhinitis is one of the annoying diseases related to inflammation of mucous membrane of the nose. Diagnosis is usually made by history and clinical examination. Several reasons can cause the symptoms of allergic rhinitis, yet the purpose of this study was to investigate the Prick skin test results to assess the sensitivity to airborne antigens in patient with Allergic rhinitis, referred to asthma and allergy clinic to Kerman. Materials and Methods: This is a descriptive-cross sectional study. It was done by study of 51 patients with rhinitis referred to asthma and allergy clinic of Kerman. Aftergetting confirmed therhinitis, prick skin test was done by 20 airborne antigens. For positive control, the histamine skin test was done for all patients. Finally the result was analyzed using the SPSS17. Result: In total of 51 subjects, 23 males and 28 females with average age of 24±8 years were studied.Of the studiedairborneantigens, the highest prevalence were belong to chenopodiaceae(41.2%),compositae (35.3%), chopodiunAlb(21.6%),salicaceae ,and animal hair (19.6%)among patient with allergic rhinitis in Kerman.out of the studied antigens, the strongest association of antigens was seen between compositae and chenopodiaceae.(p value: 0.009). Conclusion: The result of this study indicated that, encounterwith the plants and animal hairantigens can cause allergic symptoms of allergic rhinitis in the population of Kerman.
Keywords: airborne antigens, rhinitis, Kerman

14. Skin Prick Test Reactivity to Common Allergens among Allergic Rhinitis Patients

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Background: Allergic rhinitis is an extremely common disease worldwide .Allergic rhinitis is the mainly frequent allergic disease with negative influence on patients' quality of life. Allergens are very often involved in allergic rhinitis and their prevalence may vary in different regions. The purpose of this study was to assess the prevalence of positive skin test to numerous common allergens among allergic rhinitis patients in the city of Tabriz. Materials and Methods: 100 patients with allergic rhinitis were enrolled in this study. 40 allergens were tested on patients with Skin prick test. Allergens include Pollens, Molds, Mites,Domestic Animal, Tree mix, Grass mix and bird feather (Mix). Results: Of 100 patients with Allergic rhinitis referred specifically for allergy assessments, 100 cases gave positive immediate skin prick tests to at least one of 40 allergens used routinely. Prick test reactions in the skin test positive patients were most commonly seen to house dust mite (68%) , Grass mix (61%), Bird feather(55%), egg(40%) and mold mix(42%) . the skin test positive to other allergens was within the range 10% and 40%. The overall rate of sensitization to any allergen were House dust mites (68%),Grass mix (61%). Conclusions: Our work showed the importance of the House dust mites, Grass mix, Mold mix and Egg, especially the House dust mites and Grass mix .This information may be useful to clinicians managing patients suffering from allergic rhinitis.Thus; skin prick test is an important diagnostic procedure in such cases. We suggest that skin prick test for contact sensitization can be helpful in the management of allergic rhinitis.
Keywords: Skin prick test, Allergic rhinitis,

15. Assesment of Allergen-Specific IgE by Immunoblotting Method in Resistant Atopic Dermatitis Patient

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Background: Atopic dermatitis is a chronic and relapsing inflammatory disease characterized by typically distributed eczematous skin lesion with itches. The aim of study was to highlight the frequency of the allergens among the patient who are affected by resistant atopic dermatitis in the Eastern Azerbaijan. Materials and Methods: In this descriptive and analytical study the serum's level of Total IgE and frequency of specific IgE were measured by Immunoblotting method against 20 common allergens about 35 atopic dermatitis patients who visited Sina Hospital in 2010-2011. Result: The average age of patients was 29.2±14.79 years. In this study 51.4% patients were male and 48.6% patients were female .During this assessment, we have seen 97.1% of the patients who have at least 3 main criterions .Common minor criterions were : pruritus when sweating 68.6%, xerosis 54.3%, Dennie–Morgan lines 17.1% and keratosis pilaris 11.4%.The average of serum total IgE was 227.51±103IU/ml. In this study 32 patients (91%) had specific IgE against at least one allergen. The most frequent allergens related to: Cultivated rye(48.6%) ,Timothy grass (42.9%),House dust mite (22.9%), Alternaris (20%),Cat (20%), Cladosporiom (14.3%), Horse (14.3%), Birch (11.4%),Potato (11.4%), Dog (11.4%), Egg white (8.6%), Cow milk (8.6%). The frequency of positive allergens among the patients who had been studied was in: plants and fungus allergens group 53.34%animal allergens group 26.8%food allergens group19.56%.60% of patients after avoiding of the allergens which they had been sensitized to, and in some cases immune therapy, were cured. In the control group there was no positive allergen or serum total IgE elevation. Conclusion: Recognition of the frequent allergens such as : Cultivated rye, Timothy grass, House dust mite Alternaris ,Cat ,Cladosporiom , Horse, Birch ,Potato ,Dog , Egg white , Cow milk in order to remind to the patients to avoid to be confronted to these allergens and immunotherapy or desensitization is useful in this area .
Keywords: Atopic dermatitis, Allergen, Specific IgE, Total IgE

16. Production of Bovine Recombinant Beta-lactoglobulin in *Pichia pastoris* to Possible Reduction in Allergenicity

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Background: Beta-lactoglobulin (BLG) is the main whey protein in bovine milkand is one of the most important milk allergens. It is homodimeric at physiological conditions and each monomer contains two disulfide bonds (C66-C160 and C106-C119) and one cysteine at position 121(C121). This free thiol plays an important role in the heat-induced aggregation of BLG and, possibly, in its conformational stability. In a recombinant type, C121 was changed into Ser (C121S) to inhibit its aggregation. The aggregated form of BLG could play a role in anaphylactic responses, binding properties and allergenicity of the protein. Hence, the recombinant form can be used for further investigation the

function of C121. Materials and Methods: Yeast *P.pastoris* expressing a mutant bovine BLG in which C121 was changed into serin and *P.pastoris* expressing wild type (WT) of bovine BLG were cultured on YPD-agar medium (1% yeast extract, 2% glucose and agar) and were incubated for 3 days at 30 °C. A fresh colony of each cultures was grown overnight at 30 °C under shaking in YPD medium. For production of BLG, the yeasts cells were transformed from YPD medium into BMGY medium (1% yeast extract, 1.34%yeast nitrogen base, 2% glucose, 100 Mm potassium phosphate, pH 7.0). The supernatant was collected by centrifugation for further purification of the protein. The protein expression was followed by SDS-PAGE. Result: SDS-PAGE showed that WT and C121S recombinant BLG were produced. Conclusion: It seems that we have obtained considerable amount of WT and mutant BLG for further evaluation. The next step is purification of BLG and mutant BLG to test their aggregation, anaphylactic response and binding properties. In addition, the mutant BLG could be used for understanding the mechanism of allergic reaction to BLG.

Keywords: Beta-lactoglobulin, *Pichia pastoris*, Allergenicity

17. The Occurrence of Palatine Tonsil Hypertrophy in Pediatric Population with Allergic Diseases

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Background: Although tonsillectomy is the most common made surgery in pediatric population despite this triggers of tonsils hypertrophy are not recount exactly. Most studies emphasize hyperplasia itself is not a disease, but only a result of increased immunologic activity, and it does not necessary be due to inflammation or tonsillitis. A few investigations claim that allergy factors can be an indication for tonsillar hypertrophy. Allergic rejoinder appears in the mucosa of the respiratory tract and tonsils situated in the entrance of airway mucosa where encounter with antigens perpetually. Materials and Methods: Allergy of 250 childrenwere assessedclinically, checked theirsamplesoftonsils histopathology andwere compared with clinical findings statistically. The study was approved by the ethic committee of Tabriz University of Medical Sciences. Results: Inthis study 106girls(43.1%) and 140male (56.9%) were participatedwith amean age of7.44 ± 2.27years. Thepathologic findings ofthe tonsilsand allergy were significantlyassociated (p=0.03). Conclusion: In pediatric population, allergy to various types of allergens is one of the main operators for a larger grade of tonsils. Then, avoid of confront or early diagnose and cure to them can help alleviate of tonsil hyperplasia.

Keywords: Tonsil, allergy, hypertrophy, pediatric population

18. Evaluation of 40 Allergens with Prick Test on Atopic Allergic Patients

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Backgruond: In Atopic Dermatitis (AD) hypersensitivity reactions to allergens are commonly observed and are assumed to make a major contribution in the pathogenesis of the disease. Common allergens play an important role in atopic dermatitis via type I hypersensitivity reaction. Atopic dermatitis often occurs in patients who have immediate skin tests to several common allergens. The prevalence of atopic dermatitis is however quite difficult to establish since the diagnostic criteria are not applied universally and are not standard. Materials and Methods: The present study was undertaken to observe the prick test reactivity to common allergens in 67 atopic dermatitis patients. 40 allergens were tested on patients with Skin prick test. Allergens include Pollens, Mites,Domestic Animal, House dust mite, canned fish, glucose, Egg, Mold mix , Bird feather Mix and Mix grass. Results: Pollens, Domestic Animal, House dust mite, canned fish, glucose, Egg, Mold mix, Mix Bird feather and Mix grass.The most frequent allergen in prick test reactivity house dust mite.Of the67patients with atopic dermatitis patients referred specifically for allergy assessments, 67 cases gave positive immediate skin prick tests to at least one of 40 allergens used routinely.The most common allergies include : Grass mix (61%),Bird feather(56%),Timothy grass(51.1%), mite (48%),cultivated rye(44.6%),Sweet vernal grass(42.2%), glucose 40%, egg(40%), Grass mix (40%),mold mix(40%),Animal Hair(40%),Tomato(40%) ,canned fish(36%) and the skin test positive to other allergens was within the range 10% and 40%. The overall rates of sensitization to any allergen wereGrass mix (61%). Timothy grass (51.1%). Allergic to other pollens was within the range 12.2% and 26.7%. Conclusions: Since there is no cure for atopic dermatitis, treatment should mainly involve discovering the triggers of allergic reactions and learning to avoid them. Allergens may play a role in the pathogenesis of atopic dermatitis.

Keywords: Skin prick test, Allergy, Atopic

19. The Study of Food Allergens in Patients with Urticaria and Food Allergic Symptoms by Prick Test

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Background: Urticaria refers to a group of disorders involving adults and children, in which red patches and wheals happen in the skin. Urticaria is a frequent disorder that influences 15-20 % of the general population during their life. Urticaria negatively both work and social life of the patients. The etiology of urticaria continues to be uncertain.Urticaria frequently shows up a few minutes after exposure with the allergen. The aim of this study was to determine the frequency of allergens with urticaria. Materials and Methods:64 patients with food allergic were enrolled in this study. 40 allergens were tested on patients with Skin prick test. Allergens include Pollens, Molds, Mites,Domestic Animal, House dust mite, canned fish, glucose, Egg, Mold mix, Peanuts, Beans, Mix Bird feather and Mix grass. Results: Of 64 urticarial patients referred specifically for allergy assessments, gave positive immediate skin prick tests to at least one of 40 allergens used routinely. Prick test reactions in the skin test positive patients were most commonly seen toGlucose (70%), Beans (65%), Peanuts (65%), Mold Mix(55%) , Bird feather(40%), egg(40%), and Urticaria to other allergens was within the range 5% up to 36%. Conclusions: A notable proportion of cases with urticaria show sensitivity to House dust mite, Beans, Peanuts, glucose, egg, and mold mix and bird feather. Thus, skin prick test is an important diagnostic procedure in such cases. We suggest that skin prick test for contact sensitization can be helpful in the management of urticaria.

Keywords: Skin prick tests, Aallergens, Urticaria

20. Frequency of Mold Allergy in Rhinitis Allergic Patients Referred to Tabriz Specialized Clinics

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Background: Allergic rhinitis can be stimulated by severalallergens. Moldsare one of these allergens which theirproliferationrate in different geographicareasseems to beimportant. Therefore, in this study, the frequency of mold allergens in allergic rhinitis patients referred to specialized clinics of Tabriz Imam Reza hospital were studied. Materials and Methods: In this study, levels of specific IgE against four molds including Penicillium, Aspergillus, Alternaria and Cladosporium were investigated in the serum of 90 patients with symptoms of rhinitis diagnosed by physician using Immunoblotting method. Results: Ninety allergic rhinitis patients (40 men and 50 women) were recruited. Agerange of patients was between 6 to 53 years and mean age was 29.9 years. Most patientswerebetween28-31years.Frequency ofallergywas3.3% for Penicillium, 5.6%for Aspergillus, 13.3% for Alternariaand 4.4% for Cladosporium.There was a significant statistical association between age and allergic rhinitis to Alternaria.There were no other associations between any other fungal sensitization and sex and age. Conclusion: Moldscangrowandproliferate inveryhumidenvironments. As Tabriz (in the northwest of Iran) haslowhumidity climate, allergytomolds is of low rate in this area.

Keywords:Frequency, Mold Allergy, Allergic rhinitis

21. Prevalence of Allergy to Plants Pollen in Patients with Rhinitis Allergy Referred to Tabriz Imam Reza ENT Specialized Clinics, 89-90

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Background: Allergic rhinitis (AR) is the most common allergic disease with negative impacts on patients' quality of life. The prevalence and pattern of sensitization vary among different countries and populations. Inhaled allergens such as pollens are the main cause of AR that highly frequent in the environment. Identification of the most prevalent aeroallergens in each area has a very important role in diagnosis and treatment of AR. Plant pollen role as causing AR in the northwest of Iran, has not been yet well studied. Therefore, is research was aimed to investigate the prevalence of the sensitivity to pollen allergens in AR patients referred to allergic clinics of Tabriz city. Materials and Methods: This cross–sectional study was conducted on 90 allergic patients who were referred to allergic specialized clinics of Tabriz city. Specific immunoglobulin E against common pollen allergens were measured using Immunoblotting method. Results:A total of 90 cases (female: 50, male: 40, aged between 6-53 years, mean age 29.9 years) diagnosed with AR through history and clinical manifestations were recruited to study. Most patients were ages were between ages of 28-31 years. And 58.9 percent of patients had a sensitivity of at least one common inhaled pollen allergens. Immunoblotting testing results showed that Prevalence of allergy to pollen cultivated rye, timothy grass, sweet vernal grass and cocksfoot were 51.1%, 44.6%, 42.2% and 40% respectively. Allergytootherallergens was at the range12.2% -26.7%. Conclusion: according thefindingsof this study,the pollen of plants are mainallergenscausingAR inthis geographic area.The high prevalence of allergy to plants pollen seems that training to avoid contact with these factors in pollen seasons, treatment and immunotherapy could probably be helpful.
Keywords: Allergy -pollen-allergic rhinitis- Immunoblotting

22. Evaluation of TGF-β1 and VEGF Gene Expression in Pterygium Tissue of Atopic Patients

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Background: The exact pathogenesis of pterygium has not been completely elucidated. Ultraviolet light exposure, wind, dust, heat, infection, smoke, chemicals, dry eye, and pollens are associated with pterygium. Growth factors are also considered to play a key role in pterygium angiogenesis process. Transforming growth factor beta-1 (TGF-β1) and vascular endothelial growth factor (VEGF) are two of the principle mediators of fibroblast stimulation and tissue remodeling in allergic conditions. We compared the association between pterygium and TGF-β1 and VEGF gene expression between atopic patients and nonatopic individuals. Materials and Methods: After obtaining informed consents, questionnaires were used to obtain demographic and clinical data from patients who underwent pterygium excision surgery. Skin prick testing and total serum IgE measurement were done to confirm atopy in 30 consecutive patients (case group). Besides, 30 consecutive patients without a history of atopy and negative skin prick test were included as the control group. A semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) was done to determine TGF-β1 and VEGF gene expression in all patients using GAPDH gene as an internal control and the results were compared between two groups. Results: Eosinophil count and serum IgE were significantly (P-values =0.031 and 0.001, respectively) more in atopic patients compared to the control group. Besides, TGF-β1and VEGF mRNA gene expression were significantly more in atopic patients compared to non-atopic individuals (2.50 ± 1.11 vs 1.40± 0.46 (P-value = 0.0001) & 1.90±0.74 vs1.34±0.52 (P-value = 0.003), respectively). Conclusions: The excessive expression of TGF-β1and VEGF gene in pterygium tissue of patients with atopy suggests that these growth factors may play a role in severity of the pterygia pathogenesis.
Keywords: Pterygium , Atopy, TGF-β1, VEGF, Gene expression

23. Is Response to Food Allergies Regulated CXC Chemokine?

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Background*:* Food allergies are defined as adverse immunological (hypersensitivity) response to food, and as such it is not a single disease, nor is it caused by one path physiologic disturbance. They are a range of conditions such as allergic asthma, -rhinitis, -conjunctivitis, -dermatitis, food and drug allergies and anaphylaxis. Induction of T helper (Th)-2 immune response in parallel with IgE dependent eosinophil, basophil and mast cell mediated tissue damage is the characteristic feature of the allergies. Therefore, we aimed the current study to find out whether if chemokine network as recruiter system for these cell types is involved in development of food allergies. Material and methods*:* In this experimental study, blood samples were taken from 63 food allergy children and 100 healthy controls on EDTA pre-coated tubes. From the specimens, Circulatory levels for CXCL1 (Gro-α), CXCL1 (Mig), CXCL10 (IP-10) and CXCL12 (SDF-1) were measured by ELISA. The demographic information was also collected in parallel with experimental part of the study by a questionnaire which was designed specifically for this study. Results*:* Our results indicated a differential pattern of CXC chemokin expression in these children. We observed elevated levels of inducible pre-inflammatory chemokins CXCL1(Gro-α),CXCL1(Mig) and CXCL10(IP-10) while not significant enhanced constitutive CXCL12(SDF-1) level in food allergic patients.Conclusion*:* our findings suggest that CXC chemokines are crucial factor in pathogenesis of food allergies. It can also be concluded that those chemokines can be used as pivotal biological markers in diagnosis of food allergies.
Keywords: Food allergies, CXC Chemokine

24. Evaluation of the Effect of Maternal Factors on Cord Blood IgE

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Background: Allergic disorders are among the most common diseases around the world especially in children. Many factors are contributed to the pathogenesis of atopic disorders but early events during the pregnancy period are the most important. Studies have shown that level of cord blood IgE can be a predictor of forthcoming allergic disorder in children and therefore the recognition of factors which influence cord blood IgE has an important role in proper control and management of allergy. The aim of this study was to evaluate the correlation between maternal factors with cord blood IgE in a sample of Iranian population. Materials and Methods: In a cross-sectional study, 160 pregnant women randomly selected and Data on sociodemographics and history of allergic disorders were taken by mean of questionnaire. Blood samples of mothers and matched cord blood were collected and total serum IgE levels were measured by ELISA method. To rolling out the possibility of contamination with maternal blood, total IgA was checked for all cord blood samples. Results: Among mothers, 15.9% had history of atopic diseases and the mean IgE level was significantly higher in atopic group than non atopic (247 vs 99, P <0.05). In 63% of cord bloods, IgE was more than 1 KIU/ml and the mean total IgE for male and female cord blood was 2.36 and 2.32 respectively. There was no correlation between maternal IgE with cord blood IgE. The correlation between maternal factors such as age, parity, gravidity, allergens exposure and smoking with cord blood IgE was not significant. Conclusion: The results of this study show that significant number of cord blood samples has high IgE level but we found no association of maternal factors with cord blood IgE levels. Further studies need to evaluate the reasons for high level of IgE in cord bloods.
Keywords: Maternal Factors, Cord Blood IgE

25. Crucial Role of CC Chemokines MCP-1, RANTES, and Eotaxin in Children with Food Allergy

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Background*:* Food allergies are defined as adverse immunological (hypersensitivity) response to food, and as such it is not a single disease, nor is it caused by one pathophysiologic disturbance. They are a range of conditions such as allergic asthma, -rhinitis, -conjunctivitis, -dermatitis, food and drug allergies and anaphylaxis. Induction of T helper (Th)-2 immune response in parallel with IgE dependent eosinophil, basophil and mast cell mediated tissue damage is the characteristic feature of the allergies. Therefore, we aimed the current study to find out whether if chemokine network as recruiter system for these cell types is involved in development of food allergies. Material and methods*:* In this experimental study, blood samples were taken from 63 food allergy children and 100 healthy controls on EDTA pre-coated tubes. From the specimens, Circulatory levels for MCP-1, RANTES, and Eotaxin were measured by ELISA. The demographic information were also collected in parallel with experimental part of the study by a questionnaire which was designed specifically for this study. Results*:* Our results indicated a differential pattern of CC chemokin expression in these children. We observed elevated levels of inducible pro-inflammatory chemokines MCP-1, RANTES, and Eotaxin enhanced constitutive in food allergic patients.Conclusion*:* our findings suggest that CC chemokines are crucial factor in pathogenesis of food allergies. It can also be concluded that those chemokines presumably can be used as pivotal biological markers in diagnosis of food allergies.
Keywords: MCP-1, RANTES, Eotaxin, Food Allergy

26. Artemisia Vulgaris and Allergy

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Background: Artemisia Vulgaris L. is called Branjasef in Iran. It is a perennial weed and native to temperate region (e.g Iran). It is a very common plant growing on nitrogenous soils, like weedy and uncultivated areas, such as waste places and roadsides. Nearly the whole of A.vulgaris segment is used in traditional medicine. The leaves have an antibacterial action, inhibiting the growth of Staphylococcus aureus, Bacillus typhi, Bacillus dysenteriae , streptococci, E. coli, Bacillussubtilis, Pseudomonas. It is slightly toxic, however, and should never be used by pregnant women, especially in their first trimester, since it can cause a miscarriage. It is diuretic and antispasmodic. It is very effective for killing of stomach and gut worms especial ascaris. The extract from A.vulgaris has antioxidant activity. It is widely used in the Philippines for its anti-inflammatory properties. But pollen of Artemisia particular Artemisia Vulgaris L. is strong aeroallergen .this plant pollen in summer .Then its pollen bring seasonal allergy in capable individual. Recently has been shown that people with specific subtype of HLA-DRB1 have more susceptible to allergy to Artemisia vulgaris. Materials and Methods: In our search in Sabzevar, in summer, we study on 50 patients with allergy with refer to doctor for treatment of their allergy. They were tested with prick test. Then DNA extraction and HLA typing was performed for patients have allergy to Artemisia vulgaris and 20 controls. Result: It was known that 27 patients (54%) had allergy to Artemisia vulgaris L. In the group of patients, a statistically increase in HLA-DRB1*01, allele frequencies was found when compared with control group (9% vs 2% P= 0.035). Conclusion: Incidence of allergy to Artemisia Vulgaris in Europe and USA is 10-14% of whole seasonal allergy in summer, however, incidence of this allergy in Sabzevar is 54% . Therefore incidence of this allergy in this town is more common than usual. Art v1 has a tendency towards binding to HLA-DRB1*01. Individuals with HLA-DRB1*01 are more susceptible to allergy to Artemisia vulgaris.
Keywords: Artemisia vulgaris, HLA-DRB1, Prick test

27. A Study on the Morphology, Ultrastructure and Allergenecity of *Populus alba* L. Pollen grains

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Background: Along with an increase in the traffic volume and construction of the numerous parks and other green spaces by the City, the planting of trees in the city parks, streets and the sidewalks is ever increasing without any consideration of their allergenecity effects. Hence investigating the allergenecity of tree pollen is of great importance. In this study, the morphology, ultrastructure and the allergenecity effects of *Populus alba* L. (Salicaceae) pollen grains were investigated. Materials and Methods: Pollen was collected from public parks in Tehran-Iran during the flowering period and subjected to scanning electron microscopy (SEM). Pollen protein content was investigated by SDS-PAGE. For allergenecity studies, pollen extract and PBS (control) were separately injected, intraperitoneally and subcutaneously into two groups of guinea pigs. Dot blot analysis was performed for the presence of any protein allergen in the extract. SEM revealed a semitectate exine with conspicuous crevices, and its surface has a reticulate pattern that is perforated by conspicuous micropores. At the end of pollination, pollen grains of Populus were empty of their content, and the pollen surface appeared ruptured. Results: Coomassie staining of the SDS-PAGE showed several bands ranging from 17 to 97 kDa. Skin test showed a significant increase (P< 0.05) in wheal diameter in the animals immunized with pollen extract compared to the controls (buffer). The amounts of eosinophils and total IgE were also significantly increased (P<0.001 and P< 0.05, respectively) in the group treated with pollen extract compared to the controls (buffer). Immunodotblot results were positive at serum and 2°-Ab concentrations of 1:10 and 1:1000, respectively, an indication of the presence of the protein allergen in the pollen extracts of *Populus alba*. Conclusion: Overall, the results of skin tests, the content of total Ig*E* and eosinophils and immunodotblot analysis in animals treated with pollen extract indicate the allergenic activity of *Populus alba* L. pollen grains.
Keywords: *Populus alba*L., pollen grain, allergy

28. Changes in Prevalence of Asthma and Allergies in 13-14 Year Old Children of Birjand City

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

29. Increased Expression of CD69 Antigen on Human Peripheral Blood NK Cells in Patients with Allergic Rhinitis

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Background: Allergic rhinitis is an inflammatory disorder of the nasal mucosa with great morbidity and high prevalence. Natural killer (NK) cells might have a role in allergic rhinitis or may have been affected during the process of allergy. We aimed to evaluate the changes of some markers and receptors on NK cells in patients with allergic rhinitis and compare them with normal controls. Materials and Methods: Flowcytometric analysis was used with double staining of the peripheral blood mononuclear cells to examine the expressions of activation markers CD69 and CD25, and activation receptor NKG2D and inhibitory receptor NKG2A on CD56+ NK cells of 20 patients with allergic rhinitis and 20 nonatopic controls. The serum total IgE level was measured by Enzyme-linked Immunosorbent Assay. Results: The expression of CD69 antigen on CD56+ NK cells in patients with allergic rhinitis was significantly higher than that of healthy group ($p < 0.05$). No significant differences were observed between CD25, NKG2D and NKG2A expression on the surface of CD56+ NK cells from healthy controls and allergic rhinitis subjects. Our study also showed that there is no significant correlation between the expression of CD69, CD25, NKG2D and NKG2A on CD56+ NK cells and level of serum total IgE in patients and normal subjects. Conclusions: These results indicate that the expression of CD69 antigen on NK cells of patients with allergic rhinitis is increased. The high expression of CD69 on NK cells in allergic rhinitis patients suggests that these cells are activated, which might be due to the cytokines secreted from allergen-stimulated T cells or activated monocytes.
Keywords: CD69, NK Cells, Allergic Rhinitis

30. Review of Allergen Databases for Allergenicity Assessment of GMO Novel Proteins

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Genetically modified (GM) plants can be a key solution to growing demand for food in the world. In recent decade genetically modified (GM) plants are increasingly used for food production and industrial applications. Hence biosafety of GM products is one of the important necessities for commercialization of them. One of the most important issues in biosafety is being Non-allergenic. Allergy and allergens affect approximately one third of the world population. Allergens are proteins or glycoproteins that are recognized by IgE produced by the immune system of allergic individuals. Untill now many manners have introduced for allergy assessment of novel proteins. One of these manners as effective strategy is bioinformatic analysis of allergy assessment of novel proteins.
The efficacy of any specific bioinformatics analysis of the potential allergenicity of new food proteins depends directly on the nature and content of the databases that are used in the analysis. Recently, number of different allergen-related databases has been developed and each designed to meet a different need. These databases differ in content, organization, and accessibility. Until nowaround 1,600 allergenic structures have been identified. The main purpose of this study is review of allergenic databases including IUIS, AllAllergy, Allergome, Allergome, InformAll, AllergoPharma, SDAP, EVALLER, ALLERDB, Biopep and allergenicity assessment of GMO novel proteins.
Keywords: *In Silico* analysis, allergenicity assessment, Allergens, GMOs

31. A Proteomics Study in FcεRI Mediated Signaling of Mast Cells

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Background: Inflammatory and hypersensitivity disorders such as allergy and asthma are closely related to IgE-mediated antigen stimulation of mast cell/basophile lineage and induced release of pharmacologically active mediators from these cells. This investigation aimed at an improved understanding of the biochemical pathways and molecules released as a result of mast cell exocytosis through the application of a functional proteomics approach to generate from the model mast cell line, RBL-2H3.1: (i) a temporal profile for secretory proteins released from cells sensitized with IgE and activated with antigen since mediators released are directly responsible for the pathogenesis of mast cells in allergic/type I hypersensitivity disorders and present potential targets for therapeutic intervention strategies. (ii) 2-D reference map for RBL-2H3.1 cells' proteome, which may underpin future proteomic investigations on this cell and other functionally related cell lines. (iii)an expression and phosphorylation profile of proteins involved in IgE-mediated exocytosis. Materials and Methods: Secretome analysis of samples generated from IgE+Ag activated cells for 90s, 30min and 3h compared to samples from non-activated cells using LC-ESI mass spectrometry. Results and Conclusion: A profile of 299 proteins with a considerable degree of reproducibility (30-60%) between two biological replicates. Complementary sequence analysis identified 158 (~53%) of these proteins as secretory proteins. Protein identification by MALDI-TOF mass spectrometry generated reproducible 2-D reference maps for proteins from lysates of stimulated and non-stimulated cells. Maps comparing the 2-D profile of tyrosine-phosphorylated proteins in resting and activated cells identified GRP78 and GRP94 as differentially phosphorylated proteins in the latter. Comparative and semi-quantitative analysis of 2-D maps provided evidence for differential expression of 13 proteins, including a 1.5 fold increased expression for microtubule regulatory protein, stathmin 1. The latter is subject to distinct changes in its phosphorylation state at Ser16 and 38 depending on IgE receptor occupancy by ligand only or cross-linking by IgE+Ag.
Keywords: Proteomics, FcεRI, Mast Cells

32. Effect of Zaditen in Maintenance Therapy of Childhood Constipation

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Background: Functional constipation is a common and challenging problem in pediatrics. Without early diagnosis and treatment, an acute episode of constipation can lead to anal fissure and become chronic. At the time the patients are visited, they may be in an impaired cycle. Irritation, itching and discomfort may occur when pieces of soft stool trap in skin folds around the anus and may result in mucosal edema and inflammation. The aim of this study was to evaluate the efficacy of Zaditen (an antihistaminic drug) in pain relief of chronic constipation related to mucosal irritation in children with functional constipation. Materials and Methods: 73 children (male/female 1.08/1) aged 4-12 years, with functional constipation according to RomeIII criteria, who successfully treated for the first step of disimpaction; were divided into two groups randomly. Both groups received classic method of maintenance therapy for functional constipation including high fiber regimen, paraffin oil and soluble fiber(PEG).Group I(n=37) received 1 mg/day Zaditen in addition to above mentioned drugs and group two received nothing else. Both groups treated for a period of 2 weeks. Successful treatment was defined as daily passing painless soft stool in subjects. Onset of improvement, patient compliance and family satisfaction also was evaluated using a scored questionnaire.Frequency of bowel movements, stool consistency, number of fecal incontinence episodes, abdominal pain, painful defecation per week, success of treatment and side effects were determined in each group before and after treatment. Results: In group I, 30 out of 37(81%) and in group II, 22 out of 36(61%) were treated successfully ($p<.05$). Family satisfying and compliance were obviously more achieved in group I. The most common side effect of zaditen was mild and transient lethargy in some subjects. Conclusion: It seems that using Zaditen in maintenance therapy of functional constipation could be an effective treatment in children particularly in who suffer from anal irritation.
Keywords: Zaditen, Maintenance Therapy, Childhood Constipation

33. Comparative Study of Response to One-Month Course of Apoceterizine with Intermittent Treatment of Ceterizine, Loratadin and Chlorpheniramine in Allergic Rhinitis Symptoms

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Background: Antihistamines have been successfully used in treatment of allergic rhinitis. There are few studies about combination therapies with these drugs. Materials and Methods: The aim of this study was comparative assessment between combination therapy with intermittent ceterizin, loratadin and chlorpheniramine versus apoceterizine for the period of one month in patients with allergic rhinitis symptoms. This was a randomized double blind clinical trial. Fifty-four patients received combination therapy or apoceterizine in random prescriptions. Results: Based on Major symptom complex score and Total symptom complex score, there was no significant difference between two groups. Considering adverse effects of treatment, there was also no significant difference between two arms. Conclusion: Efficacy of treatment with low cost combination therapy is comparable with more expensive regimen of apoceterizine. We prescribe combination therapy instead of single agent treatment with apoceterizine.
Keywords: Apoceterizine, Ceterizine, Loratadin, Chlorpheniramine, Allergic Rhinitis

34. Allergic Fungal Sinusitis

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Background: Allergic fungal sinusitis (AFS) is a noninvasive fungal rhinosinusitis, which presents with nasal polyp and respiratory allergic symptoms. Surgical intervention has both diagnostic and therapeutic role beside medical treatment. Materials and Methods: in this study, 48 cases of rhinosinusitis with or without polyp whom did not show acceptable response to a course of 4 weeks of medical treatment, had been operated on. During surgery peanut buttery discharge was detected in one or more sinuses in 4 patients, and as AFS was probable, aggressive surgical intervention included complete opening of sinus ostium of involved sinus, complete extracting of suspicious fungal mucin and thorough sinus irrigation performed. Results: Fungal hyphae were reported in sinus mucin of all 4 patients postoperatively. Postoperative management included intranasal corticosteroids, short-term prednisolone, and regular irrigation of nose with normal saline. Monthly endoscopic evaluation was done up to 6 months postoperatively to remove crusts and allergic mucin. Conclusion: AFS is one of the uncommon causes of rhinitis as is one of the causes of refractory rhinosinusitis. Surgery has a great role in treatment protocol. Whenever rhinosinusitis is accompanied by allergic symptoms, especially typical fungal discharge in endoscopic investigations or presence of dense, heterogeneous opacity in CT images, AFS should be considered. Surgical intervention would be of diagnostic and therapeutic assistance.
Keywords: Allergic, Fungal, Sinusitis

35. Does surgery has a role in treatment of rhinitis?

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Background: Topical medication has a major role in the treatment of allergic and non-allergic rhinitis. Anatomical obstruction can limit mucosal distribution of inhaled intranasal drugs and can increase the need for systemic corticosteroids or antihistamines administration. Meanwhile due to narrowed nasal space, obstructive symptoms would not improve by only medical treatment. Materials and Methods: seventy patients with non-purulent rhinitis evaluated endoscopically and by CT images in our center for anatomical obstructions. Results: The results were: severe septal deviation and unilateral total obstruction in 8%, mild to moderate septal deviation 13%, concha bullosa 25%, unilateral or bilateral inferior turbinate hypertrophy 25% and adenoid hypertrophy in 5%. In 18%, there was more than one anatomical problem. Surgical procedures including septoplasty, turbinoplasty and adenoidectomy could be suggested at least in 18% of patients. Conclusion: Complete ENT examination and endoscopic evaluation of all patients with rhinitis symptoms is highly recommended especially in those who are refractory to medical treatment or those with severe or fixed obstructive symptoms. So surgical intervention is not only effective but also is safe and usually can be done with little morbidity and as an outpatient procedure.
Keywords: Topical medication, surgery, rhinitis

36. Samter's Triad: a Short Experience of Rhinosinusitis Management

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Background: Management of nasal symptoms in patients with Samter's triad (pulmonary asthma, rhinosinusitis & ASA sensitivity) is one of the major challenges in ENT field. The sinuses are involved more severely and the risk of failure of treatment is increased compared to other patients with chronic rhinosinusitis. Surgical interventions are 7 times more common in chronic rhinosinusitis patients with Samter's triad. To control the disease, they need more reoperations. Materials and Methods: In this article we introduce five patients with Samter's triad complaining of sinonasal symptoms and had been operated on endoscopically, in a period of two years. The minimum follow up was six months. Four patients had history of at least one surgical procedure on their sinuses. Four of them were female. Due to their diagnosis of samter's triad, surgical interventions were done aggressively. Postoperative management included short-term systemic corticosteroid, intranasal corticosteroid and montelukast administration. Results: Clinical symptoms such as obstruction and discharge improved significantly. No sign of recurrence was detected in serial nasal endoscopic exams. Olfaction of the patients was not satisfactorily improved in spite of medical and surgical managements. Conclusion: Aggressive sinus surgery, medical treatment and close monitoring of patients are basic steps in control of symptoms in Samter's triad.
Keywords: Rhinosinusitis, Samter's Triad

37. Study of Antibodies to Dermatophagoides Pteronyssinus, D. Farinae in Allergic Patients

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Background: Allergic rhinitis is the most common cause of Allergy It is an extremely common condition, affecting approximately 20% of the population.and An estimated 300 million people worldwide suffer from asthma. There can be multiple factors that contribute to the cause Allergic rhinitis and asthma, one of these is exposure to dust mite. Among house dust mites, Dermatophagoides pteronyssinus and D.farinae play an important role as allergens in relation with asthma, allergic rhinitis and other allergic conditions. Therefore in this study, we studied the sensitivity of asthmatic and rhinitis allergic patients to house dust mites. Materials and Methods: In this prospective study Sera of 101 patients with positive history of allergic diseases were studied. Serum was tested for specific IgE antibody against dust mites using RAST tests (Radio Allergo Immuno Sorbent Test). Results: Among 101 patients, 60 (59.41%) were asthmatic, 15 (14.85%) had allergic rhinitis and 26 (25.74 %) had the history of both disease. Eleven of these patients (11/101, 10.89%) had positive results for IgE antibody against mites. The sensitivity rate of patients to D. pteronyssinus and D.farinae were 7.92% and 2.97% respectively. Among the control only 7 patients (7%) had positive results for antimite IgE antibody. The sensitivity rate of the control group toward the aforementioned dust mites allergens were 5% and 2% respectively, and there was no significant difference between the results of cases and controls. Conclusion: We didn't find a higher sensitivity rates to dust mites in allergic patients compared with normal individuals. We believe that low humidity was the plausible cause of low sensitivity rate to house dust

mite in our allergic patients.
Keywords: Dermatophagoides pteronyssinus, D. farinae, Asthma, Rhinitis allergic, House dust mites.

38. Analysis of Il-17 A and Il17f Genetic Polymorphisms as Risk Factors for Allergic Rhinitis

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Background:The development of allergic rhinitis entails a complex interaction between genetic predisposition and environmental exposure to different factors that allergens are the most important. Responding molecules are; chemokine's and their receptors, interleukins and their receptors, eosinophil peroxidase and leukotriene's, among others. The interleukin-17 cytokines (IL17A and IL17F) are emerging as critical players in host defense responses and inflammatory diseases. This study investigated the association between single-nucleotide polymorphisms (SNP) in IL17A gene promoter (rs2275913, IL17 G152A) and IL17F exon 3(rs763780 IL17F 161His-Arg) and Rhinitis-related traits among the patients in Chaharmahal va Bakhtiari province. Materials and Methods: DNA was extracted using standard phenol-chloroform method. The screening of mentioned polymorphisms was performed using PCR-RFLP procedure. A case- control association study was performed (rhinitis group; n=300 and control group; n=160). Chi- square test was performed to compare proportions of subjects with different clinical features among subjects with different genotypes.(All statistical analyses were performed using SPSS). Result: There was significant association between rs2275913; IL17A and allergic rhinitis (p=0.025) but no association between rs763780; IL17F and cited disease in Chaharmahal va Bakhtiari province was found (p=0.468). Conclusions: Our data indicated that the IL17A may play an important role in the inflammatory response and promoting allergic rhinitis and rs 763780; 1L17F have no role in rhinitis in Chaharmahal va Bakhtiari province.
Keywords: Allergic Rhinitis; polymorphism; Interleukin-17A and IL17F

39. The Study and Counting the Fungi in Air Of Tehran: the Role of Seasonal Changes

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Background: Fungal spores are extended in our environment that this extension is related to geographical coordinates, humidity, temperature and the type of fungi. Although all types of fungi are not pathogenic but they can have an effective role in allergic and pulmonary illness, meanwhile some kinds of fungi can produce mycotoxin. In this research central area of the Tehran that has an area around 45/2138 hectare andcovers about 3/3 percent of this city was selected as a sample and studied to count different kinds of fungi spores in four seasons. Materials and Methods: By using pollen and spore sampler, fungi spores were counted daily and study the kinds of fungi, plates consisting media culture subrodextros agar with chloramphenichol was used. For identifying the difference between some kinds of fungi, czhapexs and BHI media was used. These plates placed daily in 1 meter and 50 centimeters upper than ground level for one hour and 30 minutes from 8-10 am and 4-6 pm.The plates was incubated between 3-10 days then, each colony were counted respectively and studying by optical microscopy. Daily CFU was determined for fungi and then slide culture was done until better distinguishing the kind of fungi and they compared based on different seasons T-test was used for identification of higher and lower values of fungi in seasons. Results: 51/302 fungi colonies from 108 plate was counted. In this study these kinds of fungi were seen: Aspergillus, Alternaria, penicillium, cladosporium, yeasts, Trichotechium, Pesudoallescheria, strilehyphae, Auerobasidium, Fusarium, candida, Acromonium, Ulocladium, Mucor and Rhizopus that %86.7 were related to Aspergillus (higher percent) and yeast 1% andFusarium 2.2% have lower percents. Meanwhile the value of daily count of fungi spores was higher in April, September and January more than other months. Conclusion: Seasonal changes and climate situations are very important factors in reducing or increasing suspended spores in air and detailed study in this field can get a spore calendar to patients who have Immunedeficiency disease and other patients who are sensitive to allergic diseases.
Keywords: Fungal spores, pulmonary illness, mycotoxin

40. Allergenicity of *Chrysanthemum Maximum Ramond* Pollens Compared to the Pollens without Flavonoid Pigments and Purified Total Flavonoid Pollen Grains

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Background: pollen grains were known as one of the main factors of allergy.Compositae pollen grains are one of the important allerginicity pollens because it contains thorny exiens and different allergens. In the present research we study the role of flavonoids pigments of the pollen grains in allergeinicity. Materials and Methods: morphology of natural pollen grains and pollens which their flavonoid had been extracted using methanol %80, were observed with optical and SEM microscopy.Sianidin test, TLC and NMR tests were applied for assuring of flavonoid lack in the pollens. The proteins extraction of pollens had been performed by PBS and cross section of two samples with SDS-PAGE. Study and purification of flavonoid with help of chromatography, IR, HandCNMR, Mass, TCL and HPLC in the presence of two standards in ruin and Apigenen.Ability of allerginicity natural pollen proteins, pollen without flavonoid and also extracted flavonoid from pollens had been compared with each other with parchment and respiratory tests and serological such as IgE measurement on the guinea pig and western immune blotting. Results: The results of tests showed that in each gram of dried pollen grains, there are 0/3gr flavonoid compound. Natural pollen morphology and without flavonoid the same from and SDS-PAGE lines in 7 KDa to 70 KDa had no any differences.Having extracted flavonoid from pollens would have any allergen factor on guineapigs.Natural allerginicity of pollen in all parchment and respiratory tests were more than pollens which were without flavonoid.The results of immunoblotting in each two tests were similar and were related to the major 66KD protein in SDS-PAGE. Conclusion: according to the results we can conclude that flavonoid as a part of anti-allergy
components can reduce the pollen allerginicity effect.

41. Generation and Characterization of Monoclonal Antibody against Lipid TransferProtein in Grape

Hosseinpour Tahereh

Background: Lipid transfer protein (LTP) is a member of the plant non_specific protein family has been identified as a relevant allergens in foods. This allergen family is particularly important in the Mediterranean area, but shows a very limited incidence in central and northern Europe. Materials and Methods: Natural LTP was purified by ion exchange and identified by mass spectrometry .BALB/c mice were immunized with gel contained 9 KD protein that is related to LTP. The splenocytes of the immunized mouse were fused with Sp2/0 Ag-14 myeloma cells. Hybridomaprocedures were done according to standard protocol. Result: ELISA was done and positive clones were selected. We obtained two proper clones. Other experiments are under investigation. Conclusion: : Produced monoclonal antibody will use to set up sandwich ELISA for the standardization of lipid transfer protein extracts intended for clinical use. This monoclonal antibody is also useful for immunoaffinity purification and immunoassays.
Keywords: Monoclonal Antibody, Lipid TransferProtein, Grape

42. Overweight Association with Increased Risk of Allergy in Iranian Adolescents

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Background: A positive association between obesity and allergic diseases has recently been suggested; however results of studies on obesity and allergic diseases are quite conflicting, and most of them are related to asthma and asthma-like symptoms. The aim of this study was to determine the association of obesity indices and the prevalence of some allergic diseases. Materials and Methods: Data from a cross sectional study on 1085 high school students in Tehran (2007-2008), using a cluster sampling, were analyzed. The association between body mass index (BMI) for age and physician-diagnosed asthma, allergic rhinitis or conjunctivitis, atopic dermatitis, food allergy and self-reported wheezing was investigated in a questionnaire study. Weights and heights were measured to calculate their BMI by trained surveyors using valid methods. The BMI number was plotted on the CDC BMI-for-age growth charts for girls and boys to obtain a percentile ranking. Over weight was defined as BMI>85th percentile for age. Logistic regression was performed to examine the relationship between percentiles of BMI for age and allergy. Results: Mean±SD age was 15.25±2.69 among girls (50.7%) and 16.11±2.32 among boys. Compared with adolescents at the lowest percentile group, Iranian adolescents at>85th percentile group showed a higher risk of allergy (OR=2.37, 95% CI 1.12-4.29; p=0.040). Iranian atopic dermatitis at>85th percentile group showed a significantly higher risk of allergy (OR=3.24, 95% CI 1.19-4.27; p=0.025). This association was not observed when children with food allergy were excluded from the analysis. Conclusion: Our results showed that being overweight was associated with an increased risk of allergy in our study population. Future clinical studies are warranted to confirm these findings.
Keywords: Allergy, obesity, allergic diseases, Adolescents

43. Quality of Life in Iranian Adults with Allergic Rhinitis

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Background: Allergic Rhinitis (AR) is a common global health problem with approximately one quarter of the world population affected. The Quality of Life (QOL) of sufferers with AR is significantly affected. While AR affect physical, psychological and social dimentions of patients life, the disease underestimated by them. The aim of the study is to evaluate the QOL of adults with allergic rhinitis. Materials and Methods: The study was conducted using a valid Rhinitis Quality of Life Questionnaires (RQLQ) which completed for each patient during clinic visit. The study was designed for adults with AR above 18 years old referred to Children's Medical Center in Tehran during 2010. The data gathered from the completed RQLQ was analyzed applying statistical methods. Results: The RQLQ filled for 110 AR patients. The age of patients in the study was between 18-55 years old (81% under 35 years old). About 62% of patients were female and 38% were male. Frequencies of mild persistent, moderate-severe persistent, mild intermittent and moderate-severe intermittent types of AR were 18%, 34.5%, 9% and 38% respectively. Completed RQLQ indicated that about 74% of the cases were suffering from moderate-severe disturbances in their quality of life (QOL). Furthermore, congestion (88%) and rhinorrhea (85%) were the most common symptoms. It is worth to mention that the correlation between congestion and QOL reduction was significant. The most common co-morbidity was as follows: sinusitis (57%), hyposmia (34%) and asthma (27%). The QOL of the patients with hyposmia were severely impaired. There was a good relevancy between sleep problems and emotional scores Conclusion: The findings indicate that allergic rhinitis has significant effect on the quality of life of the affected patients. The results showed a good relevancy between severity of symptoms and QOL scores. Moderate-severe reduction in the quality of life were found in patients with moderate-severe intermittent and persistent. Nasal congestion is associated with sleep-disordered breathing, nocturnal sleep impairment, day time fatigue and somnolence which finally lead to emotional and psychological impacts. Considering high prevalence of AR and its high impact on QOL, it is important not to underestimate the disease and its management. Therefore, early diagnosis and treatment of AR (especially nasal congestion) are necessary to be given high priority.
Key words: Quality of life; Allergic Rhinitis; Iranian Adults

44. Single Nucleotide Polymorphisms of TNFα, TGF-α, IL-4, IL-10 and IL-12 in Iranian Patients with Allergic Rhinitis

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Background: Allergic rhinitis (AR) is one of the most common allergic diseases caused by inflammation of upper respiratory airways. Cytokines could contribute to this inflammatory process. This study was performed in order to analyze the genetic profile of cytokines in Iranian patients. Materials and Methods: The allele and genotype frequencies of a number polymorphic genes coding for tumor necrosis factor(TNF)-α, tissue growth factor(TGF)-β, interleukin(IL)-4 ,IL-10, IL-12 were investigated in 76 patient with AR in comparison with 140 controls using polymerase chain reaction with sequence-specific primers. Results: The most frequent genotypes in patients were TNF-α AA at position -308(P=0.001), TGF-β TT at position 10 codone (P=0.001) and IL-4 TT at position -33(P<0.05). In contrast, the frequencies of genotypes TNF-α GA at position -238(P<0.05), IL-4 TC at position(P<0.05), IL-4 TC at position -33(P<0.05), IL-4 TC at position -590(P<0.05), IL-10 AA at position -1082(P=0.001) in the patient group were significantly lower than controls.The most frequent haplotypes in patients for TGF-β (positions -238,-308) was T/C (P<0.05) and for Il-4(positions -33,-590,-1098) was TCT and GTC(P<0.05) in comparison with controls. Conclusion: While environmental factors are important in the development of AR, genetic factors could have a critical role in the expression of the disease. As the results of our study, the genetic polymorphisms of TNF-α AA, TGF-β TT and IL-4 TT could be contribute to development of AR in Iranian patients.
Keywords: TNFα, TGF-α, IL-4, IL-10, IL-12, Allergic Rhinitis

45. Dental Materials and Allergy

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Background: Of all medical emergencies that can occur in dental offices, allergy-related emergencies are actually common. Additionally, "anaphylaxis" is the 5th most common medical emergency seen in dental offices. There is a strong link between allergy and toxicity and both affect our immune system in a negative way. Materials and Methods: A search was performed using keywords "lichen planus" and "immunology" to identify the current literature.The related articles were searched in databases including IDL, Pubmed, and Medline from 2007 to 2011. Results: The most common allergen in the dental environment today is latex. Penicillin is the most common cause of drug-induced anaphylaxis. The dental literatures are essentially devoid of research assessing implant failure as a result of allergy to titanium or the other newer materials used in fabricating these prosthetic devices and also bonding agents, disinfectants, rubber, metals and detergents. If the allergy is severe, the patient has lost, or soon will lose consciousness. The dentist should place the patient in a supine position, open the airway, and evaluate breathing. If the patient is not breathing, the dental professional must administer positive pressure oxygen via a bag–valve–mask device. Immediate discontinuation of the offending drug(s) and early administration of epinephrine are the cornerstones of treatment. Epinephrine is the drug of choice in the treatment of anaphylaxis. Next is the use of general anesthesia if the allergy was to a local anesthetic. Conclusion: To be considered safe, dental treatment needs some precautions, which sometimes are neglected by dentists. Obtaining a credible medical history is the first step for the allergic patient's diagnosis and there should be patch testing to rule out allergy.
Key words: anaphylaxis, latex, disinfectants, epinephrine

46. The Prevalence of Allergic Diseases in 1st Degree Relatives of Neonates that Hospitalized with Diagnosis of TTN in Fatemieh Hospital of Hamedan

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Background: Transient tachypnea of neonate (TTN) is one of the causes of respiratory distress in neonates. This disease has seen in preterm neonates, cesarian section ,male gender and neonates whom their mothers have an allergic disease .The goal of this study is to comparison frequency of allergic diseases in first degree relatives of neonates with TTN with healthy neonates. Materials and Methods: This case_control study was planed in two groups. The case group was the neonates who admitted with impression of TTN by neonatologist in NICU ward of Fatemieh Hospital in hamedan and healthy neonates selected as control group .Two groups of neonates matched according to weight ,form of delivery ,gestational age ,gender ,smoking in mother and maternal diabetes. The questionnaire was completed according to positive history of allergic diseases including asthma, atopic dermatitis and urticaria and allergic rhinitis in 1st degree relatives in two groups. Results: Out of 35 neonates with TTN, 3 neonates (2.3%) have positive family history of allergic diseases in 1st degree relatives.There was no significant statistically difference between two groups (P value: 0.399). Two neonates had positive family history of atopic dermatitis in1st degree relatives. There was no significant statistically difference between two groups (P value: 0.151). One neonate had positive family history of asthma in 1st degree relatives there was no significant difference between both groups (P value: 0.384). Conclusion: On the basis of this study we can suggest that positive family history of allergic diseases do not increase the risk of TTN in neonate. Repeated study in the greater groups of patients is recommended.
Keywords: Prevalence, Allergic Diseases, Transient tachypnea of neonates allergy

47. Common Allergen Agents in Iranian Patients

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Background: Allergy and allergic diseases represent a major health problem not only in industrialized societies but worldwide. Although there has been an epidemic increase in prevalence of allergic diseases in the last few decades with 10–30% of the population affected but in some countries, detection of common allergic agents in affected individuals is insufficient and/or inadequate. The aim of this study was to determine the most common allergens in Iranian subjects. Materials and Methods: One hundred subjects with skin, respiratory or gastrointestinal symptoms, thought to be due to allergy, were enrolled in this study. The total serum IgE levels were measured in all patients by elecsys 2010 and allergy to 20 critical allergens was monitoring with *EUROIMMUN*ELISA kit. Results: From the analyzed data it has been observed that 34%, 26% and 20%, of subjects had allergic to dermatophyte, pepper and to dost respecfully. Conclusion: The collected data showed that dermatophyte (D1 and D2) are must common allergic agents and also the serum IgE levels have been higher among all subjects. However, in consideration of the discrepancy of the data published for research into allergic condition, it is essential to diffuse the peculiar data of the local territory, reaching the largest possible number of interested subjects.
Keywords: Allergy, Allergen agents, Iran

ALLERGY IMMUNOTHERAPY

Oral Presentation

48. Immunological Mechanisms of Allergen-Specific Immunotherapy

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The studies on the mechanisms of specific immunotherapy (SIT) point out its targets that decide on the efficacy of SIT and hence might be used for its further improvement. Several mechanisms have been proposed to explain the beneficial effects of immunotherapy. The knowledge of the mechanisms underlying allergic diseases and curative treatment possibilities has experienced exciting advances over the last three decades. Studies in several clinical trials in allergen-SIT have demonstrated that the induction of a tolerant state against allergens in many ways represents a key step in the development of a healthy immune response against allergens. Several cellular and molecular mechanisms have been demonstrated: allergen-specific suppressive capacities of both inducible subsets of $CD4^+$ $CD25^+$ forkhead box $P3^+$ T-regulatory and IL-10-secreting type 1 T-regulatory cells increase in peripheral blood; suppression of eosinophils, mast cells, and basophils; Ab isotype change from IgE to IgG4. This review aims at the better understanding of the observed immunological changes associated with allergen SIT.
Keywords: allergy, immunetolerance, immunotherapy, T-cell subsets

49. Comparison of Immunotherapeutic Effects of a Recombinant Hybrid Molecule with an Allergenic Cocktail or *Chenopodium Album* Pollen Extract in a BALB/c Mouse Model of Type I Allergy

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Background: The objective of the current study was to assess the therapeutic potential of a recombinant hybrid molecule (rHM) alongside an allergenic cocktail from recombinant wild-type allergens as well as pollen extract on *Chenopodium album* allergy, using the BALB/c mouse model. Materials and Methods: The BALB\c strain mice were sensitized to *C. album* by intraperitoneal (i.p.) injections of alum-adsorbed allergenic cocktail. The sensitization state was established by applying aerosol challenge with 1% of allergenic cocktail. Immunotherapy procedure was followed by two subcutaneous injections of either rHM or allergenic cocktail or pollen extract with weekly interval. Humoral immune responses were monitored via measurement of specific IgE, IgG1 and IgG2a antibodies in serum. The splenocytes of immunized mice were stimulated with the hybrid molecule and cell mediated immune responses were evaluated with IL-4 and IFN-γ secretion in cell culture supernatants. In addition, mRNA expression of genes involved in immunotherapy was examined by real-time PCR. Results: The sensitized mice were labeled with high specific IgE against allergenic cocktail compared with healthy mice that received alum adjuvant in PBS. Immunotherapy with the rHM induced the highest ratio of the IgG2a/IgG1 levels compared to allergenic cocktail or *C. album* pollen extract. The rHM was able to induce high proliferative response in splenocytes. Immunotherapy whit the rHM significantly improved IFN-γ and IL-10 production, while IL-13 decreased. Interestingly, mRNA expression of GATA3 strongly decreased in rHM treated mice whereas, T-bet and Foxp3 significantly increased. Conclusions: Our results demonstrated that immunotherapy with the rHM effectively controlled allergic responses through shifting from Th2-like immune responses to a Th1-dominated immune response.
Keywords: rHM, *Chenopodium Album*, Type I Allergy

50. Effect of Immunotherapy on the Regulatory T Cell and Th17 Gene Evaluation of Expression and Clinical Manifestation in the Patients With Allergic Rhinitis

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Background: Allergic rhinitis is the most common that affecting 20% of the general population. Allergic rhinitis is defined as a disease with sneezing, rhino rhea, nasal itching and congestion after exposure to a specific allergen. Subcutaneous immunotherapy has been suggested that inhibitory response of T-reg cells via IL-10 and TGF-β is the main mechanism of mucosal immune response to allergens. The aim of this study was to evaluate changes in lymphocytic phenotypes in patients with allergic rhinitis before and after immunotherapy. Materials and Methods: Twenty three patients with allergic rhinitis ranged 10- 45 years old were visited in Allergy Clinic, Ghaem Hospital and Dr Farid Clinic. Their allergic rhinitis was confirmed via clinical criteria and sensitivity confirmation by skin prick test to common lesional aeroallergens. The main gene related to the presence of T-reg cells have been evaluated by PCR. The used primers were generally FOXP-3, TGF-β, IL-10, and GITR and Th17 lymphocytes, IL 17 primer m TH1 and TH2 and INF and IL4 primers were evaluated. Results: There was a significant difference between TGF-β gene before and after the immunotherapy (P=0.001). Also a significant difference about IL-10 was showed before and after the immunotherapy (P=0.05). No statistical significant difference was obsereved between FOXP-3, GITR, IL-4 and INF-γ (p>0.05). Conclusion: Induction of tolerance in the peripheral T lymphocytes is the main step in the subcutaneous immunotherapy. This induction is performed by the Treg cell and its promotion is done by FOXP3 and TGFβ.

Keywords: Allergic rhinitis, Treg cells, FoxP3, Immunotherapy

51. Study of Sublingual Immunotherapy in Allergic Rhinitis

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Background: No DBPCT of sublingual immunotherapy (SLIT) in the Iran have been reported. This study compared the clinical and immunological effects of high Dose Dermatophagoides farinae vaccine versus placeboes. Materials and Methods: Forty poly-sensitized patients with allergic rhinitis, all of them sensitive to mite, aged from 6 to 33 year were enrolled into the study. Twenty one patients in the active group and 19 received placebo via sublingual administration for 6 months. mRNA expression levels of IL-10, TGF-β , FoxP3 and IL-17 were measured by using real-time PCR before and after of SLIT. Clinical efficacy estimated by the reduction rate of symptom/medication scores in active group compared with placebo treatment. Results: Of the 40 randomized subjects, 6 withdrew because of non–treatment-ascribed events and of 34 subjects who completed the course of study 19 were in active group and 15 in placebo group.6 patients refused blood sampling so we had 14 subjects in each group for evaluation of immunological response. Clinical efficacy was supported by significant reduction of symptom/medication scores in active group compared with placebo group. No severe systemic reactions were noted. After 6 months' active SLIT, TGF-β mRNA expression levels were increased compared with pretreatment (P <0.01) and FoxP3 mRNA expression levels were increased compared with placebo group (P<0.05). Conclusion: SLIT with Dermatophagoides farina extracts is an effective treatment in poly-sensitized patients with allergic rhinitis.TGF-β-mediated T-cell suppression may be an important mechanism in the first 6 months of SLIT.

Keywords: SLIT, Allergic Rhinitis, IL-10, TGF-β, FoxP3, IL-17

Poster Presentation

52. Evaluation of G.I.T (General Adjuvant (G2) Immunotherapy) Therapy in, IgE Associated Allergen disease

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Background: In Allergic diseases such as seasonal Allergic, Rhinitis, Conjunctivitis, Allergic Asthma, Eczema, Urticaria, Drug & food Allergy and so on after receiving of allergen or hapten by mast cell receptors (due to IgE-bridge receptor) producing chemical mediators & ultimately,: Histamine & leukotrins & other mediators, total IgE of blood enhance by using of GIT that is the activator & ultimately class switching of IgE producing by plasma cell to other Immunoglobulin. Total IgE producing down–hilled lowered significantly.Materials and Methods: From 2009 to 2011 (2 years) 2000 patients referred to Allergy Clinic, 45 of them had clinical signs & symptoms of related disease with increased Total IgE ,(atmost: 2000 iu/ml No. 2pt's & at least 88 iu/ml No. 1pt.) in 10 groups disease (e.g skin pruritus disease (Eczema, HWH syndrome , any kinds of urticarias PAR,SAR, SAC, heavy metal dermatitis Drug & food eruption chronic Allergic sinusitis , Atopy & Angioedema, ENT. Results: Allergies. 27 of pt's are female & 18 of them are male, (age group 9y/o -to 74y/o). By git immunotherapy between 1 month (4wks) to 4 months. Clinical signs & symptoms decreased & total IgE & eosinofillic count lowered significantly & meaningful.(by inj. Of 0/1 cc s.c every week) PAR=Perrenial, SAR=Seasonal. Allergic rhinitis, SAC= Seasonal Allergic Conjunctivitis

ANGIOGENESIS & TUMOR

Oral Presentation

53. Generation and Characterization of Functional Nanobody against the Most Important Angiogenesis Cell Receptor (VEGFR-2)

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Background: Vascular Endothelial Growth Factor Receptor-2 (VEGFR2) is an important tumor-associated receptor and blockade of the VEGF receptor signaling can lead to the inhibition of neovascularization and tumor metastasis. Nanobodies are the smallest intact antigen binding fragments derived from heavy chain-only antibodies occurring in camelids. Material and Methods: Here, we describe the identification of a VEGFR2-specific Nanobody, named 3VGR19, from dromedaries immunized with a cell line expressing high levels of VEGFR2. Results: We demonstrate by FACS, that 3VGR19 Nanobody specifically binds VEGFR2 on the surface of 293KDR and HUVECs cells. Furthermore, the 3VGR19 Nanobody potently inhibits formation of capillary-like structures. Conclusion: These data show the potential of Nanobodies for the blockade of VEGFR2 signaling and provide a basis for the development of novel cancer therapeutics.

Keywords: VEGFR2, Nanobody, Angiogenesis

54. Role of angiogenesis in tumor growth and metastasis

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Angiogenesis, endothelial cell division/migration and growth from pre-existing vessels to form new capillaries, is essential in many physiological processes such as ovulation, menstruation and wound healing. Angiogenesis is pathogenic in many chronic inflammatory diseases such as diabetic retinopathy, rheumatoid arthritis, atherosclerosis and neurodegeneration. Similarly, the growth of many solid tumors is not only dependent on angiogenesis, but any uncontrolled (excessive) angiogenesis provides cancer cells with a gateway through which they can enter the circulation and metastasize to distant sites. Unregulated angiogenesis depends on decreased expression of endogenous inhibitors, and/or increased levels of angiogenic inducers including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). Angiogenic switch is a

highly complex process, which is not yet fully understood, but hypoxia (oxygen deficiency) in the tumor is an important factor, stimulating production of angiogenic inducers by the tumor cells. Hypoxia-inducible factor-1 (HIF-1), a transcription factor activated in response to intratumoral hypoxia, plays a critical role in adaptation of tumor cells to oxygen deficiency by activating transcription of target genes that regulate several biological processes including angiogenesis, cell proliferation and survival. Highly malignant tumors are able to induce strongly angiogenesis almost indefinitely and, if not successfully treated, will prove fatal.

55. Angiogenesis and Apoptosis

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Angiogenesis, the growth of new blood vessels from the existing vasculature,is necessary for normal growth and development and in the adult during wound healing and the reproductive cycles. In most adult tissues, however, the vasculature is maintained in a quiescent state by the balanced presence of both angiogenic inducers and inhibitors in the tissue milieu. For progressive growth and metastasis, cancer cells must shift this balance to favor angiogenic induction. When inducers predominate, vascular endothelial cells (VECs) become activated, proliferating and migrating toward the source of the angiogenic inducer. In the activated VECs, distinct cell signaling pathways are initiated compared with that of quiescent VECs, providing the specificity of anti-angiogenic therapies to the tumor vasculature.VEC apoptosis has been well documented in regressing vessels, and it has been shown that, in addition to activating the VECs, some inducers such as vascular endothelial growth factor also up-regulate Fas expression, thus sensitizing the cell to apoptotic stimuli. Endogenous angiogenesis inhibitors, such as thrombospondin-1 (TSP-1) and pigment epithelium-derived factor (PEDF), stimulate signaling cascades within the VECs and also induce the expression of Fas ligand in activated VECs.During cytotoxic chemotherapy, apoptosis of endothelial cells in the vascular bed of tumors precedes apoptosis of tumor cells, even when the tumor has been made drug resistant. Administration of an angiogenesis inhibitor which is not directly cytotoxic to tumor cells can increase tumor cell apoptosis and inhibit tumor growth by inhibiting endothelial proliferation and migration and/or by inducing endothelial apoptosis. Furthermore, oncogene expression and loss of tumor suppressor gene activity can at once protect tumor cells against apoptosis and increase their angiogenic output. Both of these survival advantages conferred on the tumor can be overcome by antiangiogenic therapy. They can also be overcome by cytotoxic chemotherapy administered on a low dose antiangiogenic schedule which continuously exposes endothelial cells in the tumor bed to the drug. As a result, endothelial apoptosis can be demonstrated to precede tumor cell apoptosis, and tumors regress or are inhibited, whether or not the tumor cells are resistant to the drug, and with little or no host toxicity. In contrast, cytotoxic chemotherapy administered on a conventional schedule of maximal tolerated dose followed by an off-therapy interval, becomes ineffective after drug resistance is acquired.On the basis of these experimental findings, chemotherapy of cancer may possibly be improved—i.e. decreased drug resistance and decreased toxic side-effects—by changing dose and schedule to maximize apoptosis of endothelial cells in the vascular bed of tumors.Therefore, when inhibitors predominate, the apoptotic cascade is initiated. Depleting the supply of angiogenic inducers can also induce apoptosis, and thus, anti-angiogenic therapies can target the inducer supply or directly target the VECs. Further improvement may be achieved by combining angiogenesis inhibitors with 'antiangiogenic chemotherapy.

Key Words: Angiogenesis; apoptosis; endothelial cell; antiangiogenic.

56. Anti-angiogenic/proliferative behavior of a ''4-aryl-4H-chromene'' on blood vessel's endothelial cells: A possible evidence of anti-tumor activity

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Development of therapies aimed at inhibiting the angiogenesis, in combination with classical anti-cancer therapies, is among the most intensively studied approaches to the treatment of cancer. As a continuation of the efforts to discover and develop ''4-aryl-4H-chromenes'' as novel anticancer agents, in this study, we have been synthesizing (and examining) these compounds that appear to inhibit metastasis and, in particular, angiogenesis by using successful approaches such as three-dimensional capillary tube formation as well as matrix metalloproteinase (MMP) gelatinase assay in the endothelial cell-based experimental system. Anti-angiogenic and anti-proliferative effects of chromene compound 1 were especially checked on three-dimensional culture of human umbilical vein endothelial cells (HUVECs) in collagen matrix and HUVECs proliferation assay, respectively. Compound 1 was identified to be a highly potent anti-angiogenic agent at well-tolerated concentrations. In mechanistic studies, baseline MMP activities were inhibited in the presence of compound 1, in a dose-dependent manner. Based on our data, there is this possibility that chromene compounds (especially 1) are useful in treatmentof some cancers because of their ability to both induce cancer cell apoptosis and reduce the stimulatory factors involved in HUVEC cell proliferation and angiogenesis.

Keywords: Anti-angiogenesis, Anti-proliferative, HUVEC, Chromene compounds, MMPs

57. Anti-angiogenic Properties of Soybean Bowman-Birk Protease Inhibitor Purified by Trypsin Affinity Chromatography

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Soybean Bowman-Birk protease inhibitor (BBI) is a small polypeptide with anti-inflammatory and anti-cancer properties. This study aims to prepare pure BBI using a new and efficient method and assess the anti-angiogenic potential of BBI. The polypeptide was purified from soybeans using a two-step method including ethanol extraction and trypsin affinity chromatography. The anti-angiogenic property of BBI was evaluated on capillary tube formation by human umbilical vein endothelial cells (HUVECs). Cytotoxic potential of BBI was assessed by lactate dehydrogenase (LDH) and trypan blue assay. The purified BBI was an 8 KDa homogenous polypeptide that yielded perfect anti-angiogenic effectat doses higher than 5 µg/ml. Overall; the present results suggest anti-angiogenic potential of BBI in a dose-dependent manner and provide further support for beneficial effect of BBI on cancer and degenerative diseases.

Key words: Purification; Soybean; Trypsin; Affinity chromatography; Bowman-Birk inhibitor; Anti-angiogenic

58. Anti-angiogenic strategies

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Angiogenesis has become an attractive target for therapy because of its essential role for the growth and spread of cancer. Tumors cannot grow beyond a certain size or spread without a blood supply. When the size of tumors reaches more than 2 mm, angiogenesis inevitably develops. In this situation, the new capillary network is rapidly formed, nourishing the tumor and supporting growth.

One of the advantages of anti-angiogenic therapy is believed to be the lack of induction of resistance to therapy. There are two classes of angiogenesis inhibitors, direct and indirect. Direct angiogenesis inhibitors target the microvascular endothelial cells that are recruited to the tumor and prevent them from responding to various mitogens and stimulatory factors. Indirect angiogenesis inhibitors generally prevent the expression of or block the expression/function of a tumor protein that activates angiogenesis, such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), or block the expression of its receptors on endothelial cells.

59. Hydroalcholic extract of oak (*Quercus infectoria*) inhibits *in vitro* angiogenesis

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Angiogenesis, the formation of new blood vessels, is a critical process in several chronic diseases and is essential for tumor growth and progression. Although more attention in angiogenesis is on cancer, many other non-neoplastic diseases such as psoriasis, arthritis, macular degeneration and obesity are also angiogenic-dependent. Oak (*Quercus infectoria*) is a plant which extensively used as drug and food supplement in Western parts of Iran. Although some biological properties of this plant are determined, its effects on angiogenesis are unclear. The aim of this study was to investigate the anti-angiogenic effects of oak on HUVECs model. After determination of cytotoxic concentrations, anti-proliferation and anti-angiogenic properties of hydroalcholic extract of oak and its fractions were evaluated. In addition, the effect of the extract on VEGF secretion and Matrix Metalloproteinases (MMP-2, MMP-9) expression were assayed using ELISA and gelatin zymography. Treatment with hydroalcholic extract of oak decreased endothelial cells proliferation and angiogenesis at a dose dependent manner. Hydroalcholic extract of oak showed a dose-dependent inhibitory effect on VEGF secretion and MMP-2/MMP-9 expression. Altogether, the current study indicated that oak (*Quercus infectoria)* is a potent anti-angiogenic herb which exerts its inhibitory effect mainly through down-regulation of essential mediators such as VEGF and MMPs.
Keywords: *Quercus infectoria*, angiogenesis, endothelial cell, matrix metalloproteinase.

Poster Presentation

60. Application of Monoclonal Antibodies in Angiogenesis and Fibrinolysis System Manipulation

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Background*:* Plasminogen has a central role in fibrinolyrtic system can activate through various activators (PAs) to its active form plasmin and perfoem its vital function that is fibrin clot lysis. Furthermore the fibrinolyrtic system plays a major role in angiogenesis. The fibrinolyrtic system activation control cell migration and invasion. In addition to this, plasmin regulates tumor growth. Monoclonal antibodies, as biological tools, play an important role in basic researches. Materials and Methods*:* In the first step the effects of antibodies on the activation of fibrinolyrtic system with PAs were evaluated with several methods including macroscopic observation, quantitative measurement of DD/E fragments by D-dimer assay and activation of plasminogen by S-2251 synthetic substrate (ELISA method), subsequently we studied the effect of antibodies on angiogenesis process in an in- vitro model.
Results*:* Results showed that MC2B8 that is an inhibitor of plasminogen activation in presence of plasminogen activators can inhibit angiogenesis process: A1D12 that is against N-terminal domain of Glu-plasminogen, in addition to activation of fibrinolyrtic system in presence of plasminogen activators, can activate in vitro angiogenesis process.Conclusion:Plasmin formation is a critical step in invasion and migration of endothelial cells to form new vessels. Plasmin directly participates in angiogenesis by direct fibrin and other matrix components degradation, and indirectly by activating matrix degrading metalloproteinase and angiogenic growth factors. According to the in- vitro results, MC2B8 and A1D12 monoclonal antibodies play roles in this process in a dose dependent manner.
Keywords: Fibrinolysis system, angiogenesis, plasminogen, antiplasminogen monoclonal antibody

61. Angiogenesis in Oral Cancer

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Angiogenesis is a multistep biological process which prompt new blood vessels development and tumor metastasis along with kipping new blood vessels. Internal and external positive and negative factors control this process. New blood vessels aid tumor growth with oxysion and food supply. Angiogenesis is necessary for tumor growth and metastasis. Many proteins have recognized as activator and inhibitor factors of angiogenesis. Inhibitors using is effective in reducing cancer morbidity and mortality. Angiogenesis is effective in diagnosis and treatments of many disorders such as oral cancer. In this study we discus with more detail about angiogenesis and its effect on disease diagnosis and treatment especially oral cancer and molecular changes in this process.

ASTHMA

Oral Presentation

62. Simvastatin: A Chemical Thermoplasty Agent for Airway Remodeling in Asthma?

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Background: Statin, inhibitor of 3-hydroxy-3-methylglutaryl–coenzymeA (HMG-CoA), has been linked to improved lung health, and ongoing controlled clinical trials will investigate their potensw32tial therapeutic application in asthma and COPD. Materials and Methods: To investigate role of simvastatin in murine inflammation model, BALB/c mice were sensitized and challenged with ovalbumin (OVA) and the effect of simvastatin on lung function was investigated. Simvastatin *or* simvastatin and mevalonate (MA) were injected IP before each OVA exposure. Results: OVA-exposed mice treated with simvastatin (40 mg/kg) showed a significant reduction in airways resistance ($P = 0.018$), achieving values similar to air controls (using lung physiology measurements). OVA-exposed mice treated with simvastatin and MA did not reverse the simvastatin effect on resistance. Western blotting on whole lung lysates from challenged and control groups mice showed that simvastatin increased apoptosis (caspase-3 and -7 activation) and autophagy (Atg12-Atg5 formation, increase in Beclin-1 expression and LC3-β cleavage) compared to controls. Combined treatment with mevalonate and simvastatin reversed the apoptotic and autophagic effect of simvastatin in mouse lung tissue. Conclusion: Therefore simvastatin induces both apoptosis and autophagy in whole lung tissue, with improved lung function in OVA-challenged animals. These results could be one of the first applications of apoptosis and autophagy in asthma therapy, a treatment that could complement or replace thermoplasty in future.
Keywords: Simvastatin, OVA, BALB/c mice

63. S100A8 and S100A9 Homo-, and hetero-dimers Affects Extracellular Matrix in Human Smooth Muscle with Different Down Stream Signaling

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Background: Fibroproliferative airway remodeling, including accumulation of airway smooth muscle (ASM) cells and fibroblasts, is a cardinal feature of chronic asthma. When secreted by neutrophils, monocytes or epithelial cells, the S100 protein family members S100A8 and S100A9 primarily form a pro-inflammatory hetero-dimer complex. S100A8/A9 has been implicated in promoting fibro-proliferative lung remodeling via the receptor for advanced glycated end-products (RAGE) and TLR4. However, S100A8 and S100A9 monomers can also form homo-dimers that exhibit biological activity, though the repertoire of cell responses and receptors involved are not rigorously documented. We tested the hypothesis that S100A8/A9 and homodimeric S100A8 and S100A9 complexes induce biosynthesis of ECM in human ASM. Materials and Methods: Primary human ASM cells were treated with purified human S100A8/A9 (5μg/mL, 0-96 hours), or recombinant human S100A8/A8 or S100A9/A9 (2μg/mL, 0-96 hrs). Results: Immunoblot analysis revealed that all treatments induced significant collagen A1 and fibronectin synthesis over time. Interestingly, S100A8/A9 promoted rapid ERK1/2 and SAPK/JNK phosphorylation and subsequent NF-κB phosphorylation (p65). In contrast, S100A8/A8 and S100A9/A9 exposure led to a decrease in both ERK1/2 and SAPK/JNK phosphorylation, with subsequent phosphorylation of NF-κB (P65). In a side-by-side experiment, effect of S100A8/A9 (5μg/mL, 0-96 hrs) on collagen A1 and fibronectin biosynthesis, smad2/3, JNK/SAPK, and ERK1/2 phosphorylation was compared with that induced by TGF-β1 (2.5ng/mL, 0-96 hrs). Addition of TGF-β1 led to smad2/3 and ERK1/2 phosphorylation and ECM biosynthesis while S100A8/A9 did not affect smad signaling pathway and only resulted in phosphorylation of ERK1/2 and SAPK/JNK with subsequent biosynthesis of ECM. Conclusions: These findings suggest that S100A8/A9 may target different cell surface receptors than S100A8/A8 and S100A9/A9 in human ASM. Our data also suggest that S100A8/A9 induced biosynthesis of ECM is not dependent on smad signaling pathway.
Keywords: S100A8, S100A9, Extracellular Matrix, Airway Smooth Muscle

64. Autophagy is Modulates TGF-β1 Induced Extracellular Matrix Synthesis in Airway Mesenchymal Cells

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Background: Pathologic fibrosis is linked to many diseases including idiopathic pulmonary fibrosis (IPF), airway remodeling in allergic asthma, scleroderma, chemotherapy-induced fibrosis, granulomatous lung disease, sarcoidosis, and chronic obstructive pulmonary disease (COPD). Transforming growth factor-β1 (TGF-β1) is a primary regulator of wound healing and fibrogenesis, its expression is increased in bronchial biopsy and lavage specimens from asthmatic subjects, levels correlate with the degree of fibrosis. Recent reports from several cell systems indicate TGF-β1 may induce autophagy, a bulk cellular catabolic response that mediates clearance of long-lived cytoplasmic proteins, pathogens, and organelles (like mitochondria), but its link to the wound healing effects of the cytokine are unknown. Thus, we tested whether TGF-β1 induces simultaneous autophagy and pro-fibrotic function in human airway smooth muscle (HASM) cells and human airway fibroblasts (HAF). Methods and Results:: Primary HASM cells and HAF from 3rd-4th generation bronchi of lung resection donors were treated with TGF-β1 (2.5 ng/ml). Immunoblotting revealed that within 24hrs TGF-β1 promoted sustained synthesis and secretion of collagen A1. This paralleled the time course for accumulation of autophagy markers (LC3 β lipidation, beclin1, Atg5-12 complex formation). Autophagy was also confirmed by transmission electron microscopy (TEM), which revealed the formation of double membrane autophagosomes. Moreover, we used immunofluorescence staining to confirm autophagosome-lysosome fusion and the formation of LC3 β punctae. Interestingly, these TGF-β1 effects were associated with the induction of mitophagy in HASM, as mitotracker fluorescence decreased indicating loss of mitochondria. TGF-β1 receptor (ALK4, 5, and 7) inhibitors (SB-431542, 20 μM, and SB-505124, 1 μM) prevented both fibrotic and autophagic responses to TGF-β1. Chemical autophagy inhibitors (3-methyladenine, 2.5 mM, and bafilomycin A1, 0.01 μM), shRNA silencing of key autophagy-inducing proteins, ATG5 , and using ATG3 knock out mouse embryo fibroblast decreased the TGF-β1 induced fibrotic response but was without affect on Smad2 and Smad3 phosphorylation in HASM, suggesting autophagy my regulate TGF-β1 effects in parallel with the canonical Smad pathway. Conclusions: We show for the first time that TGF-β1-induced autophagy is a required physiological response for the pro-fibrotic effects of this cytokine on human airway mesenchymal cells. This finding could be exploited for therapeutic application, as targeting manipulation of autophagy may provide an effective complementary strategy to current therapies.
Keywords: Autophagy, TGF-β1, Extracellular Matrix, HASM

65. Investigation the Effect of Breast Cancer Cell Line in a Mouse Model of Allergic Asthma

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Background: The increasing prevalence of allergies and cancer in industrial countries obligated scientist to explore the association between these two phenomena. Contradictory result were achieved from epidemiological and laboratory research. Until now no concrete relation between these two condition was found. Materials and Methods: In order to investigate the effect of allergy and cancer on each other under controlled conditions, we administrated cancerous cell line to a mouse model of allergic asthma. Results: In this study we showed that acute allergic asthma increased the number of metastatic tumors in the lungs of asthmatic mice 2.5 fold compared to healthy mice received cancerous cell line. In addition, the number of tumors in the lungs of mice with chronic allergic asthma increased seven fold compared to normal mice when they received cancerous cell line. Cancerous cell line significantly changed the composition and number of inflammatory cells in the lungs and airways of asthmatic mice from those who where allergic asthmatic or healthy mice that received cancerous cell line. Our pathological examination of the lungs reveal that combination of allergic asthma and cancer notably changed the structure of the lungs. Additionally, serum OVA specific IgG1 and IgE from allergic asthmatic mice that have cancer are significantly higher than allergic asthmatic mice.
Conclusion: Taken together our data indicate that acute and chronic respiratory allergic inflammation exacerbated lung metastasis and also cancer in combination with allergic asthma excacebate the symptoms of allergic asthma and non of these two diseases can ameliorate other diseases.
Keywords Breast Cancer, Mouse Model, Allergic Asthma

Poster Discussion Presentation

66. Chemokines Receptors Mutation and Pathogenesis of Asthma in Patients

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Background:Chemokines and their receptors are clinically important mediators, as the chemokine receptors are expressed on almost all immune cells. They play pivotal roles in pathogenesis of almost all clinical situations including asthma. Correspondingly, MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (CCL5) are among the important chemokines involved in the pathogenesis of asthma. These chemokines bind to the

CCR5 (their related receptor) on the cell surfaces. Attachment of related chemokine ligands to CCR5 plays an important role in the pathogenesis of asthma; hence, this study aimed to analyze δ32 mutations in CCR5 in asthmatic patients.Materials and Methods: This experimental study was undertaken on 162 asthmatic patients and 200 healthy controls during February to June 2008 at Rafsanjan University of Medical Sciences. The Gap-PCR method was applied to analyze the δ32 mutation in the CCR5 gene, and demographic data (eg, age, sex, occupation, socio-economic status) were collected using a questionnaire.Results:The findings of this study indicated that none of the asthmatic patients exhibited δ32 mutation in CCR5 chemokine receptor while only 3 (1.5%) of controls had the heterozygotic form of this mutation.Conclusion:Several research groups analyzed δ32 mutations in CCR5 in different diseases, including asthma. Some investigations reported a significant relation between asthma and δ32 mutations in CCR5, but there are also many reports which failed to find a relation between asthma and this mutation. Based on the results of this study and others, it seems that the δ32 mutation does not affect the pathogenesis of asthma.
Keywords:Asthma, Chemokines, CCR5 δ32, Pathogenesis

67. The Epidemiology of Asthma, Allergic Rhinitis, and Eczema amongMiddle School Students in Tabriz

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Background: Asthma, allergic rhinitis, and eczema have an extensive epidemiologic diversity in various geographic areas. The aim of this study was to investigate the prevalence of atopic syndrome and the associated risk factors among middle school students**.** Materials and methods: This cross-sectional study was performed on 1508 students in the city of Tabriz (a northwestern Turkish-populated region of Iran) in 2009. The International Study of Asthma and Allergies in Childhood questionnairewas used to collect data. The collected data was analyzed using SPSS 16**.** Results: The prevalence of cumulative and periodic wheezing and diagnosed asthma was 3.7%, 2.9%, and 2%respectively. The diagnosed prevalence of cumulative and periodic allergic rhinitis and hay fever was 17.1%, 16.0%, and13.6 **%**respectively. For cumulative and periodic nocturnal rash and eczema, the diagnosed prevalence was 5.4%, 4.7%and 7.3%, respectively. Asthma symptoms were more prevalent in the western part of the city than in the central andeastern parts, and boys were more likely to have symptoms of rhinitis and eczema than girls. Having pets and a history ofhospitalization increased the chance of eczema, and advanced maternal age was correlated with the presence of asthmasymptoms. Breastfeeding, household size, and exposure to cigarette smoke did not have any effect on the appearance ofatopic diseases.Conclusion: The small difference observed in this study between the prevalence of cumulative and periodic allergicrhinitis could be caused by the sharp recent increase in allergic rhinitis prevalence. An ecological survey in the westernpart of the city could be valuable in helping to determine the factors contributing to the higher prevalence of asthma there in comparison with the central and eastern parts. Such a survey should examine demographic, environmental, andeven ethnic variables in this geographical region and perhaps even in adjacent countries**.**
Keywords: Asthma, allergic rhinitis, eczema, students, Tabriz, Iran

68. Study of Asthma Risk Factors among Children at 2 To 8 Years of Age in Urmia District

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Background: Asthma is most common chronic disease in childhood. Evidence linking about some of asthma risk factors among children is inconsistent. The aim of this study was investigate the association between potential risk factors and risk of asthma in children at age 2-8 years in urmia district. Materials and Methods: A case-control study was conducted in the first semester of 89-90 years. Cases (n=207) were selected from asthmatic patients who referred to Motahhari children clinic and asthma, allergy pediatric center. Control groups (n=401) with 2control per case were selected from other patient without any allergic or respiratory disease and healthy children and were matched according age and gender. Data related to potential riskfactors were collected in interview with patient's relatives by International Study of Asthma and Allergies in Childhood (ISSAC) questionnaire. To reduce the existed collinearity,the associations between investigated risk factors and risk of asthma were analyzed by multiple logistic regression using backwardselection in two separate model and combined in final model. Results: Increased risk of asthma were found for preterm delivery, antibiotic use in first year of life , breastfeeding more than 24 months , Television watching for 3 or more hours per day, using feather pillow and blanket bedding, lower air conditioning , acetaminophen use in last year , consumption of dairy product, fast foods and nuts consumption for 3 or more times a week in comparison with lower intake of them per week , family history asthma and allergic sensation in first and second degree relatives. Protective effects were found for consumption of fruit, vegetables potato for 3 or more times a week. Conclusion: Change in environmental factors and sedentary life style from first year of life may prevent the development of childhood asthma.
Key words: Childhood asthma, Case-Control study, Risk factors, Iran, Urmia, ISSAC

69. Evaluation ofSerum ZincIn Patients WithAsthmaCompared WithHealthy Subjects

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Background: Asthma as a most prevalent disorder of the airways is accompanied with excessive inflammatory, oxidative and apoptotic activity. In asthmatic patient zinc status of serum and hair has been reported to be decreased. Zinc deficiency may be suspected to play a role in the pathogenesis, control, and severity of asthma because of its antioxidant, ant apoptotic, and anti-inflammatory effects.
We aimed to investigate whether there was any relationship between serum zinc levels and adult asthma. Materials and Methods: This was a case control study. We enrolled 29 asthmatic patients and 29 healthy subjects. 10 ml blood sample were drawn for measuring zinc status of serum and it was compared with 29 non-asthmatic participants. Results: The serum zinc concentration of treated patients were 0.68 ± 0.16 mg/l vs. 0.91 ± 0.11 mg/l of control group, which shows significant difference (P=0.000). There was not any difference in body mass index between two groups. Conclusion: This study's findings indicate that zinc status of serum in asthmatic patients were significantly lower than non-asthmatic. We emphasize that checking zinc levels in asthmatic patient may be useful. More interventional trials are recommended.
Key Words: Asthma, Body Mass Index, Serum zinc

Poster Presentation

70. Siglec-8 and Siglec-F, the New Targets in Asthma Treatment

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Background: Increases of eosinophils in the tissues, blood and bone marrow are a hallmark of most asthma phenotypes and, in general, elevated numbers correlate with disease severity. The dramatic reduction of eosinophils in sputum and tissue as a result of asthma treatment with corticosteroids, associated with clinical improvement, has led to the notion that eosinophils are fundamental to airway dysfunction in asthma. Several studies suggest that eosinophil apoptosis is delayed in asthma. Nowadays Eosinophil apoptosis is an important drug target for the

development of novel anti-asthma drugs that specifically target the eosinophil. Materials and Methods: In this paper we have systematically reviewed the Siglec-8 as a novel receptor on Eosinophil. Results: The term Siglecs stands for sialic acid-binding immunoglobulin-like lectins. In the past few years, seven novel human siglecs of the innate immune system have been discovered. Eosinophils express Siglec-8 in a highly cell-type restricted manner. Siglec-8 ligation appears to induce eosinophil-specific apoptosis. Studies have shown that IL-5 and GM-CSF, fail to block apoptosis and instead enhance the sensitivity of eosinophils to undergo apoptosis in response to Siglec-8 engagement. Siglec-F, which prominently expressed on mature circulating mouse eosinophils, is the ortholog of human Siglec-8 because of marked similarities in expression patterns and ligand preferences. Siglec-F antibody administration significantly reduced levels of allergen-induced eosinophilic airway inflammation and features of airway remodeling in a mouse model of chronic asthma, especially subepithelial fibrosis, by reducing the number of eosinophils and increasing the number of apoptotic eosinophils in lung and bone marrow. Conclusion: Studies suggest that Siglec-8 and mouse Siglec-F preferentially recognizes 6′-sulfo-sLex as a glycan ligand. It is worth mentioning that sulfated sialyl-Lewis X structures occur in bronchial mucins. Therefore Siglec-8-specific antibodies can represent a novel pathway for selectively clearing the airway of unwanted eosinophils.
Keywords: Siglec-8, Siglec-F, Asthma

71. Investigating the Correlation between Asthma Severity and Serum Level OfVitamin D in Asthmatic Children Compared to Healthy Population

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Background:
Vitamin-D deficiency is one of the factors affecting the immune system and also in the case of deficiency, the body is more susceptible to demonstrate illnesses relating to immune system. Asthma is the chronic inflammation of airways because of conflicts in, and over expression of immune responses due to environmental and genetic factors.
The aim of this study was to assess the 25-hydroxy Vitamin-D level in serum of asthmatic and non-asthmatic children and investigating the core relation between the asthma intensity and 25-hydroxy vitamin-D level in serum.
Materials and Methods: This is a cross- sectional study comparing the blood serum level of 25-hydroxy vitamin -D in 50 mild to moderate level asthmatic children with the 50 healthy children with no symptoms of asthma living in Kerman. We used the Elisa assay technique .results was analyzed by using t-test, chi-square and spearman correlation coefficient. Results: The mean amount of 13.6±1.1 ng/ml blood serum level of vitamin-D was measured in asthmatic children. The mean amount of the same factor was 19.2±1.8 ng/ml in healthy children. There was a reverse relation between the disease intensity and the vitamin-D blood serum level. Conclusion: According to this study it can be concluded that regarding the low serum level of vitamin-D in asthmatic children, taking enough vitamin-D during pregnancy may lead to primary prevention of asthma and reduction of its intensity. Keyword: Vitamin-D, serum level, Asthma

72. Lack of Association between Chemokine Receptor 5 (CCR5) δ32 Mutation and Pathogenesis of Asthma: A Study on Iranian Asthma Patients

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Background: Chemokines and their receptors are clinically important mediators, as the chemokine receptors are almost expressed on all immune cells. They play pivotal roles in pathogenesis of almost all of clinical situations including, asthma. Correspondingly, MIP-1α (CCL3), MIP-1β (CCL4) and RANTES (CCL5) are among important chemokines which are involved in pathogenesis of asthma. These chemokines bind to the CCR5 (their related receptor) on the cells surfaces. Attachment of related chemokine ligands to CCR5 play important role in pathogenesis of asthma, hence, this study aimed to analysis δ32 mutations in CCR5 in asthmatic patients. Materials and Methods: This experimental study was undertaken on 162 asthmatic patients and 200 healthy controls during February to June 2008 in Rafsanjan University of Medical Sciences. The Gap-PCR method was applied to analysis δ32 mutation in CCR5 gene and demographic data (age, sex, occupation, socio-economic status and etc…) were collected by a questionnaire. Results: The findings of this study indicated that none of asthmatic patients exhibited δ 32 mutation in CCR5 chemokine receptor while only 3 (1.5%) of controls had heterozigotic form of this mutation. Discussion: Several research groups analyzed δ32 mutations in CCR5 in different diseases including asthma. Some investigations reported significant relation between asthma and δ32 mutations in CCR5 but there are also many reports which failed to find a relation between asthma and this mutation. Based on the results of this study and others it seems that δ32 mutation does not affect pathogenesis of asthma.
Key words: Chemokines, CCR5, δ 32 mutation, Asthma

73. The SDF-1α 3ᵝ A Genetic Variation Increases the Susceptibility of Asthma in Iranian Patients

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Background:Chemokine/receptor axis is a predominant actor of clinical disorders, as the chemokine receptors are almost expressed on all of Immune compatible cells. They are key factors of pathogenesis of almost all of clinical situations including asthma. Correspondingly SDF-1α (CXCL12) is involved in the immune responses. Therefore, this study was designed to explore the association between gene polymorphism at position +801 of SDF-1α (CXCL12), known as SDF-1α`A and susceptibility to asthma in Iranian patients.Materials and methods*:* In this experimental study, blood samples were taken from 228 asthma patients and 189 healthy controls on EDTA pre-coated tubes. From the specimens, DNA was extracted and analyzed for SDF-1α (CXCL12) polymorphisms using PCR-RLFP in patients and controls. The demographic information were also collected in parallel with experimental part of the study by a questionnaire which was designed specifically for this study. Results*:*Our results indicated a significant difference (P<0.0001) between the *A/A, A/G* and *G/G* genotypes and *A* and *G* alleles of polymorphisms at position +801 of SDF-1α (CXCL12). Conclusion*:* our findings suggest thatSDF-1α3ᵝ A (CXCL12) polymorphism is a predisposal factor in pathogenesis of asthma. It can also be concluded that this polymorphism presumably can be used as a pivotal biological marker in diagnosis of asthma.
Keywords: Asthma, SDF-1α (CXCL12), Polymorphism

74. Determination of PEF and FEV1 Normal Volumes and Assessment of Portable Digital Peak Flow Meter in Diagnosis of Asthma in Elementary School Students of Birjand City

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Background: Allergic diseases specially asthma are among the most common disorders around the world especially in children. Every year asthma attacks cause significant number of hospitalization and death. Because of its chronic and progressive nature, early diagnosis of asthma can prevent irreversible changes and permanent inflammatory remodeling. Standard medical protocols for manegment of asthma insist on patient's training and daily recording of lung's volumes with portable devices such as digital peak flow meter by patients themselves. On the other hands, spirometery is not applicable in routine use and therefore recording of PEF and FEV1- volumes- with small electronic devices have been encourages.
Materials and Methods: In this cross-sectional study, 200 students in 7, 8 and 10 years age groups were selected by simple random sampling from different socio-economical levels in Birjand city during 2011. Demographic data and information about presence of symptoms were collected by interview and filling a standard questionnaire. PEF and FEV1 were measured three times by digital peak flow meter and the average value was selected for analysis.
Results: The average value of PEF in 7,8 and 10 years old groups were 154-169 and 200 respectively. For FEV1, average amounts were 1.07-1.17 and 1.47 respectively. There was a significant positive correlation between PEF, FEV1 and age, weight as well as height. PEF and FEV1 values were smaller in girls - than boys but the difference was not significant. 5% of students, reported wheezing and FEV1 and PEF in this group were lower than healthy group. Although this different was just statistically significant for PEF. (177,212 $p<0.05$ and FEV1 1.67 and 1.55 $p>0.05$)
Conclusion: By increasing in age, height and weight, respiratory volumes increase. PEF and FEV1 amounts in asthmatic students are lower than healthy students. The measurement of PEF by peak flow meter can be useful in identification of people with respiratory problem such as asthma.

75. Oral Findings in Asthmatic Patients

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Asthma is a common chronic disease that associated with airway obstruction, with recurrent attacks of paroxysmal dyspnea, and wheezing due to spasmodic contraction of the bronchi. The disease and its treatment can change the oral conditions of patients. Oral findings in asthmatic patients including; increased rate of dental caries (development due to prolonged use of $\beta_2$agonists inhalers and medications used for asthma treatment that contain sugar), enamel defects, increased gingivitis and increased risk of periodontal disease and more calculus, higher rates of malocclusion and increased: overjet, overbite, posterior crossbite; high palatal vault, oropharyngeal candidiasis, xerostomia(dry mouth), decreased salivary flow rate and salivary PH. A regular follow-up of oral health status is important in asthmatic patients, especially in children and adolescents.

76. Elevated Circulating cc Chemokines CCL2 (MCP-1), CCL5 (RANTES) and CCL11 (Eotaxin) in Asthmatic Patients

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Background:Chemokine/receptor axis is a predominant actor of clinical disorders, as the chemokine receptors are almost expressed on all of Immune compatible cells. They are key factors of pathogenesis of almost all of clinical situations including asthma. Correspondingly, cc chemokines are involved in the immune responses. Therefore, this study was designed to investigate the role of cc chemokines CCL2 (MCP-1), CCL5 (RANTES) and CCL11 (Eotaxin) in pathogenesis of asthma in Iranian asthmatic patients. Materials and methods: In this experimental study, blood samples were taken from 228 asthma patients and 189 healthy controls on EDTA pre-coated tubes. DNA was extracted and analyzed for CCL2 (MCP-1), CCL5 (RANTES) and CCL11 (Eotaxin) using ELISA in patients and controls. The demographic information was also collected in parallel with experimental part of the study by a questionnaire which was designed specifically for this study. Results:Our results indicated dramatic increased level of these chemokines in serum of asthmatic patients. Conclusion: our findings suggest thatcc chemokines are critical factors in pathogenesis of asthma and they presumably can be used as a pivotal biological marker in diagnosis of asthma.
Keywords: Asthma, cc chemokine, CCL2 (MCP-1), CCL5 (RANTES) and CCL11 (Eotaxin)

77. General Prevalence of asthma related symptoms in Iran: a Meta Analysis

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Background: Asthma affects more than 300 million peoples around the world and is the most common chronic diseases among children. The prevalence of asthma differs in different parts of the world even in different regions of a country. Information about the prevalence of asthma and other allergic diseases is a key factor to imply proper strategies for prevention and management of allergic disorders which can save bodies and pennies. Several epidemiologic studies have been performed in different parts of Iran but the results are fairly different. The aim of this study was to get estimation about national prevalence of asthma in Iran by performing a meta-analysis. Materials and Methods: Data about the prevalence of asthma and asthma related symptoms were recruited from domestic and international scientific databases. Studies were included if they report the prevalence of asthma, symptoms of asthma and its risk factors in Persian or English by using ISAAC questionnaire. There was considerable heterogeneity in methodology of studies and therefore the pooled estimated effect calculated with Random effect model. Sub group–analysis was also conduct.
Results: Eighteen relevant prevalence studies were included for meta-analysis. The pooled prevalence for total population was obtained as 5.570 ($CI_{95\%}$; 4.414- 6.726), 15.758 (CI95%; 12.091 -19.425) and 18.944(CI95%; 6.028 - 31.861) for asthma, wheezing and nocturnal cough respectively. The pooled prevalence for 6-7 and 13-14 years age groups was obtained as 3.489 (CI95%; 1.926 to 5.052) and 4.921 (CI95%; 3.273 - 6.570) respectively. Similar results were found for the pooled prevalence of male and female. Conclusion: The result of this study shows that prevalence of asthma and its related symptoms in general is fairly low although there are some areas with high prevalence. More studies are needed to reveal the reason for high prevalence in some parts.
Keywords: Prevalence of asthma, Iranian population, .systematic review, meta-analysis

78. IL-6 G-174C Lower Producer Variants are Associated with Reduced Asthma Pulmonary Capacities

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Background: IL-6 is a regulatory cytokine that has been shown to be a cofactor that potentiates IgE production and elevated levels of circulating IL-6 were observed in both symptomatic and asymptomatic asthmatic subjects. In this study we evaluated role of IL-6 G-174C polymorphisms in lung functions. Materials and Methods: ARMS-PCR method and IgE serum level by ELISA technique were considered for evaluation of gene polymorphism and allergy on 81 asthmatic patients and 124 normal subjects. Pulmonary function tests (PFT) in a standard fashion were performed for all subjects. Results: IL-6-174C allele was associated with reduced FEF25-75 value in asthmatics ($p=0.045$). IgE levels in asthmatic patients was significantly higher than normal controls ($p<0.05$). Conclusions: It is clarified that FEF25-75 is a sensitive marker of

pulmonary abstraction of asthma. Low producer of and IL-6 (-174C) alleles was associated with reduced FEF25-75 and decrease in respiratory capacity and thereby more pulmonary obstruction. These results are incompatible to assumed roles of proinftammatory cytokines to progress asthma inflammation. Further studies would be more elucidative.

Keywords: Asthma, Cytokine, polymorphism, Pulmonary Function Test

79. Effects of Antibiotic Consumption and Developing Childhood Asthma at 2-8 Years of Age

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Background: Antibiotic exposure in prenatal life and early childhood is one of a possible risk factors might be associated with increasing risk of asthma prevalence and other atopic disorders over recent decades. The present study aimed to investigate the association between antibiotic exposure and the risk of developing childhood asthma.
Materials and Methods: A case - control study was undertaken between March and September 2010 in Urmia district north west of Iran. Subjects were children aged between 2-8 years old. Cases were asthmatic children diagnosed based on GINA criteria (n=207) and controls were children without asthma symptoms (n=414) using 1:2 sampling method. Cases and controls were matched for age and gender. Clinical data including Antibiotic exposure was collected by a questionnaire which completed by interviewing with parents/guardians. Results: Antibiotic consumption during the first year of life increased the risk of asthma symptoms at 2-8 years of age (crude OR: 2.30; 95% CI 1.53 - 3.50, p<0.001 and adjusted OR: 2.01; 95% CI 1.30 - 3.08, p=0.001). Conclusions: Using Antibiotic increases risk of asthma among 2 - 8 years old children. There are strong evidences about this positive association and any potential conflicts were removed from finding in this association.

Keywords: Antibiotic consumption, childhood asthma, urmia district

80. Effects of Acetaminophen Consumption in Asthmatic Children

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Background: Acetaminophen exposure might be associated with increasing risk of asthma prevalence and other atopic disorders un different period of life. The present study aimed to investigate the association between acetaminophen exposure and the risk of developing childhood asthma. Materials and Methods: A case - control study was undertaken between March and September 2010 in Urmia district north west of Iran. Subjects were children aged between 2 - 8 years old. Cases were asthmatic children diagnosed based on GINA criteria (n=207) and controls were children without asthma symptoms (n=414) using 1:2 sampling method. Cases and controls were matched for age and gender. Clinical data including Acetaminophen exposure was collected by a questionnaire which completed by interviewing with parents/ guardians. Results: Using Acetaminophen during the first year of life had no any significant effect on the risk of asthma (p=0.19), but amongst 2-8 years old children, Acetaminophenuse during the last year had significant effect (p<0.001). There was also a dose-response association between Acetaminophen consumption and risk of asthma (OR: 3.8; 95% CI; 2.15 6.59for once per 2 to 3 month and OR: 4.2; 2.50 - 7.3 for at least one per month). Conclusions: Using Acetaminophen increases risk of asthma among 2-8 years old children. However stronger evidences are required to design evidence-based guidelines to reduce acetaminophen consumption following post - vaccination and other febrile disorders.

Keywords: Acetaminophen, childhood asthma

81. Association Between duration of breast feeding and asthma at children between at age 2 -8 years

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Background: Asthma is most common chronic disease in childhood. Various results were found linking breastfeeding and childhood asthma. The aim of this study was investigate the association between duration of breastfeeding and risk of asthma in children at age 2-8 years in urmia district. Materials and Methods: A case-control study was conducted in the first semester of 89-90 years. Cases (n=200) were selected from asthmatic patients who referred to Motahhari children clinic and asthma, allergy pediatric center. Control groups (n=400) with 2 controls per case were selected from other patient with irrelevant allergic disease and healthy children and were matched according age and gender. Information related with duration of breastfeeding and other affected factors were collected using ISSAC phase II risk factors questionnaire in interview with patient's relatives. The associations between Breastfeeding pattern with risk of asthma were analyzed by multiple logistic regression after adjust for affected variables using STATA 10 software. Results: Duration of breast-feeding for 2 first years of life was associated with a reduced risk of asthmahowever lasting it for higher than 2 years significantly associated with an increased risk (OR:2.45,95%CI:1.22 - 4.94,Pvalue:0.01). Exclusive breast-feeding for less 6 months and using artificial feeding before 6 months increased risk of asthma (OR:1.34 ,95%CI:0.75-2.40, OR:1.20 ,95%CI:0.74-1.65respectively)at2-8years,Butitwasn'tsignificant. Conclusion: Lasting of breastfeeding for 2 first year of life cannot increase risk of asthma and exclusive breast-feeding for first 6 months of life has protective effect against asthma at 2-8 years.

Keywords: Childhood asthma, Case-Control study, Duration of Breastfeeding, Risk of asthma

82. Effect of Fruits, Vegetables Consumptions in Childhood Asthma

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Back ground: There is growing evidence have shown protective effects of fruit and vegetables at lower risk of asthma. The aim of study was to evaluate the association between fresh fruits and vegetables consumption in dietary pattern and the risk of childhood asthma. Materials and Methods: A case - control study was performed among children at 2 - 8 years. Cases (n=207) were selected from asthmatic patients who referred to Motahhari children clinic and asthma, allergy pediatric center. Control groups (n=401) with 2 control per case were selected from other patient without asthma and other irrelevant disease and healthy children. Data related to fruits, vegetables intakes and other related variables were collected in interview with patient's relatives using questionnaire. The associations between dietary patterns and risk of asthma were analyzed by multiple logistic regressions using STATA 10 software. Results: Asthmatic patients had lower intake of fresh fruits, vegetables and potatoes consumptions than control group. Protective effects were found for consumption of fruit, vegetables potato for 3 or more times a week (OR_{fruts}:0.36 , 95% CI :0.19 - 0.68, P value: 0.002, $OR_{vegetables}$:0.74, 95% CI :0.51 - 1.07 , P value : 0.11). Conclusion: Our findings indicate that 3 or more intake of fruits and vegetables per week have a protectiveeffect on the risk of childhood asthma.

Keywords: childhood asthma, diet, Urmia district

83. HistopathologicalStudyin Lung Tissue ofRats AsthmaThatWereTreatedWithTheophylline

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Background: Over 150million people worldwideareafflicted by asthma. Sudden increase indeathsdue toasthmaisessentialforunderstandingthe disease unknownmechanisms. Hyperthrophy in smooth muscle duct respiratorymucosalsecretions and increase of mucosal secretions into the respiratory duct are among the causes of contractionof the distributed respiratory ducts. Materials and Mathods: A total of 30adultmale ratswererandomly selectedanddivided into3 groups. Healthycontrol groupreceivedonlysalineduring thestudy. Thenexttwogroups ofrats were sprayed by citric acidwithfogging nozzlesfor a week to get asthma. After verification of asthma and counting neutrophils (during 1 week blood sampling) onegroupofratsthat were consideredascontrol were injected theophylline 100mg/kg (4 weeks)and second group wasasthma was considered as asthma control. After thetreatmentperiod,animalswereanesthetized and lung fixed in 10% formalin. The histopatological studies of lung after preparing cross sections and coloring with H&E and *toluidine blue* was done by light microscope. Results: inthisstudy in asthmatic rats thelarge number ofrespiratorybronchioles been hurt lung fibrozed, hyalinized thealveolihemorrhagein thealveolarsacs and bellowscompoundsmucus observed. Lymphoidcells, particularly mast cells,aroundbronchialandmacrophages in thealveolar sacssignificantlyincreased. Micetreatedwiththeophylline, had beensomewhatrestored.The size ofthelymphnodulesaround bronchialhas been reduced. The number of mast cells significantlyreduced,mast cells in end bag grown up. Theirquantity did notchange muchcompared withtheasthmaticgroup. But comparedwith the control groupshowed asignificant change. This is indication of inflammation and swelling of tissue.
Conclusion: The results ofthe presentstudysuggests resulting tissuedamageinasthmatic rats due to citric acid ispartlyrestored by injection of theophylline.
Keywords: Athmic, lung, rat, theophylline

84. Prevalence of Asthma and Allergies among 6-7 Year Old Children in Birjand City: An ISAAC Study

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Background: Asthma and other allergic disorders are common health problems around the world especially in children and have a negative impact on quality of life. Allergic diseases are one of the leading causes of school absence as well as reduction in children performance at school. Epidemiologic Studies have shown that the prevalence of asthma and other allergic diseases have risen over the past decades. Many factors have been reported that contribute to this increase including genetic factors as well as environmental factors such as lifestyle, infections and diet. Preventing and controlling allergic diseases require information about the prevalence, risk factors and triggers which can be vary in different countries. The objective of this study was to determine the prevalence of asthma and other allergic diseases among 6-7 year old children in Birjand city. Materials and methods: In a cross-sectional survey in 2011, all school children age 6-7 years in Birjand city were evaluated based on ISAAC protocol. Persian version of ISAAC core questionnaire was completed by parentsin total 3070school children (M/F ratio=0.88) were participated in this study. Results: The response rate was 91%. The prevalence rate for Wheezing, physician diagnosis asthma, exercise wheeze, rhinitis and eczema was 15.5%, 2.2%, 3.2%, 12.4% and 8.2% respectively. except eczema, prevalence of other symptoms was higher in boys than girls but the differences was not significant compared with females although it was not significant. Conclusion: Our study shows that prevalence of rhinitis and wheezing is high among school children in Birjand city. Further studies should be performed to determine risk factors of allergic disorders in this area.
Keywords: Prevalence, ISAAC, Birjand

85. The Effect of Carvacrolon Serum Cytokines and Endothelin Level of Ovalbumin Sensitized Guinea pigs

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Background: Different pharmacological effects of carvacrol including relaxant effect, its inhibitory effect on muscarinc and histamine (H_1) and stimulatory effect on β-adrenoceptors have been demonstrated on guinea pig tracheal chains in previous studies. In the present study, the effect of carvacrol on blood IL-4, IFN- γ and endothelin levels of sensitized guinea pigs was examined.Materials and Methods: Five groups of guinea pigs sensitized to ovalbumin (OA) were given drinking water alone (group S), drinking water containing three concentrations of carvacrol (40, 80 and 160 µg/mL) and dexamethasone. The blood IL-4, IFN- γ and endothelin levels of sensitized and control guinea pigs were evaluated (n=6, for all groups). Results: Blood IL-4 and IFN-γlevels ($p<0.001$ for both cases) as well as endothelin ($p<0.01$) were increased but IFN-γ/IL-4 ratio decreased ($p<0.05$) in sensitized animals compared to controls. Treatment of S animals with dexamethasone ($p<0.01$) and two higher concentrations of carvacrol ($p<0.001$ for both cases) significantly decreased IL-4 level. Treatment of S animals with dexamethasone did not changed IFN-γlevels but treatment with high concentration of carvacrol significantly increased its level ($p<0.001$). In addition, IFN-γ/IL-4 ratio was significantly increased in S groups treated with dexamethasone ($p<0.05$) and two higher concentrations of carvacrol ($p<0.001$ for both cases). Treatment of S animals with dexamethasone ($p<0.01$) and all concentrations of carvacrol also significantly decreased endothelin level ($p<0.01$ to $p<0.001$). Conclusion: These results showed that carvacrol caused reduction of IL-4 and endothelin but increased IFN-γ and IFN-γ/IL-4 ratio in the blood of sensitized guinea pigs. The results also suggest more specific effect of carvacrol compared to dexamethasone due to the absence of the effect of later on IFN-γ.
Keywords carvacrol, asthma, sensitization, cytokines, dexamethasone

86. Mental Health Survey of Mothers with Asthmatic Children

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Background: Asthma is one of the main problems ofhealth in the world that has long treatment and the mental health of asthmatic children and their families is affected. Furthermore, caring for a child with asthma as a chronic illness combines the demands of parenting with the emotional and physical burdens of protection of the child's chronic illness. The objective of this investigation is evaluation of maternal mental health that their child has asthma. Materials and Methods: In this study, between the dates of December 2009 to June 2010, 80 mothers who had 7-12 aged children with asthma diagnosis completed a questionnaire including question about somatic symptoms, anxiety, social dysfunction and severe depression (GHQ.28). Results: The results showed that mothers of asthmatic children compared with the community cut-off point, suffered from depression ($p<0.001$); whereas GHQ scales in three levels of asthma control in children (i.e. controlled, partly controlled and uncontrolled) were be same. These results reveal that caring for a child with asthma has an impact on the mother's mental health and exacerbates risk for depression among them apart from the control level of the disease. Conclusion: These finding suggests that the clinician's awareness of maternal depression is important for guiding effective intervention. Besides, the presence of a psychotherapist for diagnosing and treating depression in mothers of children with asthma would enhance child well-being both psychological and physical.
Keywords: Mental Health, Asthmatic Children

AUTOIMMUNE DISEASES

Oral Presentation

87. Calcitriol-Treated Dendritic Cells: Phenotypic and Functional Properties and Their Effects on EAE

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Background*:* Regarding the crucial role of dendritic cells (DCs) in management of immune response, immunomodulatory compounds which affect DCs function, seems to be useful in treatment of autoimmune diseases. The aim of this study was to investigate the in vivo effects of calcitriol (active form of vitamin D3) on DCs and the ability of calcitriol treated DCs to prevent the EAE (Experimental Autoimmune Encephalomyelitis) as animal model of MS (Multiple Sclerosis). Materials and Methods*:* Calcitriol was regularly injected (IP) to C57BL/6 mice and their spleen DCs were separated using the magnetic beads separation technique. The phenotypical and functional properties of DCs were studied by flow cytometry and mixed lymphocyte reaction (MLR) respectively. The DCs after pulsing with Myelin Oligodendrocyte Glycoprotein (MOG), were injected before EAE induction into syngeneic mice and the disease was followed up. Histopathological studies on CNS tissues were also done to determine the extent of cellular infiltration. Spleen DCs from solvent treated mice were used as control. Results*:* The expression of CD86 and MHCII as maturation markers and costimulatory molecules, as well as allogeneic T cell stimulation in MLR in comparison with control groups, were significantly decreased while CD11b expression as marker of Th2 inducer DCs were significantly increased. Statistical analysis showed that immunization of mice with calcitriol treated DCs before EAE induction caused significant decrease in severity of disease and cellular infiltration in CNS tissues compared with control animals.
Conclusion*:* Calcitriol shows potent in vivo regulatory effects on maturation and function of DCs. The calcitriol treated DCs (Tolerogenic DCs) possess the ability to downregulate the severity of EAE (as animal model of multiple sclerosis) and decrease the rate of cellular infiltration to CNS.
Keywords: Dendritic cell, calcitriol, Experimental Autoimmune Encephalomyelitis (EAE)

88. Immune Modulatory Effects of Vitamin D on Patients with Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus is an autoimmune disease that is complicated by autoantibody mediated organ involvements. In SLE immune tolerance breakdowns and some environmental and genetic factors are involved in the pathogenesis of SLE. In recent year's disorders in regulatory suppressive lymphocytes has been suspected. Vitamin D has been implicated as an environmental immunomodulator factor which targets various immune cells and may play a role in immune regulation via increasing and activating regulatory cells and its related molecules. Immunosuppressive effects of vitamin D have been investigated in various studies, however the mechanism by which it induces its modulatory effects is not well understood. In this investigation we studied the mechanisms by which vitamin D may regulate the immune responses in SLE patients. Materials & Methods: Study group comprised of 25 SLE patients, and 20 age and sex matched controls. Lymphocytes cultured with 1,25 dihydroxyvitamin D3, and CD4+CD25+FOXP3+ cells were analyzed by flowcytometry methods before and after incubation. RNA was extracted and after cDNA synthesis the expression levels of FOXP3, IL-17, IL-6, IL-12, IFN-γ, TGF-β genes was studied by Real-Time PCR method. Results:1,25 dihydroxyvitamin D_3 increased CD4+CD25+FOXP3+ cells, and the expression levels of FOXP3, TGF-β, and IFN-γ genes increased, while IL-6, and IL-17 decreased significantly in SLE patients. Conclusions: 1,25 dihydroxyvitamin D_3 modulates inflammatory immune resposes in lupus patients by increasing the regulatory T cells subsets.
Keywords: vitamin D, regulatory cells, Th17, lupus

89. The Effects of IFNβ on Denderitic Cells Polarization and MBP Related Autoreactive T cells Responses

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Background: One proper method to improvement MS treatment is skewing autoreactive T cells response from pathogenic Th1/Th17 to Th2 response. Dendritic cells play a crucial role in the conduction and polarization of T cells response. The aim of this study is evaluation of potential of IFNβ on DC maturation and polarization of autoreactive MBP related T cells. Materials and Methods: From 5 volunteer people, peripheral blood monocytes were collected and converted to Immature DC. Then, these cells were pulsed to MBP. After that, IFNβ was added in culture as DCmaturationfactor. Flowing DC immunephenotyping on day7, these cells were adjusted to autologus T cells in order to determination of T cell polarization status. Results: The usage of this compound caused increaseing DC maturation indices (D80, CD83, CD86 and HLADR) and decreasing of CD14 compared with control group. The more production of Il-10 compared with Il-12 suggested that DCs shifted to DC2. The more decreasing of pathogenic IFN-ϒ and IL-17 cytokines compared with IL-4 indicated that more polarization of T cells to Th2. Conclusion: This approach may be a useful strategy to control MS.
Keywords: Multiple sclerosis, Denderitic cell, T cell response, MBP, IFNβ.

90. The Effects of Histamine on Denderitic Cells Polarization and MBP Related Autoreactive T Cells Responses

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Background: One proper method to improvement MS treatment is skewing autoreactive T cells response from pathogenic Th1/Th17 to Th2 response. Dendritic cells play a crucial role in the conduction and polarization of T cells response. The aim of this study is evaluation of potential of histamine on DC maturation and polarization of autoreactive MBP related T cells. Materials and Methods: From 5 volunteer people, peripheral blood monocytes were collected and converted to Immature DC. Then, these cells were pulsed to MBP. After that, histamine was added in culture as DCmaturationfactor. Flowing DC immunephenotyping on day7, these cells were adjusted to autologus T cells in order to determination of T cell polarization status. Results: The usage of this compound caused increaseing DC maturation indices (D80, CD83, CD86 and HLADR) and decreasing of CD14 compared with control group. The more production of Il-10 compared with Il-12 suggested that DCs shifted to DC2. The more decreasing of pathogenic IFn-ϒ and IL-17 cytokines compared with IL-4 indicated that more polarization of T cells to Th2. Conclusion: This approach may be a useful strategy to control MS.
Keywords: Multiple sclerosis, Denderitic cell, T cell response, MBP, Histamine

91. The Effects 1,2 Dimethyl 3,4 A-Naphtaquinon(DMNQ) on Denderitic Cells Polarization and MBP Related Autoreactive MBP Related T Cells Responses

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Background: One proper method to improvement MS treatment is skewing autoreactive T cells response from pathogenic Th1/Th17 to Th2 response. Dendritic cells play a crucial role in the conduction and polarization of T cells response. The aim of this study is evaluation of potential of DMNQ on DC maturation and polarization of autoreactive MBP related T cells. Materials and Methods: From 5 volunteer people, peripheral blood monocytes were collected and converted to Immature DC. Then, these cells were pulsed to MBP. After that, DMNQwas added in culture as DCmaturationfactor. Flowing DC immunephenotyping on day7, these cells were adjusted to autologus T cells in order to determination of T cell polarization status. Results: The usage of this compound caused increaseing DC maturation indices (D80, CD83, CD86 and HLADR) and decreasing of CD14 compared with control group. The more production of Il-10 compared with Il-12 suggested that DCs shifted to DC2. The more decreasing of pathogenic IFn-ϒ and IL-17 cytokines compared with IL-4 indicated that more polarization of T cells to Th2. Conclusion: This approach may be a useful strategy to control MS.
Keywords: Multiple sclerosis, Denderitic cell, T cell response, MBP, 1,2 Dimethyl 3,4 α-naphtaquinon (DMNQ)

92. In Vitro Immunomodulatory Effects of Sodium Benzoate on IL-17 and IL-21 Production in Mononuclear Cells of Multiple Sclerosis Patients

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Background: Sodium benzoate, a metabolite of cinnamon and a safe food additive, has been shown to protect mice from experimental allergic encephalomyelitis by induction of Th2 and Treg lymphocytes. Recently the prominent role of Th17 in the pathogenesis of multiple sclerosis has been noticed. Therefore, the present study was undertaken to investigate the in vitro effect of Sodium benzoate on IFN-γ, IL-17 and IL-21 production by peripheral blood mononuclear cells of twenty MS patients along with twenty healthy controls. Materials and Methods: After stimulation of lymphocytes by myelin basic protein or ConA as mitogen in the presence or absence of sodium benzoate, the production of IL-17 and IL-21 in supernatant was measured using ELISA technique, and the mRNA levels of these two cytokines were measured using quantitative real-time PCR. Results: Levels of IFN-γ, IL-17 and IL-21 mRNA expression were non-significantly higher in patients than controls. Adding sodium benzoate to the culture of peripheral mononuclear cells caused a reduction in both IFN-γ and IL-17 expression and protein production and an increase in IL-21 expression and production. These results were not significant. Conclusion: According to published articles, sodium benzoate deviates the immune system response through Th2 subset. Therefore a reduction in IFN-γ expression is acceptable. In addition, this substance decreased IL-17 and increased IL-21. IL-21 is produced by Th2 cells in addition to Th17 cells, therefore after deviation of immune system to Th2 activity, it is predictable to see higher amount of IL-21 in culture with SB compared to the culture that SB is absent.
Keywords: Immunomodulatory, Sodium Benzoate, IL-17, IL-21, Multiple Sclerosis Patients

93. 17-β Oestradiol Induces the Generation of Regulatory T Cells from Naïve CD_4^+ T Cells

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Background: The identification of regulatory T cells (Treg) as key regulators of immunologic processes in the control of the immune system by preventing auto reactive responses has opened an important era in the prevention and treatment of autoimmune diseases. Elevated Treg cell number during pregnancy have been found to correlate with high E2(17β-oestradiol) levels which could propose a relationship between the numbers of Tregs and E2 levels during pregnancy . The aim of this study was to evaluate the effect of female hormones, such as E2 on the generation of Tregs from naïve peripheral $CD_4^+CD_{25}^-$ T cells. Materials and Methods: Using MACS separation kit, naïve CD_4^+ CD_{25}^-T cells from healthy female Peripheral blood mononuclear cells (n=4) were isolated by negative selection. The isolated T cells were stimulated with TGF-β1 and different concentrations of E2 (3.6×10^{-9} M (preovulatory level) and 1.3×10^{-7} M (pregnancy level)) in the presence of anti-CD3 / anti-CD28 for 4 days. The induction of the Tregs was investigated through staining with antibodies against human CD4, CD25 and FOXP3 and analyzed by flow cytometry. The expression of programmed death-1 molecule (PD-1) as a marker for suppressive activity of induced Tregs was analyzed by flow cytometry. The proliferative capacity and Cytokine secretion of induced Tregs were analysed through XTT assay and ELISA, respectively.Results: After 4 days of incubation, E2 at preovulatory Phase level induced comparable frequencies of CD25 (88 % vs 80%) and FOXP3 (31% vs 35%) as TGF- β 1 induced cells. Almost all induced T cells expressed PD-1 and these cells showed no potential for proliferation in response to PHA. Our primitive results showed that E2-treated cells have more potential to release IL-10 than TGF- β1treated cells. E2 (preovulatory level) showed Comparable results as TGF- β1 treated cells,When analysing IFN-$\gamma$and TNF-α I cell supernatant. Conclusion: Our data shows the potential of 17 β -oestradiol as a good candidate for *invitro* generation of Tregs, which may contribute to developing novel therapeutic approaches.
Keywords: anti-CD3, anti-CD28, Regulatory T cells, TGF β1 and 17 β oestradiol.

94. The Effect of Induced Hyperglycemia on the Expression Level of TLR4 Gene during a Time Course Induction of Diabetes Type 1 in Mature Wistar Rats

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Background: Type 1 diabetes (T1D) is a chronic autoimmune disease which results from autoimmune destruction of insulin-producing pancreatic beta cells. Diabetic nephropathy (DN) is the most important outcome of microvascular implications in diabetes and currently the most common cause of end-stage renal disease in Western countries. Numerous studies have shown that the inflammation induced by hyperglycemia is the main mechanism of the pathogenesis of diabetic nephropathy (DN). The relationship between inflammation and diabetic nephropathy progression included the processes and complex molecular networks. There is compelling evidence that the innate immune system plays a key role in early mechanisms triggering diabetes. Toll-like receptors (TLRs) are the key receptors that activate the innate immunity following the recognition of Danger-associated molecular patterns (DAMPs) that involved in DN. Materials and Methods: In this study, the time course expression of TLR4 in the kidney cortex tissue of rats following induced hyperglycemia was studied. Hyperglycemia was induced in male wistar rats with intraperitoneal (I.P.) injection of Streptozotocin. In different time points (4, 6, 8 and 20 weeks) post diabetes type 1 induction, rats were euthanized and cortex kidney tissues was removed for further analysis. One of cortex kidney samples was fixed in Formaldehyde (10%) for histological examinations. The fixed samples were then embedded in paraffin, stained with Periodic acid Schiff (PAS), and observed under the light microscope. Histological results showed an increased level of glomerular volumes in diabetic rats as compared to the control group. RNA was isolated from another kidney cortex sample from the same rat followed by cDNA synthesization using oligo-dT primers. Exon specific TLR4 primers were used to amplify TLR4 cDNA. After performing RT-PCR, the expression level of TLR4 mRNA was quantified by real time quatitative PCR (qPCR). Results: Expression level of TLR4 as quantified by qPCR and statistically analyzed results showed a significant up-regulation of TLR4 transcripts during the time course after diabetes induction as compared to the control healthy rats. Conclusion: Increased understanding of the role of these molecules may provide insight into site-specific immunoregulatory mechanisms in the kidney cortex. Enhanced TLR4 expression might be linked to the development and maintenance of pathogenic conditions in kidney cortex.
Keywords: Hyperglycemia, TLR4, Diabetes Type 1, Wistar Rats

95. Polymorphism of FOXP3 Gene in Multiple Sclerosis

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Background: Multiple sclerosis (MS) is a demyelinating disease of central nervous system. The dysregulation of immune self-tolerance is considered to be a key element in the autoreactive immune response in MS. The *FOXP3* transcription factor is predominantly expressed by the Treg cell lineage and appears to act as a master regulator for cytokine production and cell–cell contact-dependent inhibition of T effector cell activation. Associations have been reported between *FOXP3* gene variants and some autoimmune diseases. The aim of this study was to investigate the possible association between single nucleotide polymorphisms (SNP) in the *FOXP3* gene and predisposition to MS. Materials and Methods: This study comprised 85 MS patients and 80 controls, who were genotyped for the SNP rs 3761549. DNA extraction from peripheral blood mononuclear cells was performed using DNA extraction kit. The desired fragment was amplified by thermocycler and the amplicons were verified by agarose gel electrophoresis. RFLP analysis was performed using AluI restriction enzyme and the digestion results were assessed using agarose gel electrophoresis. Results: The rs 3761549 (GG) was found in 76 (89.4%) of MS patients and in 68 (85%) of controls, AA was found in 2 (2.35%) of MS cases and in 8 (10%) of controls, AG was found in 7 (8.23%) of MS cases and in 4 (5%) of controls. Conclusion: Based on our knowledge, this is the first study to investigate the association of FOXP3 SNP of rs 3761549 with MS. Although the exact role of Foxp3 and FOXP3 gene variations in MS is still not clear, the present data support the importance of variations in the *FOXP3* gene region as an ethiologic factor of MS. This study showed that most of population in study has rs 3761549 (GG) genotype; But the significant differences were found in two other genotypes,rs3761549 (AA) and rs 3761549 (AG). The results showed the relation between rs 376154(AG) and the risk of MS.
Keywords: Multiple sclerosis, FOXp3, Polymorphism

96. Adoptive Transfer of InVitro Differentiated Th17 Cells To Induce EAE

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Backgeround: T cells, in particular $CD4^+$ helper T cells, have been implicated in many aspect of autoimmune disease. A novel subset of $CD4^+$ helper T cells, IL-17 producing subset, called Th17, which develop via cytokine signals distinct from Th1/Th2 lineage, has been recently identified and shown to play critical function in inflammation and autoimmunity. Experimental Autoimmune Encephalomyelitis (EAE), and inflammatory autoimmune disease of the CNS, is the rodent model of multiple sclerosis. Initially IFN-γ-producing Th1 cells, and recently IL-17-producing Th17 cells with specificity for myelin antigens, have been implicated in EAE induction, but whether Th17 cells are encephalitogenic has been controversial. In present study, we generated MOG specific Th17 cells in-vitro and then induced EAE by adoptive transfer of these cells to show the pathogenic role of this lineage. Materials and Methods: Naive T cells were isolated from spleen and lymph node of MOG_{35-55} immunized C57/BL6 mice. Naïve T cells were cultured in Th17 differentiating medium (contain IL-6 and TGf-β), in the presence of peptide MOG_{35-55} and Anti-CD28 antibody as stimulators, after 48h, IL-23 were also added. At day 5 or 6 polarized cells harvested and tested for expression of intracellular IL-17 and IFN-γ by flowcytometry and used for adoptive transfer. Results: After polarized differentiation process, at least 12% of cultured cells were become IL-17 positive. Mice that have received polarized T cells, developed clinical EAE. Conclusion: Th17 cells can be considered as a potential tool for inducing EAE in C57/Bl6 mice.
Keyworld: Th17, adoptive transfer, EAE

97. The Roles of CD_8^+ T Cell Subsets in Multiple Sclerosis

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Multiple sclerosis (MS) was first described by Charcot and Vulpian in 1866. MS is an autoimmune demyelinating and progressive degenerative disease of the central nervous system.This disabling disease is characterized by multi-focal demyelination, axonal loss, activation of glial cells and infiltration by immune cells. The etiology of MS is unknown, but many findings indicate a central role for the "immune system" in the disease pathogenesis, and both genes and environmental factors influence the risk of developing disease. The strongest known genetic risk-factor is the DRB1_1501–DQB1_0602 haplotype which encodes for the human leukocyte antigen (HLA)-DR2 and DQ6 molecules thus implicating HLA class II molecules and CD4 T cells in MS pathogenesis. Also arguing against a sole pathological role for CD4+ T cells in MS, selective depletion of these cells did not lead to disease amelioration .in contrast,depleting all lymphocyte populations, significantly reduced relapse rate and new lesion formation In addition CD8+ T cells typically outnumber CD4+ t cells in in acute and chronic lesions of patients with multiple sclerosis and the CD8+ T cell subsets shows more evidence of antigen-driven activation than do CD4+ T cells in the CNS and blood of patients with multiple sclerosis. However, CD8 T cell differentiation results in several subsets of effector and regulatory CD8 T cells that could be differentially implicated in the mechanisms contributing to tissue damage or by contrast ,reducing new lesion formation . Here we review evidence supporting a role for distinct CD8 T cell subsets in the pathogenesis of MS.
Key words: CD8+ T cells, CD8+ T cell subsets, multiple sclerosis, regulatory T cells

98. MICB Gene Expression on Peripheral Blood Mononuclear Cells and Susceptibility to Multiple Sclerosis in North of Iran

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Background: Multiple sclerosis (MS) is an autoimmune multi factorial degenerative disease with detrimental affliction on central nervous system. MHC class I chain-related gene A, B (MICA and MICB) are non classical human leukocyte antigens that can affect on some diseases and also on transplantation. The purpose of this study was to evaluate the MICA and MICB mRNA expression in multiple sclerosis patients. Materials and Methods: In this study, we evaluated MICA and MICB MRNA expression in the peripheral blood mononuclear cells by reverse transcriptase - polymerase chain reaction (RT-PCR) in MS patients and normal controls. Results: The results of this study showed that 32.6% of patients with progressive clinical outcome over expressed MICB genes in comparison with controls (p=0.002). Conclusion: It is concluded that the high expression of MICB gene in MS patients is an important criterion of MS disease that it may be due to the interaction between MICB and its receptor on CD_8^+T or NK cells.
Keywords:MICA, MICB, Multiple Sclerosis

99. Clinico-Pathologic Features of Systemic Lupus Erythematosus in Local Patients – A Comparison with National & Regional Studies (Pakistan, India, Iran & Bangladesh)

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Background: Systemic Lupus Erythematosus (SLE) is a clinically and serologically diverse autoimmune disease, with multiple autoantibodies found in patient serum and disease spectra ranging from subtle symptoms to life-threatening multi-organ failure. Objectives: To study the clinico-pathologic features of Systemic Lupus Erythematosus in Pakistani patients at the time of presentation and compare them with the results of similar studies conducted in Pakistan, India, Iran & Bangladesh. Material and Methods: This cross sectional observational study was conducted at

the Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi from January 2011 to June 2011. Patients with a clinical diagnosis of SLE were included in the study on fulfilling ACR classification criteria (1997). Main outcome measures were clinical and laboratory features at presentation and their comparison with results of earlier studies to identify any changes in pattern of clinical and laboratory presentation over time. Results: Seven male and fifty five female patients with an age range of 16-47 years (mean 34.5 years) and 8-65 years (mean 31.7 years) respectively were studied. Arthralgias/arthritis was the most common presentation (58%), followed by generalized weakness/fatigue (42%), fever (32%), oral ulcers (24%) and Raynaud's phenomenon (24%). Laboratory results showed positive antinuclear antibody in 97% of patients followed by anti double stranded DNA antibodies (anti dsDNA antibodies: 76%), anti extractable nuclear antigen antibodies (anti ENA antibodies: 24%) and reduced complement in 62% of patients. The percentage of patients at the time of diagnosis who presented with mild, moderate and severe disease was 20%, 62.5% and 17.5% respectively. A significantly lower percentage of Indian patients presented with haematological features compared to the results from Pakistani studies. In case of patients reported in studies from Iran and Bangladesh, a higher percentage of patients was reported with neuro-psychiatric & hematologic features at presentation. Conclusion: The most common combination of clinical features at presentation differ markedly in the studies from India, Pakistan, Iran and Bangladesh and clinical suspicion of SLE in patients from these countries should rest on the commonest clinical features as determined by the studies involving local data.

Keywords: SLE, clinic-pathologic features

100. Study of Inflammatory and Immunologic Test in Patients with Chronic Spinal Injury

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Background: chronic spinal cord injury (SCI) patients have elevated ESR and CRP due to common situations like pressure ulcers, urinary tract infections and atherosclerosis. Also ESR and CRP are elevated in rheumatologic disease like rheumatoid arthritis and spodyloarthropathies. In this study we tested ESR, CRP, RF, Anti ccp, HLA B27 and Wright in SCI patients. Materials and methods: in a cross sectional study 317 SCI patients admitted for periodic checking up in khatamalanbia hospital in Tehran in 2010-2011 history taking performed and questioned for arthralgia, pressure ulcers, urinary catheterization and ischemic heart disease. Then arthritis considered in physical examination. ESR, CRP, RF, Anti ccp, HLA B27 and Wright test examined for each patients. Renaults:309 patients were male and 8 patients were female. Age Average was 48.47 years. With range of 23 to 81 years old. 92.4 percent was paraplegic and 7.6 percent was quadriplegic. The most injury level was in thoracic spine (60.6 percent). Post injury periods range was 2 to 35 years. 87.7 percent had spinal injury 23 years ago. 18.9 percent had arthralgia, 61.5 percent urinary catheterization, 3.8 percent ischemic heart disease and 2.2 percent had arthritis. ESR was elevated in 36.8 percent, positive CRP in 44.1 percent, positive RF in 5.8 percent, positive HLA B27 in 2 percent and Anti ccp in 2 percent and positive Wright test in 0 percent. There was no correlation between arthralgia and arthritis with elevated ESR and positive CRP. There was no correlation in urinary catheterization and positive CRP but there was significant correlation between urinary catheterization and elevated ESR (P=0.00). Also there was no significant correlation between arthralgia and arthritis and positive RF, Anti ccp and HLA B27. Conclusion: some of these results were like other studies and some studies not compatible with these results. The positive RF frequency was the same as general population but HLA B27 was lower than Iranian average.

Keywords: chronic spinal cord injury, ESR, CRP, RF, Anti ccp, HLA B27,

Poster Discussion Presentation

101. Gold Nanoparticles Ameliorates Experimental Autoimmune Encephalomyelitis in C57BL/6 Mice

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Background: Multiple sclerosis is the most abundant central nervous system inflammatory disease, which is due to the reaction of auto reactive T cells with own myelin proteins. Experimental autoimmune encephalomyelitis (EAE) is used as an animal model of this disease. Nanogold particles have immunomudulatory properties. Materials and Methods: Gold nanoparticles about 13-15 nm in size were prepared by trisodium citrate reduction procedure. 32 C57BL/6 male mice were divided into 4 groups: 1-Control, 2-EAE without treatment, 3-High dose nanogold treated EAE and 4-Low dose nanogold treated EAE. EAE was induced in Groups2, 3 and 4 by subcutaneous injection of MOG and CFA and intraperitoneal injection of pertussis toxin. Mice in group3 and 4 received a daily intraperitoneal injection of 400ng/kg and 40ng/kg nanogold respectively for 10 days. Clinical and weight assessments were performed daily. On day 25 animals were sacrificed. The brain tissues were stained for histological studies. Spleen cells were analyzed using flowcytometer and Real-time PCR. Brdu assay was used for splenocyte proliferation. Results: Our results showed significant mean weight increase and Clinical score decrease in group3 and 4 comparing with group2. Histological studies revealed lower lymphocytic infiltration and demyelination in group3 and 4 compared to group2. Splenocytes proliferation showed reduction in group3 and 4 in comparison to group2. The percentages of spleen $Foxp3^+$ cell population in $CD4^+$ cells reduced in group3 and 4 compared to group2. Expression of transcription factor and cytokines related to Treg and Th2 showed increase in group3 and 4 compared to group2.
Conclusion: It seems that nanogold particles may alleviate disease condition in EAE mice through reducing inflammatory immune responses. Significant reduction of spleen Treg cells might be due to emigration of these cells to CNS for local suppression of immune responses. Moreover it seems nanogold drives the polarization of Th2 and Treg subsets.

Keywords: Gold Nanoparticles, Experimental autoimmune encephalomyelitis (EAE), C57BL/6 Mice

102. Effect of Sexual Hormones on Cytokines Production in the Pathogenesis of Systemic Lupus Erythematosus

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Background: Systemic Lupus Erythematosus (SLE) is a chronic inflammatory disease that can affect various organs. One of the most important factors that are involved in the pathogenesis of SLE is sexual hormones and cytokines disorders. Sex hormones affect the function of the mammalian immune system, and their expression is different in patients with SLE than in healthy subjects. Sex hormones play a role in the pathogenesis of autoimmunity. Evidence suggests that female hormones, particularly estrogenes and prolactin, may contribute to the pathogenesis of SLE through induction or suppression of some cytokines. This paper focuses on hormonal-related cytokine changes observed in SLE patients compared to normal subjects. Materials and Methods: The study group comprised 20 SLE patients and 20 sex and aged matched healthy subjects as control group. 10^{cc} blood was collected from each participant in EDTA. Lymphocytes isolated using Ficoll-Hypaque. Isolated lymphocytes were cultured In the presence of estrogen, DHEAS and Prolactin the for 24 hours and then RNA extracted using Trizol. Then cDNA synthesized using M-MuLV RT enzyme. Finally using specific primers and probes the expression levels of Th1, Treg and Th17 related genes; such as IFN-γ, IL-6, IL-17, Foxp3 and TGF-β were analyzed by Real-time RT- PCR based on TaqMan method. Results: Our results showed that DHEAS shifts naïve Th cells to Th1 cells rather than Th2 cells and increased cytokines related to Th1 such as IFN-γ. Also this hormone has anti inflammatory properties and down regulates IL-6 production. Prolactin and estrogen has been shown to stimulate the production of IFN- γ and to increase

production of IL-6 and IL-17. Conclusion: It seems that DHEAS may alleviate disease condition in SLE patients through reducing inflammatory immune responses, but however estrogen and prolactin shifted immune balance toward Th17 and inflammation.
Keywords: Sexual Hormones, Cytokines, Systemic Lupus Erythematosus (SLE)

103. Interleukin-17A and Interleukin-17F Serum Levels in Patients with Relapsing-Remitting Multiple Sclerosis

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Background: Multiple sclerosis (MS) is an inflammatory condition of the central nervous system, with genetic and environmental factors having a role in its etiology. The condition is characterized by demyelination, acute inflammation, and chronic and acute lesions in the central nervous system. Human and experimental studies have shown that T-helper cells and pro-inflammatory cytokines have a major role in the pathogenesis of MS. Recent researches have shown that IL-17 secreting T cells have a role in inflammation and demyelination of the central nervous system. In the present study, the role of IL-17A and IL-17F in the immunopathogenesis and follow-up of the MS disease was evaluated. Materials and Methods: Thirty-five MS patients were included in the present study. The subjects were selected from the patients referring to the Department of Neurology at Neurology Research Center, Tabriz University of Medical Sciences. Blood samples were taken from 35 MS patients and 35 healthy individuals as controls. ELISA was used to determine IL-17A and IL-17F serum levels. Results: A statistically significant increase was noted in the serum levels of IL-17A and IL-17F in MS patients compared to the controls ($P < 0.05$); however, there was no significant relationship between the serum levels of these cytokines and Expanded Standard Disability Stated Scale (EDSS) and disease Progression Index (PI). Conclusion: The results of the present study confirm the results of previous studies which have indicated an increase in expression and serum levels of IL-17A and IL-17F in MS patients compared to healthy individuals. The results of the present study might have a great role in pathophysiology, immunotherapy and follow-up of MS by indicating the role of these cytokines in the ever-increasing MS disease.
Key words: Multiple Sclerosis; Relapsing-Remitting; Th17; IL-17A; IL-17F

104. 1, 25-Dihydroxyvitamin D3 Ameliorates Experimental Autoimmune Encephalomyelitis in C57BL/6 Mice

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Background: The experimental autoimmune encephalomyelitis (EAE) is an animal model for human multiple sclerosis. Vitamin D has several reported immunomodulatory properties including the reduced generation of pro-inflammatory $CD4^+$ Th1 cells and the increase in levels of the anti-inflammatory Th2 subset. Less clear has been the impact of vitamin D on the pro-inflammatory Th17 subset, and whether and how vitamin D may preferentially drives the polarization of one of the T helper subsets. Materials and Methods: 24 C57BL/6 male mice were divided into 3 groups: 1-Control, 2-EAE without treatment and 3- 1,25-dihydroxyvitamin D3 (Vitamin D3) treated EAE. EAE was induced in Groups2 and 3 by subcutaneous injection of MOG and CFA and intraperitoneal injection of pertussis toxin. Mice in group3 received intraperitoneal injection of 40 ng/kg vitamin D3 daily for 10 days. Clinical and weight assessments were performed daily. On day 25 animals were sacrificed. The brain tissues were stained for histological studies. Spleen cells were analyzed using flowcytometer and Real-time PCR. Brdu assay was used for splenocyte proliferation. Results: Our results showed significant mean weight increase and Clinical score decrease in group3 comparing with group2. Histological studies revealed lower lymphocytic infiltration and demyelination in group3 compared to group2. Splenocytes proliferation showed significant reduction in group3 in comparison to group2. The percentages of spleen $Foxp3^+$ cell population in $CD4^+$ cells reduced in groups3 compared group2. Expression of transcription factor and cytokines related to Treg and Th2 showed significant increase in group3 in comparison to group2. Conclusion: It seems that 1,25-dihydroxyvitamin D3 may alleviate disease condition in EAE mice through reducing inflammatory immune responses. Significant reduction of spleen Treg cells might be due to emigration of these cells to CNS for local suppression of immune responses. Moreover it seems 1, 25-dihydroxyvitamin D3 drives the polarization of Th2 and Treg subsets.
Keywords: experimental autoimmune encephalomyelitis (EAE), C57BL/6 Mice, Multiple Sclerosis

105. A Study of CD4+Foxp3+Treg andCD8+Foxp3+Treg Cells in Patients with Rheumatoid Arthritis

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Background: Two subsets of effectors TCD4+ cells are categorized; T_H1 and T_H2 which differ in their cytokine profile. RA is defines as a T_H1 dominant diseases. Tregs are a rather new group of T cells that are demonstrated to adjust other immune cells including T_H1 and T_H2. Foxp3 is a lineage-determining factor for Treg cells. Several subsets of $Foxp3^+$regulatory T cells have been ever identified; $CD4^+Foxp3^+$Treg and $CD8^+Foxp3^+$Treg are the main cell population in circulation. Materials and methods:Peripheral blood mononuclear cells (PBMC)c were obtained from 31 patients with rheumatoid arthritis (RA) and 21 healthy controls. Monoclonal antibodies including anti-CD4 and anti-CD8 and anti-Foxp3 were used and the staining process was performed. Flow cytometry were applied for evaluation the markers. Results: The percentage of $CD4^+Foxp3^+$Treg cells was 1.03% ± 0.28 % in RA group and 1.25% ± 0.3% in control group (P=0.010). The percentage of $CD8^+Foxp3^+$ Treg cells in RA group was 0.79% ± 0.18 %, and 0.63% ± 0.16 % in control group (P=0.002). The WBC and Lymphocytes population in RA group were higher than control group (P=0.001). In addition the percentage of TCD4 Lymphocytes was 31.8 ± 5.6 % in the RA and 34.46 ± 3.6% in control group (P=0.064) and TCD8 was 22.97±4.1% in RA and 20.99±2.47% in control group with (P=0.054). Conclusion: These data demonstrate that altered frequency of Treg cells might be involved in the pathogenesis of RA. This may be a contributory factor in the susceptibility to RA (T_H1 dominant), or it may achieved during the progression of the disease.
Key words: Regulatory T cells, Rheumatoid Arthritis, Foxp3, CD4, CD8

Poster Presentation

106. The Measurement of Serum Ferritin Levels in Patients with Autoimmune Diseases

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Background: Ferritin, the major intracellular iron storage protein, is normally present in the serum in concentrations directly related to iron storage. The expression of ferritin is regulated at both the transcriptional and posttranscriptional levels by iron, cytokines, hormones, and oxidative stress. Ferritin also may be elevated in conditions not reflecting iron stores such as acute inflammatory diseases, infections, and malignancy. The role of ferritin in the pathogenesis of autoimmunity is under investigation. The objective of the present study is to explore the evaluation of ferritin concentrations in patients with autoimmune diseases. Materials and Methods: The study was carried out in a group of 41 patients who had autoimmune diseases. The concentration of ferritin in the serum was. Ferritin levels (gender and age adjusted) were measured in 41 serum samples by immunoturbidimetric assays. Results: Hyperferritinemia was documented in 10 of 41patients (24.3%). Also hyperferritinemia was associated with male gender. Conclusion: Ferritin plays multiple roles in the immune system. Our study showed that hyperferritinemia is common in autoimmune diseases. Further investigation into the mechanisms of ferritin in this group of diseases is warranted.
Keywords: autoimmune diseases, Hyperferritinemia, serum

107. Prevalence of Celiac Disease in Patients with Recurrent Abdominal Pain Referred to the Medical Center in Tooba, Sari

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BACKGROUND: Celiac disease (CD) is an autoimmune disease characterized by immunemediated inflammatory damage of the small intestinal mucosa, precipitated by the ingestion of gluten-containing foods.The aim of this study is to evaluate the prevalence of CD in patients with recurrent abdominal pain (RAP). Materials and METHODS: In a cross sectional study, blood samples were collected from 311 patients with RAP screened for CD, IgA anti-Tissue Transglutaminase (TTG) and anti-endomysium (EMA) antibodies that measured by enzyme linked immunosorbent assay. Diagnosis of CD was confirmed by endoscopy and duodenal biopsy that was scored according to the Marsh classification in subjects who had elevated titre of anti-TTG and EMA. Results: A total of 311 patients (114 males, 197 females) with RAP were studied. 17 subjects showed positive anti-TTG and EMA (3 males and 14 females, mean age 37.5 years). All subjects with positive serology except six of them were found to have small bowel biopsies in compatible with gluten sensitive enteropathy. One of 17 had Marsh I, 1/17 Marsh II, 3/17 showed Marsh III lesion and the other patients (6/17) had non pathologic lesion. Conclusion: The prevalence of CD in patients with RAP is estimated 1.6% (nearly 2 times more than normal population) in north of Iran. Also, the results confirmed that evaluation of CD in patients with RAP is useful in early screening.
Keywords: Celiac, Tissue Transglutaminase, endomysium, Abdominal Pain

108. Th1/Th2 Cytokines in Patients with Graves' Disease with or without Ophthalmopathy

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Background: The aim of this study was to determine the Th1 and Th2 serum cytokines, in patients with Graves' disease (GD) with or without ophthalmopathy and to compare their cytokine levels with those of normal control subjects. Materials and Methods: Serum levels of cytokines and autoantibodies including Interferon-gamma (IFN-γ), Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-10 (IL-10), thyroid stimulating hormone receptor antibody (TRAb), thyroid peroxidase antibody (TPOAb) and Thyroglobulin antibody (TgAb) were measured by enzyme linked immunosorbent assay in 34 patients with GD and in 33 normal controls. Patients *were also divided* in *two subgroups*: 18 cases with *ophtalmopathy* and 16 cases without *ophtalmopathy.* Cytokine and antibody responses were analyzed in both groups. Results: Compared with control subjects, patients with GD had elevated levels of IL-2 and IL-10 ($P<0.05$). IFN-γ level was lower in the patients than in the controls and *no*significantdifferences were foundbetween patients and controlsin terms of IL-4. There was no statistically significant difference in cytokine levels between those with or without ophtalmopathy. Conclusion: Quantitative-cytokine analysis demonstratesthat mixed Th1 and Th2 cytokines may be associatedwith the pathogenesis of disease.
Keywords: Cytokines; T cell; Graves' disease; *Ophtalmopathy*

109. The Immunoregulatory Role of sHLA-G in MS Disease

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Background: HLA-G is a non-classical HLA-class Ib molecule with multiple immunoregulatory properties. HLA-G is expressed not only as a membrane bound molecule on the surface of cells, but also as a soluble moiety in body fluids. The major isoforms of HLA-G present in serum are soluble HLA-G1 and HLA-G5 which are generated by shedding or proteolytic cleavage of the membrane bound isoform and by secretion of a soluble isoform, respectively. Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system(CNS) of autoimmune origin.We have investigated the presence of non-classical soluble HLA-G molecules(sHLA-G) in serum of Relapsing-Remitting patients of Multiple Sclerosis (MS) and the possible relationships between disability degree(EDSS) and duration of disease. Materials and Methods: sHLA-G were tested in serum from twenty five from RRMS patients, thirty healthy control by enzyme-linked immunosorbent assay (ELISA) method. Results: sHLA-I serum levels were higher than in early stage MS disease and the patients who have not treatment for 2-4 years compared with control subjects. In MS patients no statistical relationship was documented the disability degree as expressed by EDSS and sHLA_G.Moreover , there was no significant association between the duration of MS. Conclusion:ELISA results obtained that sHLA-G1 and HLA-G5 molecules can exist in the blood of healthy donors and defined the role of sHLA-G in the regulation of immune responses and in the pathogenesis, as well as the usefulness of sHLA-G as a diagnostic and prognostic biomarker in pathological conditions.
Keywords: HLA-G, Multiple sclerosis, ELISA

110. Relationship between IL-17 Levels and Ulcerative Colitis

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Backgrounds: UC (Ulcerative Colitis) is an autoimmune disease which characterized by a chronic inflammation of the intestine. Several genetic, biologic and environmental factors in the pathogenesis of this disease have been proposed so far. We investigated the role of IL-17 as one of the pro-inflammatory cytokines on onset or progression of UC. Materials and Methods: IL-17 serum level was detected in 85 UC patients and 256 controls using ELISA technique. Results: Mean serum levels of interleukin 17 in patients and controls were 22.89±57.16 (pg / ml) and 9.69±22.77 (pg / ml) respectively which showing significant difference between the cases and controls (p value: 0.003). Conclusion: This result suggests an important role of IL-17 in the pathogenesis of UC.
Keywords: Interleukin 17, Ulcerative colitis

111. Anti-Inflammatory Properties of ThymoquinoneAlleviateExperimental Autoimmune Encephalomyelitis in C57BL/6 Mice

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Background: Multiple sclerosis is the most abundant central nervous system inflammatory disease and Experimental autoimmune encephalomyelitis (EAE) is used as an animal model of this disease. Thymoquinone, a component derived from the bioactive constituent of Nigella sativa, has been investigated for its anti-oxidant and anti-inflammatory activities on both in vitro and in vivo models. Its anti-oxidant/anti-inflammatory effect has been reported in various disease models, including encephalomyelitis, diabetes, asthma and carcinogenesis. Moreover, thymoquinone could act as a free radical and superoxide radical scavenger. Materials and Methods: 24 C57BL/6 male mice were divided into 3 groups: 1-Control, 2-EAE without treatment and 3- thymoquinone treated EAE. EAE was induced in Groups2 and 3 by subcutaneous injection of MOG and CFA and intraperitoneal injection of pertussis toxin. Mice in group3 received intraperitoneal injection 10 mg/kg thymoquinone daily for 10 days. Clinical and weight assessments were performed daily. On day 25 animals were sacrificed. The brain tissues were stained for histological studies. Spleen cells were analyzed using flowcytometer and Real-time PCR. Brdu assay was used for splenocyte proliferation and Griess reaction was performed for detection of serum level of NO. Results: Our results showed significant mean weight increase and Clinical score decrease in group3 comparing with group2. Histological studies revealed lower lymphocytic infiltration and demyelination in group3

compared to group2. Splenocytes proliferation showed significant reduction in group3 in comparison to group2. The percentages of spleen $Foxp3^+$ cell population in $CD4^+$ cells reduced in groups3 compared to group2. Expression of transcription factor and cytokines related to Treg and Th2 showed significant increase in group3 in comparison to group2. Conclusion: It seems that thymoquinone may alleviate disease condition in EAE mice through reducing inflammatory immune responses. Significant reduction of spleen Treg cells might be due to emigration of these cells to CNS for local suppression of immune responses.
Keywords:Multiple sclerosis, Experimental autoimmune encephalomyelitis (EAE), Thymoquinone, C57BL/6 Mice

112. CD_4^+ CD_{25}^+ FOXP3$^+$Regulatory T Cells in Systemic Lupus Erythematosus Patients

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Background: Systemic lupus erythematosus is a chronic, systemic autoimmune disease characterized by loss of tolerance to self antigens. An increasing number of studies indicate that a subset of CD_4^+ T cells with regulatory capacity (Tregs), which constitutively express CD25, can function to control autoimmune diseases. Regulatory T cells are naturally occurring $CD_4^+CD_{25}^+$ $FOXP3^+$ T cell subsets that exhibit powerful suppressive properties. Depletion of regulatory T cells has been shown to cause autoimmune diseases in animal models.
The aim of this study was to quantify regulatory T cell subsets and the mean fluorescence index (MFI) of Tregs in the peripheral blood of SLE patients in comparison to normal controls.cMaterils and Methods: Participants in this study were 20 SLE patients and 20 age and sex matched controls. Lymphocytes were stained with CY5 labeled anti-CD4, PE labeled anti-CD25, and FITC labeled anti-FOXP3. Then flow cytometery was used to determine the CD_4+ CD_{25}+ FOXP3+ regulatory T cells and the MFI of these cells. Results: CD_4+ CD_{25}+ FOXP3+ T cells and MFI of them were decreased in SLE patients when compared to healthy controls. Conclusions: Regulatory T cells are diminished in SLE patients.
Keywords: Regulatory T cells, systemic lupus erythematosus

113. Imbalance between Effecter Th17 and Regulatory T Cells In the Pathogenesis of Nephritis in SLE Patients

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Background: Systemic Lupus Erythematosus (SLE) is an autoimmune multi system inflammatory disease that causes the deposition of immune complexes (ICs) in various organs such as Kidney. Nephritis is the inflammation of kidneys influenced by the presences of ICs at various sites of the glomeruli. Glomerular pathological findings of lupus nephritis were originally categorized in six classes from class I to VI. Among the classes of Glomerulonephritis, class IV has the highest ratio of mortality and prevalence. New investigations suggested that activation of autoimmune lymphocytes such as Th17 and defective function of regulatory T cells have important role in the pathogenesis of SLE nephritis. So we investigated the expression of cytokines and molecules related to these two types of lymphocytes in glomrulonephritis class IV SLE patients in comparison to non nephritis SLE patients and healthy subjects. Materials and Methods: The study group was comprised of 20 glomerulonephritis class IV SLE patients, 20 sex-age matched SLE patients without kidney involvement and 20 sex-age matched healthy subjects as control group. 10 ^{cc} bloods were collected from each participant. Lymphocytes isolated using Ficoll-Hypaque and immediately RNA extracted from lymphocytes using TRIZOL, then c-DNA synthesized using M-MuLV RT enzyme, finally with specific primers and probes the expression levels of Foxp3, TGF-β,IFN-γ, IL-6 and IL-17 genes were analyzed by Real time RT-PCR based on TaqMan method. Results: Our results showed that the expression levels of IL-6, IL-17, IFN-γ and Foxp3 genes in SLE patients with glomerulonephritis class IV SLE were significantly higher than SLE patients without kidney involvement group, but there was no significant difference in TGF-β expression between two groups. Conclusion: We found that the expression level of cytokines related to Th1 and Th17 cells (IFN-γ, IL-17) and inflammatory cytokine IL-6 and also Treg related gene, Foxp3, increased in glomerulonephritis class IV SLE patients.
Keywords: Th17, Regulatory T Cells, SLE

114. Quantitative Analysis of CXCR1and CXCR2 Gene Expression in Peripheral Blood Cells of Patients with Multiple Sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) that develops in genetically susceptible individuals after exposure to environmental triggers. The onset of the symptoms of multiple sclerosis is often associated with the breakdown of blood–brain barrier. CXCL8 is a prototype chemokine of the C-X-C family. There is evidence that shows the role of CXCL8 as a key player in neutrophil transmigration into the brain. CXCR1 and CXCR2 are receptors for CXCL8 and other CXC chemokines. CXCR2 is the most important ligand for chemotaxis, and CXCR1 mediates activation of neutrophils. The purpose of this study was to quantitative analysis of CXCR1 and CXCR2 peripheral white blood cells (WBCs) mRNA gene expression in Multiple sclerosis patients along with healthy controls. Materials and Methods: We measured CXCR1 and CXCR2 mRNA expression in peripheral WBCs of forty-nine relapsing-remitting MS (RRMS) patients and sixty controlsbyQuantitativeRealtimePCR. Results: There were no significant differences in expression of CXCR1 and CXCR2 mRNA in peripheral WBCs of MS patients and healthy controls (P= 0.148 and P= 0.220, respectively). CXCR1 mRNA expression in the peripheral white blood cells was positively correlated with the CXCR2 mRNA expression in patients and control group (rs= 0.327, p= 0.015 and rs= 0.860, p<0.001, respectively). Conclusion: We didn't find any significant differences in CXCR1 and CXCR2 gene expression between MS patients and healthy subjects. To clarify the role of these chemokine receptors in pathogenesis of MS, further comprehensive investigations of them as well as their chemokines ligands in larger samples is needed.
Keywords: CXCR1, CXCR2, Multiple Sclerosis

115. Study of Binding Antibodies to Therapeutic IFN-β in Multiple Sclerosis Patients: A Comparison between Three IFN-B Products, Cinnovex , Betaferon, and Rebif

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Background: Multiple sclerosis (MS) is a demyelinating disease of the central nervous system, which mainly affects young adults. Interferon-beta (IFNβ) was the first disease modifying drug to be approved for the treatment of multiple sclerosis (MS). Some MS patients treated with (IFNβ) develop antibodies to the drug. Anti-Interferon-beta antibodies can reduce both bioactivity and clinical efficacy of IFNβ. Objective: Evaluation of Binding Antibodies (BAbs)to therapeutic IFN-β in Multiple Sclerosis Patients treated with Cinnovex , Rebif , betaferon. Method: Sera were

tested from 120 patients with relapsing-remitting MS. Patients were treated with IFNβ-1b (Betaferon, n = 40), IFNβ-1a (Cinnovex, n = 40), or IFNβ-1a (Rebif, n = 40) for Over 6 months. IFNβ binding antibodies were tested by Enzyme-Linked Immunosorbent Assay (ELISA). Results: Patients were considered positive for BAb , that they had a positive sample with an optical density (OD) 1.2<. BAb was found in 30 patients treated with IFN-beta. BAb was positive for 35.7 % for Betaferon, 26.8 % for Cinnovex, and 21.95% for Rebif.
Conclusion: The three IFNb preparations have different degrees of immunogenicity and IFN-β1b molecule is more immunogenic than the IFN-β1a molecule.
Key words: Multiple Sclerosis, interferon-beta, binding antibodies, ELISA Assay.

116. Relationship of Plasma Interlukin-18 Concentration with Atherosclerosis in Patients with Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is associated with atherosclerosis as an important cause of morbidity and mortality in patients. Recent studies indicated the higher concentration of circulatory interlukin (IL)-18 in SLE patients than in healthy population. IL-18 acts as a proinflammatory and proatherogenic cytokine. Our aim was to evaluate the relationship of plasma interlukin-18 concentration with atherosclerosis in patients with SLE. Materials and Methods: 60 patients and 30 healthy controls (age and sex matched) were selected. Disease information gathered using SLEDAI form. Serum concentration of IL-18 was measured in both groups. IMT of carotid artery was determined by ultrasound for patients and controls. Results: Plasma concentration of IL-18 and IMT were significantly higher in SLE patients than age-matched healthy controls. SLE patients with IL-18 concentration in the top tertile compared with the bottom tertile had higher disease activity. No significant correlation was seen between IL-18 concentration and signs of atherosclerosis. Conclusion: In SLE patients a high IL-18 level reflects activity of the disease and is not related with vascular atherosclerosis.
Keywords: Interlukin-18, Atherosclerosis, Systemic Lupus Erythematosus (SLE)

117. The Association between Down's Syndrome and Systemic Lupus Erythematosus: A Case Report And Review Of Literature

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Background: Down's syndrome (DS) is a genetic disorder associated with trisomy of chromosome 21. There is a raised incidence of autoimmune diseases among DS patients. However it seems that association between DS and systemic lupus erythematousus (SLE) is not common. We reviewed 4 previous case reports and discussed if the immune disorders in Down's syndrome patients can predispose them to SLE as an autoimmune disease. Case presentation: A 17 year old male with Down's syndrome (DS) who had systemic lupus erythematosus(SLE) is described. His first presentation was chest pain causing by pericardial effusion. This patient fulfilled 6 of the revised criteria for the classification of SLE: malar rash, arthritis, pericarditis, leucopenia, positive ANA and positive anti ds DNA. He is on remission under treatment with prednisolon. Discussion: According to our literature review in medical sources, 4 Down's syndrome cases with SLE presentation had been reported. Characteristics of these 4 cases are compared. Considering this fact that enhanced autoantibody production and apoptosis have very important roles in pathogenesis of SLE may lead us to conclude that DS patients have some immune disorders that predispose them to develop SLE more than healthy population. To prove this claim more clinical and immunological studies are needed.
Keywords: Down's Syndrome, Systemic Lupus Erythematosus

118. The Beneficial Effects of Therapeutic Plasma Exchange on the Frequency, Proportion and Function of the Most Important Subsets of CD_4+ T Lymphocytes in the Immuno-Pathogenesis of Multiple Sclerosis: Regulatory T Cells and Th17 Cells

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Plasma exchange is used increasingly as an individual therapeutic decision in the treatment of severe, steroid resistant relapses of Multiple Sclerosis (MS). However, its mechanism of action in this CD4+T cell mediated autoimmune disease remained unknown. Clarifying the effects of therapeutic plasma exchange on Regulatory T cells as the major controllers and Th17 cells as the main promoters of MS, may help us to use this procedure as a disease modifying treatment in remission phase for reducing the rate and severity of future attacks. In this regard, we hypothesized that plasma exchange provides the immune system an exceptional break for de novo recognizing of myelin auto-antigens in a tolerogenic manner, by depleting the body of inflammatory mediators that acts as providers of co-stimulatory signals for the adaptive immune system. This may lead to an increase in the frequency and function of regulatory T cells and in contrast, a decrease in the frequency and function of Th17 cells. To investigate the reality of this hypothesis, for the first time in the world, we are going to compare the frequency, proportion and function of these cells before the first and after the last session of therapy in a group of 20 Relapsing-Remitting MS patients under the course of therapeutic plasma exchange. The plan of techniques for investigating this issues will be flowcytometric assays for the frequency and ratio of lymphocyte subsets, Real-time PCR for the assessment of the expression levels of lymphocyte subsets specific transcription factors, and co-culture inhibition assays for evaluation of the inhibitory function of regulatory T cells on the autologous responder T lymphocytes. We are now at the beginning of this study. So the results and conclusion about our hypothesis will be reported in the future.

119. The Relation between Some Biochemical Parameters by Balancing Pro-Oxidant-Antioxidant in Rheumatoid Arthritis Disease

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Background: Rheumatoid arthritis is mainly characterized with non-exclusive inflammation of local joints or joints inflammation, morning stiffness. In rheumatoid arthritis patients, increased free radicals of oxygen (ROS) act as mediators of tissue damage. This point emphasizes on the necessity of applying appropriate methods for examining tissual oxidative condition and antioxidant compounds capabilities in patients with rheumatoid arthritis. Then, we considered its relation with biochemical parameters. Materials and Methods: In surveying 100 patients with rheumatoid arthritis, the index rate of oxidant was compared in case group and control group using independent T Test. Results: Due to the fact that P Value <0. 001, we observed a meaningful difference, and the result of misbalancing oxidant-antioxidant in the group with rheumatoid arthritis favored the increase of oxidant. In examining biochemical parameters in patients with rheumatoid arthritis, urea has decreased while uric acid content has increased. By examining the relationship between biochemical parameters and oxidant-antioxidant balance we observed that there was a meaningful difference in patients with rheumatoid arthritis compared to control group in urea content which has a reverse relationship with changes of oxidant content. Conclusion: Due to the results from PAB Test as well as much less time and cost performing this test compared to the other current tests, and noting the fact that this test examines tissual oxidative conditions not only unilaterally but also bilaterally by considering the both content dimension of oxidants and antioxidants, the current test seems to be as suitable in evaluating pathogens and pre-advice of disorders accompanying oxidative damages particularly in the case of rheumatoid arthritis. Moreover, there would be the possibility of applying PAB in various diseases occurring in veterinary involving rheumatoid arthritis and osteo-arthritis in horses.
Key words: rheumatoid arthritis, Peroxidant-antioxidants balance (PAB), Biochemical factors

120. Management of Antiphospholipid Syndrome in Pregnancy

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Background: Recurrent early miscarriages, late fetal loss, and maternal thrombosis are clinical characteristic ofobstetric antiphospholipid syndrome (APS). Antiphospholipid (aPL) antibodies (i.e., lupus anticoagulants and anticardiolipin [aCL] antibodies) are laboratory criteria of this syndrome. A lot of obstetric complications such as preeclampsia, fetal growth restriction and premature delivery are seen with APS. The effect of unfractionated or low-molecular-weight heparins in combination with low-dose aspirin to prevent of recurrent obstetric complications was evaluated in this study. Materials and Methods: 80 patients with past history of obstetric complication related to APS were evaluated for pregnancy outcome after treatment. Data were collected for complication of drug uses, pregnancy and fetal outcome and analysis with SPSS soft ware. Result: Live births were improved to about 80% of patients with treatment, but failure in 20% of the cases. Abortion in 50%, preterm labor in 25% and stillbirth in 15% of cases with failure were occur. 5% of patient had postpartum hematoma related to anticoagulant agent and there was no fetal complication among patient with term delivery. Conclusion: Despite good results with this treatment we need to improve outcome of these pregnancies with changes of drugs, dosage, duration, and timing of administration.
Keywords: APS, heparin, low dose aspirin

121. $CD8^{low}$ and $CD8^{high}$ T Cells Profiling in Multiple Sclerosis Patients

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Background: Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system manifested morphologically by inflammation, demyelination , axonal loss and gliosis. The inflammatory lesions are characterized by massive infiltration by a heterogeneous population of cellular and soluble mediators of the immune system. Cumulating evidence points to a key role for CD_8+ T cells in this disabling disease. CD8 expression level was believed to be constant on the surface of human peripheral blood T cells. However, it was shown that peripheral blood lymphocytes may be divided by the level of CD8 expression, into $CD8+^{high}$ and $CD8+^{low}$ T cells. Now it is well established that the $CD8^{low}$ population of CD8+ T cells demonstrates an activated effector phenotype while the $CD8+^{high}$ T cells have been reported to have regulatory function. Materials and Methods: In this report we used a flow cytometric assay to compare the frequency of these two subsets in multiple sclerosis patients with healthy age- and gender-match controls. Thirty-one MS patients (20 female, 11 male; mean age: 38.3±8.4) with clinically definite MS, according to the McDonald's criteria (McDonald et al., 2001) (19 RRMS, 6 PPMS & 6 SPMS) were studied. All patients met our predefined inclusion criteria of not being treated with any kind of IFN-β and receiving no corticosteroid for at least 3 prior months and were at acute phase.Eighteen ethnically matched individuals (10 female, 6 male; aged: 37±7.4), who had no history of MS in their families, were used as healthy controls. All patients and controls were of Iranian Caucasian origin.PBMCs isolated from MS patients and controls were stained with anti-CD8- Samples and analyzed using a FACScalibur flow cytometer.
Results and conclusion: We found that CD8+ T cells and $CD8+^{low}$ T cells significantly increased in SP and PPMS patients ($p<0.0002$ and $p<0.004$ respectively). These results demonstrate the role of $CD8^{low}$ T cells as activated effector cells in progressive form of multiple sclerosis.
Keywords: $CD8^{low}$, $CD8^{high}$, Multiple Sclerosis

122. A Rapid and Simple Method for Purification of Myelin Basic Protein from Bovine Brain White Matter

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Backgeround: Myelin basic protein (MBP) is a basic protein which accounts for about 30% of the total protein content of the myelin in the central nervous system. MBP is thought to be involved in the pathogenesis of multiple sclerosis and other neurological disorders and induces experimental allergic encephalomyelitis (EAE) in animal models. In the present study a simple and efficient procedure has been developed which produce reasonable purified MBP from bovine brain white matter in a short time. Materials and Methods: Bovine brain white matter was hemogenized with H2SO4, the pellet was discarded by centrifugation and the pH of resulting supernatant then neutralized. Potential neutral and acidic protease activities were reduced by adding PMSF and Beta-mercaptoethanol to all reagents. The supernatant was loaded on to a cationic exchange column packed with CM-Sepharose equilibrated with sodium acetate buffer containing NaCl. MBP was eluted by linear NaCl gradiant. Eluted fractions analyzed for MBP content by SDS-PAGE. Results: In this method we purified the 18.5-kDa isoform of MBP from bovine brain white matter that is predominant in humans and cattle. The SDS-PAGE revealed that isolated MBP has considerable purity in this manner. Conclusions: The proposed method is simple and rapid for preparation of large amounts of pure MBP. The pure MBP can be used for EAE induction in rodents.
Keywords: MBP, Purification, Cation exchange chromatography, Multiple Sclerosis

123. The Prevalence and Incidence of Biopsy-Proven Lupus Nephritis in China: A Systematic Review of Chinese Literature

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Background: It is clear that environmental and genetic factors interactions result in the development of systemic lupus erythematous (SLE) or lupus nephritis (LN). Studies of the incidence of LN in China have yielded conflicting results, maybe due to China's vast territory, with a large population, and many ethnic groups. The aim of the present study was to perform a systematic literature review of studies associated with prevalence and incidence of biopsy-proven LN in China. Materials and Methods: All articles between 1975 to 2011 were searched with the key words “lupus nephritis, Chinese”, “renal biopsy, Chinese” or “systemic lupus erythematous, Chinese” in the database of Pubmed & Medline, also searched with the same key words without Chinese in the database of China National Knowledge Infrastructure (CNKI). And reviewed papers with validity criteria, Data of patients with biopsy-proven lupus nephritis were extracted and analyzed. Study selection. Standard: selected patients in mainland China; patients number ≥ 100 cases; the method of lupus nephritis (LN) defined by renal biopsy; Diagnostic criteria: The classification of patient's pathological histological type of renal disease was according to the WHO criteria of histological classification of glomerular defined in 1982 and revised in 1995. Characteristics of the studies included in the analyses. Pearson correlation coefficients were calculated to assess the linear association between the prevalence and incidence of LN and geographical distribution. Results: Our literature searches yielded 41 studies which met the study validation criteria. The 41 fulfilled the selection criteria for inclusion in the meta-analysis during the period 1979 to 2009, were selected and reviewed, included a total of 34621 patients with renal biopsy and 3736 patients with biopsy-proven lupus nephritis. Based on the forty-one studies, LN accounted for 2.37% to 25% of all biopsies and accounted for 27.2% to 80.65% of renal biospsies done for secondary causes of glomerular disease. The male-to-female ratio was about 1: 6. The mean age of SLE onset was 47.9 years (21-76). The included session mainly was from 1995 to 2009. The geographic latitude of the forty-one research center was from 45.45° north to 21.11° north and geographical longitude from 87.36°east to east 126.41°east. Study location had a significant effect on the intercept, with studies from the southern part having the higher overall mean intercept ($p=0.001$, $R=-0.561$). This indicates that, at least indirectly, environment affected the relationship between the epidemiology of LN and the geographical distribution in mainland China. Conclusions: Our meta-analysis of 3736 patients with LN showed the prevalence and incidence of LN in China to be associated with sex and graphical distribution. The overall rate of incidence of lupus nephritis to all biopsies or second glomerular diseases tends to significantly increase in response to decreasing geographic latitude (º) from the northern to southern part of China. These important clinical questions should be addressed by future prospective studies.
Keywords: Lupus Nephritis, China, Systematic Review

124. Toll-Like Receptor 9 Is Involved in the Activation of Peripheral Blood Basophiles of Patients with Systemic Lupus Erythematosus

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Background: Activated basophiles maybe play a critical role in the pathogenesis of nephritis in systemic lupus erythematosus (SLE). But what mediated the activated of basophiles in patients with SLE still not clear, especially in the in the early phase of disease flare in the absence of autoreactive IgEs. SLE as a systemic autoimmune disease, bacteria and virus may have a role in its pathogenesis. Toll-like receptor (TLR) 9 is able to activate innate immune cells in response to bacterial or viral unmethylated CpG DNA sequences, suggesting maybe the importance of TLR 9 stimulation in SLE. To asses whether or not TLR 9 is involved in the activation of peripheral blood basophiles of patients with SLE. Materials and Methods: After basophilic differentiation of KU 812 induced by IL-3, these cells were transfected with NF-κB–luciferase and β-galactosidase reporter vectors, then stimulated with 10% serum of active SLE patients (n=6), ODN M362 and ODN TTAGGG plus serum of SLE patients (n=6), respectively. Then, TLR 9 expression was assessed by flow cytometry and NF-κB activity was tested with the dual luciferase reporter assay system. Also, 15 patients had active SLE (SLEDAI score > 4) and 17 had inactive disease (SLEDAI score ≤ 4) were included and their disease activities were assessed by Disease Activity Score of SLEDAI. Twenty healthy subjects constituted the control group. TLR 9 expression on peripheral blood basophiles which identified as $Fc\varepsilon RI\alpha^{+}CD203c^{+}CD123^{+}$ cells was assessed by flow cytometry. Results: TLR 9 relative fluorescence units (RFUs) expression of KU-812 were significantly unregulated and activity of NF-κBsignificantly increased in both active and inactive SLE group, comparing to healthy controls (all $p<0.05$). And blocked with ODN TTAGGG lead to a decreased activity of NF-κB comparing to both active and inactive SLE group ($p<0.05$). Also, expression of TLR 9 on peripheral blood basophiles of patients with active and inactive were higher than healthy controls, but not significantly (all $p>0.05$). Conclusions: In this study, we provided first evidence that TLR 9 is overexpressed by peripheral blood basophiles of patients with SLE, and TLR9 is involved in the activation of basophiles, suggesting that bacterial or viral containing unmethylated CpG DNA sequences exposure could induce inflammatory responses in SLE.
Keywords: Toll-Like Receptor 9, Systemic Lupus Erythematosus

125. Serum Interleukin-23 (IL-23) and Interleukin-33 (IL-33) Concentrations in Patients with Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is a long- term autoimmune systemic disease that identified by inflammatory responses which mainly affecting joints and surrounding tissues. Although the etiology of RA is unknown, but cytokines that produced by different cells such as lymphocyte, monocyte, endothelial and epithelial cells are believed to play major roles in the induction and propagation of the inflammatory conditions. During recent years researches have been revealed that IL-23 stimulates particular T-cells to produce IL-17 which has a major role in autoimmune inflammation. Furthermore, IL-33 is the 11th and most recently discovered IL-1 cytokine family which is expressed in normal and diseased synovium but diseased tissue displays higher level of expression. A limited number of studies have focused on IL-23 and IL-33 in RA. The aim of this study was to measure the levels of IL-23 and IL-33 in the serum of patients with RA, before treatment, three month after treatment and compare to patients with osteoporosis as well as healthy volunteer controls. Materials and Methods: We measured the serum levels IL-23 and IL-33 of 30 RA patients, 15 patients with osteoporosis and 16 age and gender matched healthy controls by using ELISA assay. Results: The serum IL-23levels of the RA patients before treatment 1419.7±252.7 (pg/ml) were significantly higher than the Il-23 level three months after treatment748.1±209.7 (pg/ml) P=0.009 and the control group 634.07±204.3 (pg/ml) P= 0.007. Serum levels of IL-33 were significantly higher in patients with RA before treatment 5.47± 0.142 (pg/ml) versus three months after treatment 4.34±0.072 P=0.001, and control subjects 4.53±.076 (pg/ml) P=0.001. There were significant differences between patients with RA and osteoporosis before treatment in IL-33 levels 5.47± 0.142 versus 3.65 ± 0.08 P=0.002.Conclusion: Our results showed that IL-23 and IL-33 are highly active in RA and these cytokines might be closely connected to pathogenic mechanisms of the disease.
Keywords: Rheumatoid arthritis, Interleukin-23, Interleukin-33, Osteoporosis, Cytokines

CANCER IMMUNOLOGY

Oral Presentation

126. Expression of Interleukin-11 (IL-11) and IL-11 Receptor-α (IL-11Rα) in Human Gastric Adenocarcinoma

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Background: Gastric cancer is the second cause of cancer death worldwide including Iran with the high mortality rate of 39%. Previous studies indicated that interleukin-11 (IL-11), a member of IL-6 cytokine family, was correlated with the regulation of tumor progression, cellular growth and differentiation in several malignancies. Our objective was to clarify the role of IL-11 and IL-11Rα in gastric adenocarcinoma. Materials and Methods: In this descriptive-analytic study, 85 gastric and duodenal biopsy specimens from Imam Khomeini Hospital and Tooba Clinic of Sari were enrolled. Based on the histo-pathologic and endoscopic assessments, samples were first divided into two groups of tumoral (n=45) and non-neoplastic tissues (n=40). The non-neoplastic samples were further classified into four groups: Normal Esophago-gastro-duodenoscopy (NEGD) (20%), Gastritis (35%), Duodenal ulcer (Du) (22.5%) and Gastro-esophageal Reflux Disease (GERD) (22.5%). The grades of tumor samples were defined. Using the SYBR Green Real-time PCR method, the expression levels of IL-11 and IL-11Rα genes were measured for all study subjects. Results: Tumor samples were found to be in three different grads (grade I: 17.8%, grade II: 66.7%, and grade III: 15.5%). Both IL-11 and IL-11Rα genes were significantly up-regulated in tumoral mucosa compared to non-neoplastic mucosa (*$p<0.05$*). Enhanced IL-11 and IL-11Rα expression was shown in Du group compared to NEGD group (*$p<0.05$*), but not in gastritis lesions. In GERD, we found increased expression of IL-11Rα gene but no change in expression of IL-11 gene. No altered expression of these genes observed among different grades. Conclusion: These findings suggest that IL-11 and IL-11Rα expression may be associated with the development of gastric adenocarcinoma, but not with gastritis lesions. We identify IL-11 and IL-11Rα may not play key role in tumor differentiation.
Keywords: IL-11, IL-11Rα, Gastric Adenocarcinoma

127. Association between Inducible Nitric Oxide Synthase (iNOS) and Gastric Cancer

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Background*:* Inducible nitric oxide synthase (iNOS) plays a central role in the pathway of reactive oxygen and nitrogen species metabolism especially in the presence of *Helicobacter pylori* infection. Occurrence of C150T polymorphism in exon 16 of the iNOS gene has a critical effect on enzyme activity. This study was performed with the aim of determining whether iNOS C150T polymorphism was associated with increased susceptibility to gastric cancer. Materials and Methods: Genomic DNA was extracted from 159 patients with gastric adenocarcinoma (mean age of 62.14± 12.6) and 170 healthy controls with age mean of 58.93±14.2, who were conformed to the patients group regarding age, sex and ethnic background. Gastric cancer was diagnosed based on clinical parameters, endoscopy findings and pathology verification. Genotyping was done by PCR-RFLP method. Odds ratios and 95% confidence intervals were obtained from logistic regression models adjusted for potential confounding factors. Results: There was no significant difference in frequency of T allele between patients with gastric cancer and controls (25.5% vs 24.7%, p=0.78). In addition, the genotype frequencies of C150T polymorphism of iNOS in two groups were not differed significantly (p=0.21). However, stratification of patients in two groups, with or without *H. pylori* infection, revealed significant differences in allele and genotype

frequencies. The presence of T allele significantly increased the risk of gastric adenocarcinoma up to 2.38 times in patients infected by *H. pylori* (95% CI; 1.44-3.92, p=0.0006). Conclusions: The results of this study suggest that the iNOS-150 T allele is a potential genetic marker for susceptibility to gastric adenocarcinoma in Iranian population.
Key words: Nitric oxide, iNOS, Polymorphism, Gastric cancer, *H. pylori*

128. Inhibitory Effects of a Single Chain Antibody against Prostate Stem Cell Antigen (PSCA) on Prostate Cancer Cell Lines
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Background: Prostate stem cell antigen (PSCA) is a highly glycosylated cell surface antigen related to the Thy1/ Ly6 family of glycoproteins. Limited expression in normal tissues and overexpression in some human malignancies has offered PSCA as an ideal target for immunotherapy in PSCA expressing tumors. Single chain fragment variable (scFv) antibodies have shown great promise in the replacement of intact monoclonal antibodies in different medical areas. In the present study, we intended to evaluate the inhibitory effects of a PSCA specific scFv antibody on the proliferation of PSCA expressing cell lines. Materials and Methods: A highly diverse library of phage-displayed scFv antibodies was used to select specific scFvs against an immunodominant epitope of PSCA. The DU-145 cells (PSCA expressing cell line) and LNCaP cells (PSCA negative cell line) were treated with different concentrations of the selected scFv for 24 and 48h. The inhibitory effects of the scFv were assessed by cell proliferation (MTT) assay. Results: The results showed that after 24h treatment, all concentrations of the selected scFv (except for 100scFv/cell concentration) resulted in the significant inhibition of the proliferation of DU-145 cells (25-50%, P<0.05). It was also shown that after 48h treatment, all concentrations of the scFv could significantly inhibit the growth of DU-145 cells (25-50%, P<0.05). None of the scFv concentrations resulted in the inhibition of growth in LNCaP cells. Discussion: Our results showed that the selected scFv inhibited the growth of PSCA expressing (DU-145) cells, while no inhibitory effect was observed in LNCaP cells. The comparison of cell growth of DU-145 cells after 24h and 48h treatment showed that the scFv was more effective in the inhibition of cell growth at 500scFv/cell concentration after 24h treatment. Our results provide an evidence for the usefulness of anti-PSCA scFv for the treatment of PSCA expressing cancers including prostate cancer.
Keywords: Single Chain Antibody, PSCA, Prostate Cancer Cell Lines

129. Intracellular CTLA4 Molecule and FoxP3+ Treg Cells are Elevated in Patients with Laryngeal Squamous Cell Carcinoma (SCC)
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Background: Approaching the causes of immune suppression may provide useful clues for immunotherapy in cancer patients. CTLA4 is the most crucial intrinsic molecule for lymphocyte self regulation, and regulatory T (Treg) cells, the well known cells which provide the extrinsic inhibitory signals for lymphocytes in different physiologic circumstances. This study investigates the percentages of CD4+T, CD8+T, and CD19+B cells, expressing surface (Sur) and Intracellular (In) CTLA4 molecule, and also the prevalence of Treg cells (CD4+CD25+FoxP3+) in patients with laryngeal squamus cell carcinoma (SCC). Materials and Methods: 45 patients and 27 healthy individuals were enrolled. Flow cytometry analysis with fluorochromes-labeled antibodies, BD FACSCalibur flow cytometer, and CellQuest Pro software package was used to evaluate the samples. 1% paraformaldehyde and 1% saponin were used to fix and to permeabilize the cells before intracellular staining of CTLA4 and FoxP3. Results: Results indicated the higher percentage of SurCTLA4+CD8+ lymphocytes in the patients. The average percentages of three lymphocyte subsets with InCTLA4 expression were very higher than surface expression, and significantly higher in patients for all three subsets. A significant higher percentage of FoxP3+Treg cells was also observed in patients. Analysis revealed the association of Treg cells with the higher lymphnode-involvement (N) stage and tumor size, as well as, SurCTLA4+CD4+ lymphocytes with the highest tumor grades. Conclusion: In conclusion, these data indicate that an increase in the number of Treg cells and accumulation of CTLA4, especially in the intracellular storage compartments of main lymphocyte subsets, in patients with laryngeal SCC are among the factors that may inhibit immune system and might have roles in initiating or progression of the disease. The results suggest that laryngeal cancer is an appropriate choice for immunotherapy regimen based on CTLA4 and FoxP3+Treg cells. Keywords: CTLA4, FoxP3+ Treg Cells, SCC

130. Increase of FoxP3$^+$ Regulatory T (Treg) Cells in Patients with Epithelial Ovarian Cancer
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Background: Ovarian cancer is the fifth leading cause of death from malignancy in women and has the highest fatality to case ratio between all gynecologic malignancies. Regulatory T (Treg) cells ($CD4^+CD25^+FoxP3^+$ T cells) are a subset of T lymphocytes with great inhibitory impact on immune response. Objective: This study evaluates Treg cell percentage, among CD4+ T cells, in peripheral blood of patients with epithelial ovarian cancer in comparison with healthy donors. Subjects and Methods: Forty women suspicious to epithelial ovarian cancer (17 out of 40 finally included as the confirmed cases) and 20 healthy subjects were enrolled in the study. 6-8 cc peripheral blood was collected before operation. Cells from mononuclear ring were stained at the surface, for CD4 and CD25 molecules, following by fixation, permeabilization and intracellular staining for FoxP3. After processing and flowcytometry analysis, prevalence of Treg cells was determined as the percentages of $CD25^+FoxP3^+$ cells among $CD4^+$ lymphocytes. Results: The results indicated that Treg cell percentage is significantly higher in ovarian cancer patients than controls (5.7±3.1 versus 2.8 ±1.4, P=0.002). A trend toward higher Treg cells was observed in higher stages of ovarian cancer (III+IV) in comparison to lower stages (I+II) (6.5 ±3.18 vs 4.44±2.69, P=0.2). A near-significant association of Treg cells was also observed with high levels of tumor marker CA-125 (>100) than lower levels (≤100) (6.44±3.04 vs. 4.18±2.92, P=0.18). Conclusion: Increase of Treg cells in ovarian cancer might participate in immune suppression in these patients. The near-significant association of Treg cell increase with higher stages, as well as, higher levels of CA-125 tumor marker suggests the possible impact of Treg cell increase on the cancer progression and, consequently, patients' survival. In overall, our finding suggests the use of Treg cell–targeted immunotherapy for ovarian cancer patients.
Keywords: Regulatory T (Treg) Cells, Ovarian Cancer

131. Overexpression of the BRCA2 Pathway Gene *EMSY* in Primary Breast Cancers; a Study Using Tissue Microarray
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Background: The EMSY gene has been anticipated as a driver of the third core of the 11q13-q14 amplicon that encodes a BRCA2-binding partner protein. Its over-expression can inhibit BRCA2 functions leading to BRCA2 inactivation in non-hereditary breast and ovarian cancers. The amplification of EMSY has been associated with an increased risk of relapse as well as decreased survival in breast cancer patients.Materials and Methods: In the present study a set of samples from patients with primary operable invasive breast cancer was evaluated for the expression of EMSY protein by immunohistochemical staining of 116 breast carcinomas in a tissue microarray. The level of expression of EMSY was then associated with clinicopathological features and patients survival. The series included patients 79 years of age or less (mean = 52 years) with a long-term follow-up of 2-180 months (mean=47 months) whom diagnosed between 1996 and 2010. Results: The staining pattern of expression was either nuclear (18% of cases), cytoplasmic (35%), or both nuclear and cytoplasmic (23%), whereas 24% of tumors were considered negative for EMSY. Univariate analysis showed a positive association between EMSY expression and lymph node metastasis (p-value=0.035). The

EMSY positivity was also significantly associated with increased tumor size (p-value= 0.045). Conclusion: Amplification of EMSY inhibits activation of BRCA2 leading to impairment of DNA damage repair. Therefore, expression of EMSY protein would be related to an increased risk of Lymph Node metastasis and larger tumor size.
Keywords: BRCA2, *EMSY*, Primary Breast Cancers, Microarray

132. Multi-drug Resistant Leukemic Cells Highly Express HLA Class I Molecules, and Single-chain Fv Diabody Specific to HLA-A Overcomes Drug Resistance in These Cells

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Many tumor cells become resistant to commonly used cytotoxic drugs due to the overexpression of ATP-binding cassette (ABC) transporters. Specifically, p-glycoprotein (MDR-1) is frequently up-regulated in chemotherapy-resistant tumor cells, which is associated with poor prognosis. On the other hand, human leukocyte antigen (HLA) class I molecules are known to be significantly down-regulated in advanced tumor cells to escape from immune surveillance. However, the relationship between MDR-1 expression and HLA expression is not fully understood. Recently, we have developed a recombinant single-chain Fv diabody specific to HLA-A and demonstrated that this agent mediates cell death in HLA-overexpressing lymphoid tumor cells but not in normal cells. Here, we investigated the expression levels of HLA class I in chemo-resistant leukemic cells and evaluated the therapeutic potential of single-chain Fv diabody specific to HLA-A, C3B3-DB. Chemotherapy-resistant cells were established by sub-culturing myeloid leukemia cell line HL60 and Burkitt`s lymphoma cell line BLTH in increasing doses of vincristine (VCR), and the cells were named HL60-R and BLTH-R, respectively. MDR-1 is strongly expressed in HL60-R and BLTH-R cells both at the mRNA and protein levels, but not in the parental cells. Interestingly, expression levels of HLA class I molecules are 8 times higher in HL60-R and BLTH-R cells than in the parental cells, suggesting that MDR-1 modulates cell surface expression of HLA by its transporter function. Next, we examined the cytotoxic activity of C3B3-DB on these chemo-resistant cell lines. C3B3-DB induced apoptosis in HL60/VCR and BL-TH/VCR cells and these chemo-resistant cell lines were more sensitive to C3B3-DB than the parent cells. Combination of C3B3-DB with chemotherapeutic agents such as VCR and daunorubicin (DNR) resulted in enhanced cytotoxicity against HL60-R and BLTH-R cells. Importantly, pretreatment of these chemo-resistant cell lines with C3B3-DB reduced expression levels of MDR-1 and increased drug retention in these cells as detected by flow cytometry and confocal microscopy. Furthermore, the combination of C3B3-DB with VCR significantly blocked the cell cycle at the G2 phase compared with VCR alone. Similar results were obtained with primary acute myeloid leukemia cells from 2 patients, resulting in up-regulation of both HLA class I and MDR-1 molecules at relapse phase compared at diagnosis. These results suggest that C3B3-DB enhances cytotoxicity of chemotherapeutic agents and provides a novel approach for overcoming drug resistance in hematological malignancies.
Keywords: MDR, HLA Class I Molecules, Single-chain Fv diabody

133. Targeting Cancer Stem Cells by Gene and Immune Therapy

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In accordance to novel theories a fraction of cancer cells referred to as “Cancer Stem Cells, CSCs” act as the primary cells in charge or repopulating tumors after treatment. These cells have distinct characteristics from differentiated cancer cells, are resistant to chemotherapy and generate a series of tumor cells involved in the composition of the tumor. Indeed, CSCs, play a resembling role to the normal stem cells, but in the context of tumor development. Therefore, in theory, elimination of CSCs can result in a significant tumor regression. In this presentation, we will review a number of immunotherapy and gene therapy strategies for targeting cancer stem cells. Our team has developed an oncolytic virus capable of distinguishing CSCs on the basis of expression of CD133, a widely accepted marker for CSCs. In this proposal we explain our data about the effectiveness of this virus in causing a robust tumor regression in a number of tumor models including hepatocellular carcinoma (HCC) and colorectal cancer. We offer in-vitro and in-vivo data showing a regression in malignant capabilities of HCC and colon cancer cells upon elimination of CD133+ cells and the specificity of the virus for these cells. It is important to pay attention to the fact that this strategy, if proven to be successful in further experiments, has the potential to be beneficial for treatment of a number of malignancies including cancers of liver, pancreas, brain, ovary, lung, colorectal etc. On the other hand, from a biological standpoint, testing of this virus in-vivo answers a significant concern in the field of cancer stem cell biology, that is, the clinical relevance of CSCs to tumor therapy. Additionally, we will briefly review other cancer immunotherapy strategies targeting CSCs.
Keywords: Cancer Stem Cells, Gene Therapy, Immune Therapy

Poster Discussion Presentation

134. Characterization of Peptides Derived from Extracellular Portions of NGEP-L by Using Ab Production and their Investigations on Tissues of Prostate Cancer

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Background: Prostate cancer is one of the leading causes of cancer deaths among men. NGEP-L, is a prostate-specific gene is only expressed in normal prostate and prostate cancer. We identified for the first time, extracellular NGEP-L domains by using softwares and experimental studies and are producing Abs against the domains. We showed location of NGEP-L in tissue prostate (normal,benign prostatic hyperplasia and prostate cancer) on microarray tissues. Materials and Methods: 2 peptides (peptide 1and 2) of extracellular NGEP-L domains were designed by using softwares. The peptides were conjugated with BSA and conjugated peptides were showed with SDS-PAGE. Two female rabbits were immunized for each one peptide conjugated with BSA. The titrations of Abs were performed by ELISA. 150 paraffin-embedded prostate tissues of prostate cancer and benign prostatic hyperplasia were collected from hasheminejad hospital and we investigated location of NGEP in prostate tissues on microarray tissues by immunohistochemistry. Results: We used SDS-PAGE for showing the conjugated peptides, it was showed smear of peptide/BSA-sulfo-SMCC complex in SDS-PAGE located upper than BSA band (66 kda) and BSA-sulfo-SMCC complex. 2 female rabbits were immunized with peptide 1, after third booster had higher ($P<0.0001$) of OD (means= 1.337±0.144 &1.104±0.289) than before injection (means= 0.152±0.105, 0.134±0.084). 2 female rabbits were also immunized with peptide 2 after third booster had higher ($P<0.0001$) of OD (means= 0.77±0.363 & 0.89±0.315) than before injection (means= 0.189±0.123, 0.204±0.145) with similar titrations. We investigated the location of NGEP-L in prostate tissues on microarray tissues. We have shown the NGEP locate in apical of membrane epithelial cell prostate. Conclusion: The expression of NGEP-L in many cancer samples and its localization in the plasma membrane demonstrate that NGEP-L will be a potential target for prostate cancer immunotherapy. Therefore, Ab production against putative extracellular portions of NGEP should be done to need in the immunotherapy of prostate cancer.
Keywords: NGEP-L, Ab Production, Prostate Cancer

135. Evaluation of Dendritic Cell-Based Tumor Vaccine in Eliciting Different Types of T Cell Responses

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Background: Nowadays, dendritic cell (DC) vaccines are a promising method for immunotherapy of cancers. DCs through polarizing T cells into variety of different subsets like Th1, Th2, Th17 and Treg cells can modulate immune responses. There are many evidences that immune responses related to Th1 and also Th17 cells is effective in battle against cancer. *Listeria monocytogenes* (L.m) is an intracellular bacterium and DCs activated by L.m antigens can polarize Th1 immune response and have a potent antitumor property. In current study we evaluated the effect of repeated doses of L.m-activated DCs on the type anti-tumor immune responses. Materials and Methods: WEHI-164 cell-line were implanted subcutaneously in the right flank of Balb/C mice. Bone marrow-derived DCs were harvested after 7 days of culture in the presence of GM-CSF and IL-4. On day 5, tumor cell lysate was added to immature DCs and after 4-6 hours Lipopolysaccharide (LPS), L.m lysate, CpG-ODN were added. One, two or three doses of matured DCs were injected around tumors at 7, 10 and 13 days after tumor implantation, respectively. Estimation of cytotoxic activity of splenocytes, tumor growth rate and survival rate were done. Also, T-bet, GATA-3, RORγt and FoxP3 gene expression were analyzed in tumor area, draining lymph nodes and spleen. Results: Our results showed that administration of three doses of L.m-matured DCs led to a significant increase in cytolytic activity of splenocytes and increased expression of T-bet and RORγt in compare to one dose. But expression of GATA-3 and FoxP3 decreased after three doses of vaccine. Also, we showed that tumor growth rate significantly decreased via increasing doses of DCs vaccine. Conclusion: We concluded that repeated doses of L.m-matured DCs may lead to induce Th1 and Th17 cells as anti-tumor immune responses and reduce Th2 and Treg cells that ultimately enhance immune responses against cancer.
Keywords: Dendritic Cell, anti-tumor immune responses, *Listeria monocytogenes*

136. Differential Effects of Vitamin A on TLR9-Induced Proliferation of Normal and Leukemic B-Cells from CLL Patients

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Background: VitaminAandits metaboliteshavevariousbiologicalfunctionsin regulation ofproliferation, differentiationandsurvival of immune cells. It inhibitstheproliferation of normalBcellsstimulatedby theBCR/CD38 orCD40/interleukin-4 (IL-4), but augments theproliferation ofB cellswhicharestimulatedby the TLR9 agonistCpG. Leukemic B cells from patients with chronic lymphocytic leukemia (CLL) are inhibited by vitamin A through downregulation of Bcl-2 expression. However no data is available on the effect of vitamin A on leukemic CLL B-cells following stimulation with CpG. Materials and Methods: In this study, the proliferative effect of All-trans retinoic acid (ATRA) in combination with CpG was investigated in B-cells isolated from 24 CLL patients and 8 normal subjects by 3H Thymidine incorporation assay. B-cells were enriched from peripheral blood mononuclear cells by magnetic beads activated cell sorter (MACS) and immunophenotyped by flow cytometry. Induction of apoptosis was investigated by Annexin V binding assay. Results: Our results showed while ATRA enhanced the proliferative effect of CpG in B-cells from normal subjects, it displayed the opposite effect in B-cells from CLL patients (p=0.001). The inhibitory effect of ATRA was more pronounced in progressive (n=6) compared to non-progressive (n=18) patients and also in immunoglobulin variable region (IGHV) unmutated (n=8) compared to IGHV mutated (n=16) patients, though the differences did not reach statistical significance due to the low sample size. The inhibitory effect of ATRA was not due to the induction of apoptosis in leukemic cells. Conclusion: Our findings indicate differential effects of ATRA in leukemic CLL cells and normal B-cells and imply its potential therapeutic implication in CLL patients.
Keywords: Vitamin A, TLR9, CLL Patients

137. CD200 Expression Level on Chronic Lymphocytic Leukemia B Cells Correlates with foxp3 + Regulatory T Cells Frequency in These Patients

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Background: CD200 plays a key role in regulation of the immune system and has been shown to be up-regulated in different tumors including chronic lymphocytic leukemia (CLL). Despite some investigations of the CD200 expression in various tumors, little is known about its correlation with regulatory T cells. In current study, CD200 expression level was investigated in Iranian patients with CLL in comparison to normal B cells and its correlation was studied with foxp3+ regulatory T cells level in these patients. Material and Methods: CD200 expression level was examined on peripheral blood leukemic B cells obtained from 21 CLL patients and peripheral blood B cells isolated from 8 age matched normal subjects by Flow cytometry. This technique was also used to determine frequency of foxp3+ regulatory T cells in the same CLL patients. Results: Our results demonstrated significant up-regulation of CD200 in B-CLL in all patients compared to normal B cells (p=0.006). Also CD200 mean florescence intensity (MFI) showed a significant over-expression in progressive (n=8) versus indolent (n=13) clinically subtypes (p= 0.012) of CLL patients. Moreover, we demonstrated that CD200 expression level is highly correlated with frequency of foxp3+ regulatory T cells (r=0.7, p=0.007) of CLL patients. Conclusions: Our results indicate up-regulation of CD200 in CLL suggesting involvement of this molecule in low immune responsiveness in these patients and probably its association with disease progression.
Keywords: CD200, Chronic lymphocytic leukemia, Regulatory T cell

138. Synergistic Effect of Combined Radiation Therapy and Dendritic Cell Vaccine inMurine Fibrosercoma Tumor

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Background: Although radiation therapy has been proven to bevery efficient in local control of a wide variety of cancers, it has serious limitations. Immunotherapy offers one of the most promising new strategies for tumor treatment. Neither radiation alone nor dendritic cells (DCs) inoculation can successfully control the tumor growth. Radiation induces tumor cell apoptosis, resulting in the release of tumor antigens and danger signals, which are favorable for DC capturing antigens. Hence, the strategy of combined irradiation and activated DC vaccine may be a novel approach for treatment of solid tumor. Materials and Methods: Tumor cells (WEHI-164 a Balb/c derived fibrosarcoma cell line) were injected subcutaneously to Balb/c mice. Bone marrow cells were cultured with GM-CSF and IL-4 for 5 days. Listeria monocytogenes antigens and tumor lysate were added to DC culture for another 2 days. 7 days after tumor challenge localized single does (10Gy) irradiation was applied. Activated DCs were injected SC around the tumor the day after irradiatrion. Tumor size was monitored every other day.Anti-tumor response was evaluated by intra Cytoplasmic INFγ staining of splenic lymphocyte using flow cytometery and cytolytic activity using LDH cytotoxicity assay. Results: Combined therapy causedsignificant tumore size reduction, increased intra cytoplasmic INFγ and enhanced cytotoxicity in immunized animal, as compared to irradiation alone; especially when activated Listeria monocytogenes DCs was used. Conclusion: In the present study combination therapy using activated Listeria monocytogenes DCs and local irradiation, induced noticeable INFγ production, efficient antitumor cytotoxicity against poorly immunogenic tumor as well as tumor regression. Combination therapy and can help to minimize radiation adverse effects. Therefore co-administration of local irradiation and activated DCs may be a promising strategy for inducing a potent response to radio

sensitive tumors.
Keywords: Radiation Therapy, Dendritic Cell Vaccine, Murine Fibrosercoma Tumor

139. Anti Sortilin Antibody Induces Apoptosis in Ovarian Cancer Cell Lines

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Background: Ovarian cancer is one of the most lethal gynecologic malignancies. Developing novel and more effective anticancer drugs against it must be sought. Sortilin (NP002950.3) is a multifunctional protein capable to bind different ligands and is located in different part of cells such as plasma membrane. Global gene expression profiling of ovarian carcinoma tissues has shown a nearly four-fold increase of Sortilingene expression in compare to normal ovary at transcript level. We hypothesized targeting of over expressed Sortilin may cause apoptosis in ovarian cancer cells. Materials and Methods: Tissue samples including seven ovarian carcinomas and five normal ovaries were obtained from Imam Khomeini Hospital. Western blot was used to study the overexpression of Sortilin at protein level. Three novel monoclonal antibodies against extracellular domain of Sortilin were produced. Reactivity of antibodies against Sortilin, was assessed using flow cytometry. The cytotoxicity assay performed using *in vitro* incubation of antibodies with ovarian cancer cells considering annexin-V FITC binding. Results: Western blot analyses showed the overexpression of Sortilin in ovarian cancer tissues with low expression of Sortilin in normal ovaries. Flow cytometry analysis showed specific binding of antibodies to Sortilin expressed on the surface of ovarian cancer cells. *In vitro* incubation of ovarian cancer cells with anti-Sortilin antibodies induced apoptosis in a time- and dose-dependent manner. Conclusions: In conclusion,induction of apoptosis in ovarian cancer cells by anti-Sortilin antibodies may represent Sortilin as a survival factor in ovarian cancer. Thus, Sortilin might be considered as suitable target in therapy of this cancer.
Keywords: Ovarian carcinoma, Sortilin, Monoclonal antibody, Apoptosis

140. High Gene Expression of CXCL8 Is Associated With the Bleeding and High Prothrombin Time and High Partial Thromboplastin Time in Patients with Acute Myeloid Leukemia

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Background: Patients with Acute myeloid leukemia (AML) are at risk of bleeding. The risk factors for different severities of bleeding are poorly studied. 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 aim of this study is to evaluate gene expression of CXCL8 and its association with clinical features in peripheral blood mononuclear cells of patients with AML. Materials and Methods: We investigated the mRNA expression of CXCL8 peripheral blood mononuclear cells of twenty seven patients with newly diagnosed AML by Quantitative Real time PCR. Furthermore, for each patient evaluated clinical features such as FAB subtypes, peripheral white blood cells (WBC) count, blast count, hemoglobin value, platelet count, symptoms of AML, prothrombin time (PT) and partial thromboplastin time (PTT). Results: Expression of CXCL8 mRNA in AML patients with bleeding increased more than 10- fold compared to other symptoms of AML(P=0.003). As well as, there was a significant positive correlation of CXCL8 expression with prothrombin time (rs= 0.452, P= 0.030). Expression of CXCL8 in AML patients with high PTT or high PT increased more than 5- fold compared to AML patients with low PTT or low PT (P=0.04 and P=0.019 respectively). Conclusion: Our data showed that, increment of CXCL8 expression associated with bleeding symptom and high PTT or high PT in AML patients. IL-8 contributes to the CD44-induced differentiation of acute monoblastic leukemia and elevated in the thrombocytopenic patients with AML. This AML subtype is associated with coagulation abnormalities and bad prognosis. Our results seem to support the notion that IL-8 may be important in AML pathogenesis and clinical outcome.
Keywords: CXCL8, Bleeding, Prothrombin, Thromboplastin, Acute Myeloid Leukemia

141. CXCR1 and CXCR2 mRNA Expression Negatively Correlate with CD34 Positivity in Peripheral Blood Mononuclear Cells of Patients with Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) is considered to be clonal disorder involving early hematopoietic progenitor cells. Cellular expression of CD34 plays a relevant role in acute leukemia, and the CD34 represents a heterogeneous group of leukemias characterized by varying clinical, genetic and biological features. CXCR1 and CXCR2 are two-cell surface G protein–coupled receptors for CXC chemokines, which are involved in a wide range of biological processes with relevance for hematologic malignancies, including cell trafficking, angiogenesis, cell growth control and immunomodulation. The aim of this study is to investigate the association of CXCR1 and CXCR2 gene expression and immunophenotypein peripheral blood mononuclear cells (PBMCs) of patients with AML. Materials and Methods: We evaluated the mRNA expression of CXCR1 and CXCR2 by Quantitative Real time PCR and flow cytometric expression of antigens CD2, CD3, CD4, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD38, CD45, HLA-DR, TdT and Glycophorin A in peripheral blood mononuclear cells of thirty-one patients with newly diagnosed AML. Results: We found the negative correlation of CXCR1 and CXCR2 expression with CD34 positivity (rs= -0.462, P= 0.030 and rs= -0.542, P=0.006, respectively). The CD34 positive cell subset showed decreased expression of CXCR1 and CXCR2 receptors (P= 0.049 and P= 0.016, respectively). Conclusion: It seems that expression of CXCR1 and CXCR2 more down-regulated in immature myeloblasts and CD34 positive cell subsets are more sensitive to down-regulation of these receptors. Down-regulation of CXCR1 and CXCR2 may be important in cell growth control and AML pathogenesis.
Keywords: CXCR1, CXCR2, CD34, Acute Myeloid Leukemia

142. Expression Profile of Th1 and Inflammatory Cytokines in Adipose-Derived Stem Cells (ASCs) of Breast Cancer Patients

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Background: Adipose derived stem cells (ASCs) are important for their presence in tumor microenvironment, adjacent to the tumor cells. They are known with tumor promoting effects through production of different cytokines and proangiogenic factors. Herein, we investigated the expression of TNF-α, IL-6, IFN-γ, IL-12 and IL-18 gene transcripts in ASCs of twenty one women with breast cancer. Results were analyzed comparatively in ASCs isolated from healthy women. Materials and Methods: ASCs were isolated from fragments of breast adipose tissue after mincing and incubating with collagenase. Expression of extracted mRNAs was determined using quantitative real-time PCR (qRT-PCR). Results: Relative Quantitation (RQ) of IL-12, TNF-α and IFN-γ were more in control group compared to patients. IL-12 was expressed 6.7-fold and TNF-α 11.6-fold more in healthy individuals. These differences were not statistically significant except for the expression of TNF-α mRNA between patients and controls (P value = 0.03). In contrast, IL-18 and IL-6 were expressed 1.5- and 1.8-fold more in patients than controls, respectively (P value > 0.05). Conclusion: Data of this study conclude that presence of resident ASCs in breast tissue may change anti-tumor immune responses through the expression of different types of cytokines.

Keywords: Th1, ASCs, Breast Cancer Patients

143. CD137 Costimulatory Signal Induce the Expression of Activatory Receptor of NK Cells after Exposure to Target Cell

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Background: CD137 (4-1BB) is a costimulatory molecule expressed on a varieties of activated immune cells, including T cells and NK cells but the extent of its contribution to NK-cell activity is not clear. Here we investigated the effect of CD137-CD137L interaction on the expression of stimulatory and inhibitory receptors of activated NK cells. Materials and Methods: The MCF-7 breast cancer cells were infected by recombinant non-replicative adenovirus vectors expressing 4-1BBL transgene or GFP (as control) in advance. Peripheral blood human NK cells were negatively purified, activated with sub-optimal concentrations of IL-2 and IL-15 and cocultured with the infected MCF-7 cells. The expressions of activation markers, NKG2D, NCR2, CD69 and also NKG2A inhibitory receptor were assessed afterward. Results: There were 2-3 times more NK cells with upregulated expression of both activatory and inhibitory receptors after activation with the cytokines in the presence of tumor cells compared to the cytokines only. Provision of CD137 signal by the infected MCF-7 increased the expression of NKG2D from 35% to 42%, NCR2 from 23% to 39%, and CD69 from 50% to 59%; however, it did not affect the expression of NKG2A. Conclusion: CD137 costimulation differently governs the expression of activatory and inhibitory receptors of NK cells. It seems it primarily enhances the expression of activatory markers rather than downregulate the inhibitory receptors like NKG2A.
Keywords: CD137, NK Cells, MCF-7 breast cancer

144. Investigating the Effect of 4-1BBL and CD80 Co-stimulation on T Cell Activation against Breast Cancer Cells

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Background: Boosting the immune response against triple negative breast cancers is one of the goals of immunotherapy approaches as conventional therapies mostly failed to eradicate these cancers. Harnessing costimulatory effects of B7-1 (CD80) and 4-1BB ligand (CD137L) could better trigger lymphocytes to kill many types of tumors and control the expansion of regulatory T cells. Therefore, in this study we investigated the effect of simultaneous costimulation of CD80 and 4-1BBL on lymphocytes' activation and proliferation after facing with different breast cancer cell lines. Materials and Methods: MCF7 and MDA-MB-468 breast cancer cells were transduced with recombinant adenoviruses encoding 4-1BBL and CD80. After 48 hours of infection, cancer cells overexpressing the transgenes were co-cultured with CFSE laballed PBMCs. Lymphocytes' proliferation and intracellualar IFN-ɣ expression in proliferating T cell subsets were examined 3 and 5 days post culture. Results: Both cancer cells were found susceptible to adenoviral infection and more than 90% of the cells expressed CD80 and/or CD137L. Further to the enhanced lymphocyte growth, flow cytometry analysis showed more IFN-ɣ+ CD4 and CD8 lymphocytes in the cultures with cancer cells expressing both costimulatory ligands. In fact, the percentage of IFN-ɣ+ T cells augmented from 2.46% to 3.23% and from 2.61% to 4.45% after co-cultured with MCF7 and MDA-MB-468, respectively. CD4+IFN-ɣ+ and CD8+IFN-ɣ+ populations grew 0.36% and 0.78 % in cultures with MCF7 while in the co-culture with MDA-MB-468, CD4+IFN-ɣ+ and CD8+IFN-ɣ+ populations grew 0.71% and 1.45%, respectively. Conclusion: This data showed the advantages of CD80 and 4-1BBL co-stimulation to improve T cell function against triple negative breast cancer cells similar to some other tumors and suggests the possible widespread efficacy of the "costimulation enhancement immunotherapy" in cancer.
Keywords: 4-1BBL, CD80, Breast Cancer Cells

145. Significance of Immune Regulatory Function of Adipose Derive Stem Cells Isolated From Ovarian Cancer

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Background: Adipose derived stem cells (ASCs) are important for their presence in tumor microenvironment. Many reports have showed the immunoregulatory effects of MSCs and their role in down regulating antitumor immune responses but the roles of ASCs isolated from adipose tissue of omentum in ovarian cancer have not been studied yet. Materials and Methods: We isolated adipose derived stem cells (ASCs) from adipose tissue of omentum using collagenase to investigate the expression of SDF-1, CXCR4, IP-10, CXCR3, RANTES, CCR5, MCP-1, IL-10 and IL-4 by quantitative Real Time PCR (qRT-PCR) and flow cytometry methods. IL-10 concentration in culture supernatant of ASCs and patients' serum was detected by ELISA. Results: Our results showed that SDF-1, CXCR3 and IL-4 had lower mRNA expression and IP-10, IL-10 and CXCR4 had more mRNA expression in ASCs that isolated from ovarian cancer patients compared to ovarian benign cyst samples (P value> 0.05). Comparison analysis by flow cytometry showed that RANTES, CCR5, MCP-1, IP-10 and CXCR4 expressed more in ASCs of patients with ovarian cancer than those with benign cysts. These differences were not statistically significant except for CCR5 and IP-10 (P value< 0.05). Also, IL-10 concentration in culture supernatant of ASCs and serum of ovarian cancer patients was higher than samples from patients with benign cyst (P value > 0.05). Conclusion: These data show that ASCs in adipose tissue of omentum may have crucial roles in ovarian cancer through expression of different cytokines such as IP-10, IL-10, RANTES, MCP-1 and CXCR4.
Keywords: ASCs, Ovarian Cancer, Real Time PCR, flow cytometry

146. Expression of FXYD3 and TNF-α in tissues and lymph nodes of patients with Laryngeal Squamous Cell Carcinoma

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Background: FXYD3 is a member of the FXYD family proteins which are tissue-specific regulatory subunits of the Na,K-ATPase. It has been shown that FXYD3 expression changes in cancerous tissues. TNF-α, an inflammatory cytokine, has been shown to have both anti-tumor and tumor-promoting effects and its expression level alters in cancer.It has been demonstrated that FXYD3 has reverse correlation with the expression of TNF-α. Materials and Methods: mRNA expression of FXYD3 and TNF-α gene transcripts in Laryngeal Squamous Cell Carcinoma (LSCC) tissue samples and lymph nodes of patients with different cancer stages were detected by real-time quantitative RT-PCR. Expression of FXYD3 and TNF-α gene transcripts were compared between different clinicopathological characteristics such as tumor size, stage, differentiation, metastasis, lymph node involvement, and prelaryngeal involvement. Besides, the correlation between the expression levels of two genes was also evaluated.Results: There was a significant relation between the expression level of TNF-α and different extents of tumor differentiation but not other indicated factors. TNF-α expression level in poorly differentiated tumors was significantly lower than both moderately and highly differentiated samples (P= 0.039 and 0.045 respectively). No significant association was found in FXYD3 expression and the factors assessed. Data on lymph nodes showed no significant association between FXYD3 and TNF-α mRNA expression levels and the factors assessed. Conclusion: It seems that laryngeal squamous cell carcinoma has no correlation with the mRNA expression of both FXYD3 and TNF-α. Reaching a definite conclusion requires further investigation on a larger number of patients.
Keywords: FXYD3, TNF-α, Laryngeal Squamous Cell Carcinoma

147. Disseminated Infection with *Aspergillus Fumigatus* Enhanced Tumor Growth and Severity in Tumor Bearing Mice Model

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Background: Invasive aspergillosis increases in chronic immunosuppressive diseases such as cancer. There is little information about the mechanisms by which *Aspergillus* infection affects the immune regulation and microenvironment of cancer cells. Hence, this study was aimed at investigating the effect of invasive aspergillosis on immunosurveillance, metastasis, and prognosis of cancer in tumor-bearing mice. Materials and Methods: After implantation of mouse mammary tumor in BALB/c mice, they were infected with *Aspergillus* conidia intravenously. For comparison, groups of mice were experimentally infected with *Aspergillus* conidia or implanted with tumor cells separately. Seven days after *Aspergillus* infection, the serum levels of tissue inhibitor of metalloproteinase-1 (TIMP-1) were measured by ELISA, and subsequently regulatory T lymphocytes were analyzed by flow cytometry. The survival of animals and mean tumor size were then determined. Results: Our results indicated that tumor sizes in mice increased significantly after infection with *Aspergillus* conidia. Moreover, invasive aspergillosis enhanced the population of regulatory lymphocytes and level of TIMP-1. Conclusion: This study supports the idea that massive *Aspergillus* infection could stimulate tumor growth and increases the possibility of a bad prognosis. As a result, treatment of *Aspergillus* infection could be considered an important issue for efficient cancer therapy.
Keywords: Invasive aspergillosis, Tumor, TIMP-1, Regulatoary T cells

148. IL-17A Is Elevated in Sera of Patients with Ovarian Papillary Serous Cystadenocarcinoma

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Background: Ovarian cancer is the fifth leading cause of cancer deaths among women and most of the times it does not result in symptoms until the cancer has spread extensively. Currently there is no definitive screening test for early ovarian cancer diagnosis. Papillary serous cystadenocarcinomas are the most common form of malignant ovarian cancer making up 26 percent of ovarian tumors in women. The 5 years relative survival rate for this type of ovarian cancer is around 21%. Previous studies have shown that IL-17 mRNA is expressed in a large group of ovarian cancers. In this study, we aimed to determine the significance of IL-17A in sera of patients with ovarian papillary serous cystadenocarcinoma as a possible diagnostic tool. Materials and Methods: The concentration of IL-17A was measured in sera of 26 patients with ovarian papillary serous cystadenocarcinomas and was compared with that of 60 normal healthy age-matched women by ELISA assay. Results: Fifteen (58%) out of 26 patients showed elevation of IL-17A in their sera while none of the normal healthy women showed IL-17 elevation. The mean concentration of IL-17A among patients was found to be 1.25 ± 2.25 pg/ml. Interestingly, the mean level of IL-17A among poorly differentiated tumors was 3.33 ± 2.36 pg/ml and was significantly higher than that of moderately (0 pg/ml) and well differentiated (0.14 ± 0.38 pg/ml) tumors (p=0.002). There was also an increase in the IL-17A among patients whose fallopian tubes were involved (2.19 ± 3.32 pg/ml) compared to that of patients with fallopian tubes free of tumor (0.19 ± 0.51 pg/ml) but due to the low number of data points the difference did not reach statistical significance. There was no difference in the IL-17A concentration regarding vascular and lymphatic involvement and stage of the tumors. Conclusion: Our study is the first report on the elevation of IL-17A in sera of ovarian papillary serous cystadenocarcinoma patients and in general in ovarian cancer. Considering the high percentage of IL-17A positive patients and the absence of IL-17A in sera of normal healthy age-matched women this cytokine can be a candidate for early diagnosis of this type ovarian tumor.
Keywords: IL-17A, Sera, Ovarian Papillary Serous Cystadenocarcinoma

149. CXCR4 is Expressed at Low Level on Atrophic Lesions and its Expression is Increased Along with Progression to Metaplasia and Adenocarcinoma

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Background: Stromal derived factor-1 (SDF-1 or CXCL12), a member of the alpha chemokines (CXC) and the ligand for the CXCR4 receptor, has been shown in the past to be an effective chemoattractant for various CXCR4-expressing cells. SDF-1 is secreted by stromal and endothelial cells in bone marrow, lung, skeletal muscle, liver, kidney and brain. It is therefore important for metastasis of cancer cells to these organs. Recent studies have shown that CXCR4 plays an important role in cancer development and tumor growth, apoptosis inhibition, angiogenesis promotion, cellular proliferation, invasion and cancer metastasis in many cancers. Materials and Methods: Herein, we studied the expression of CXCR4 on gastric samples from patients with precancerous lesions (atrophy, metaplasia and dysplasia) and gastric adenocarcinoma as well as human gastric carcinoma epithelial cell line, AGS, by employing RT-PCR, immunehistochemistry (IHC) and Fluorescence Activated Cell Sorting (FACS) techniques. Results: RT-PCR data show that CXCR4 is highly expressed on AGS cells. This was confirmed by IHC and FACS as CXCR4 is detected in cell membrane and cytoplasm of AGS cell line. More importantly, we found that CXCR4 is strongly expressed on primary gastric cancer cells from patients, but not on normal gastric cells from normal individuals (as detected by IHC staining and RT-PCR).Furthermore, our data show that CXCR4 is expressed at low level on atrophic lesions and its expression is enhanced along progression to metaplasia and adenocarcinoma.
Conclusion: we present evidence that CXCR4 is expressed on gastric carcinoma and thus CXCR4 may be a suitable marker for diagnosis of gastric cancer. In addition, we demonstrate for the first time that CXCR4 expression is enhanced as premalignant lesions progress to malignant tumors, indicating that targeting CXCR4 could be a new approach for treatment of gastric cancer.
Keywords: CXCR4, Atrophic Lesions, Metaplasia, Adenocarcinoma

150. Different Fractions of Listeria Monocytogenes Proteins Have Distinguishable Potential to Activate Dendritic Cells

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Background: Dendritic cell (DC)-based immunotherapy strategies appear promising as an approach to successfully induce an antitumor immune response. There are still different factors such as production of well-matured dendritic cells in vitro which should be optimized. Dendritic cells maturation occurs when they expose to external stimulators such as pathogen associated molecular patterns. Our Previous studies demonstrated that listeria monocytogenes, particularly its protein components,transform immature dendritic cells into immunogenic cells which increase anti tumor responses. In this study we fractionated protein components of listeria monocytogenes to obtain a set of proteins which has the most potential for stimulating dendritic cells maturation. Materials and Methods: Listeria monocytogenes was sonicated and was exposed to stepwise precipitation by increasing amounts of ammonium sulfate to obtain four distinct fractions. Bowne marrow derived dendritic cells (BMDC) were prepared from *BALB/C* mice and were exposed to fractions. After 24 hours, Denritic cell maturation markers such as CD86, CD80, MHC and IL-12p70 production were evaluated. Results: Bone marrow derived dendritic cells matured by different fractions prepared by ammonium sulfate showed distinguishable potentials to induce expression of CD86, CD80, MHC and IL-12p70 production by dendritic cells. Conclusion: We conclude that some protein fractions induce DCs maturation much better than others. This set of proteins can be fractionated even more to determine the most immunogenic proteins. Finding such good maturation ligands will improve dendritic cell-based immunotherapy. Keywords: Listeria Monocytogenes Proteins, Dendritic Cells, *BALB/C*

151. Correlation between Tumor Progression and Gene Expression of Different Subpopulation of T Lymphocytes in an Experimental Model of Murine Melanoma

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Background: The tumor microenvironment includes a complex network of different cell types including Tumor cells, Monocytes, Dendritic cells, fibroblastes and subpopulations of T-lymphocytes. The purpose of this study was to explore whether there is a linkage between the infiltration of different subsets of T cells and tumor progression in an experimental model of murine melanoma. Materials and Methods: After induction of tumors in mice, tumors and draining lymph nodes were extracts at different times during tumor progression. A panel of immune related genes was analyzed in 10 tumor and lymph node specimens. We quantified functional clusters of genes associated with Th1 (T bet, IFN gama) and Treg (FOXP3. IL10) using Real-time PCR. Results: Real-time PCR analysis of gene expression in tumor microenvironment revealed decreased expression of Th1 related genes, suggesting reduced in immune system activity during tumor progression. Conclusions: Along with the decrease of effective immune cells and increase of suppressor cells, a pro tumoral microenvironment is performed, which contributed to the tumor progression. Novel therapeutic strategies that emerge are designed to change the pro-tumor microenvironment to one favoring acute responses and potent anti-tumor activity.
Keyword: Gene expression, Real-time PCR, Tumor micro-environment.

152. The Evaluation of T-Cell Subpopulations in Breast Cancer Tissues

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Background: Breast cancer is the most common cancer in women. Cancer progression is a complex process involving host–tumor interactions through multiple molecular and cellular factors of the tumor microenvironment. The tumor microenvironment includes a complex network of immune T-cell subpopulations (Th1, Th17, Treg, Th2). In this study, we analyzed different subsets of helper T cells in human breast cancers. Materials and Methods: mRNA was extracted from 10 samples of frozen breast cancer tissues and 10 samples of normal frozen breast tissues, by RNeasy mini kit (QIAGEN). Then cDNA was synthesized by QuantiTect Rev. Transcription Kit (QIAGEN). The expression of specific transcription factor T-bet (Th1), GATA3 (Th2), RORC (Th17) and FOXP3 (Treg) was measured by Real-time PCR. β-actin housekeeping gene was used to normalized the expression of genes. Results: The primary results show that the expression of FOXP3, GATA3 transcription factors is up-regulated in breast cancer tissues. In contrast, the expression of T-bet and RORC is down-regulated. Conclusions: Based on achieved data, evaluation of T cells subpopulations along with tumor progression is suggested.
Keywords: Breast cancer, Tumor microenvironment, Th1, Th2, Th17, Treg

Poster Presentation

153. Lack of Association between CCR5-Δ32 Mutation and Acute Lymphoblastic Leukemia: A Study from South-Eastern of Iran

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Background: Chemokines play crucial roles in pathogenesis of Acute Lymphoblastic Leukemia (ALL) through their corresponded receptors. It has been demonstrated that the popular mutation in CCR5 gene (CCR5-Δ32) lead to mal-expression of the receptor and affect its function. The aim of this study was to determine the rate of CCR5-Δ32 mutation among Iranian ALL patients. Materials and methods: In this study, blood samples were obtained from 50 ALL patients and 100 healthy controls. CCR5-Δ32 mutation was evaluated using Gap-PCR technique. Results: Our results showed that only 1 out of 50 and 1 out of 100 of ALL patients and healthy controls, respectively, had hetrozigotic form of CCR5-Δ32 mutation, while other evaluated ALL patients and controls had the wild form of gene. Conclusion: According to these findings, it can be concluded that CCR5-Δ32 are not associated with ALL in Iranian patients.
Keywords: CCR5-Δ32 mutation, Acute Lymphoblastic Leukemia, Chemokine

154. TGF-B1 Mediated Apoptosis Is Associated to SMAD Dependent in Burkitt Cell

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TGF-b1 can elicit various cellular responses including inhibition of cell growth, migration, differentiation and apoptosis. In addition, TGF-β1 is able to induce apoptosis in certain lymphomas. In the present study the role of Smads, Bax, Bcl-xl and Bcl_2 was characterized in Burkitt´s B-lymphoma cell line. Apoptosis was detected after exposure of TGF-β on Raji cell lines and evaluated by flow cytometry using Annexin V, RT–PCR, and Western blot analysis. FACS analysis showed that apoptosis could be observed after 24 h ofTGF-β trearment and was continued after 48 h. TGF-β down regulated the BCL-xl and Bcl-2 while the Bax was upregulated. Furthermore, mRNA of Smads 6 and 7 showed the significant upregulation. The results indicated that alteration in gene expression and protein level may determine the induction of apoptosis pathway in this lymphoma cell line exposed to TGF-β.
Keywords: TGF-B1, Apoptosis, SMAD, Burkitt Cell

155. Evaluation of Lymphocyte Subsets and their β2AR+ Percent in Peripheral Blood And Tumor-Infiltrating Lymphocytes of Patients with Colorectal Cancer

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Background: Activation of β_2-adrenergic receptors (AR) on CD4(+) T lymphocytes has been shown to inhibit Th1-cytokine production and cell proliferation. Tumor infiltration by IFN-gamma producing Th1 cells promote tumor control, and associate with an improved prognosis for patients with cancers. This study has aimed to determine the β2AR+ percent in peripheral blood cells (PBMCs) and tumor-infiltrating lymphocytes (TILs) of patients with colorectal cancer. Materials and Methods: In this study, patient's PBMCs were obtained just before surgery and TILs were isolated from fresh colorectal cancer (CRC) tissue. PBMCs from healthy volunteers were also evaluated as a control group. Flow cytometry was performed for evaluation of the different lymphocyte subsets and the surface β2AR expression in the three groups: Healthy controls' PBMCs, patients' PBMCs and patients' TILs. The concentration of cytokines in the cultured cells supernatants were also measured by ELISA. Results: $CD4^+IFN\text{-}\gamma^+$ lymphocytes (assumed Th1) were significantly lower in patients PBMCs and TILs than in controls PBMCs (P<0.001 &P<0.001 respectively). There is no difference between groups for $CD4^+IL\text{-}4^+$ lymphocytes (assumed Th2). $CD4^+IL\text{-}17^+$ lymphocytes (assumed $\beta2AR^+$ Th17) were significantly higher in controls PBMCs than those in patients PBMCs and TILs (P<0.001&P<0.005 respectively). β2AR+ percent of Th1 cells were significantly higher in patient's PBMCs and TILs than in controls PBMCs, but no significant difference were seen for β2AR+ percent of Th2 cells. Conclusion: In this study, we demonstrated the higher percentage of β2AR+ Th1 cells in patient's PBMCs and TILs. We also demonstrated that there is no difference between groups for β2AR+ Th2 cells. Collectively, these results suggest that different

percent of β2AR Th1 and Th2 cells in cancer patients and controls, providing a potential way for altering the anti-tumor immunity in CRC patients.
Keywords: Beta-2 Adrenergic Receptor, Tumor-infiltrating Lymphocytes, Colorectal Cancer, Th1, Th2

156. The Anti-Cancer Activities of Some Synthetic Benzochromene Compounds against Seven Human Cancer Cell Lines

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Background: Cancer is a disease characterized by the uncontrolled growth of abnormal cells that forming malignant tumors, and invade to other parts of the body. Cell division is a complex process that is normally tightly regulated. Normal cells are in balanced cycle; but the tumor cells growth is uncontrollable. The *tumor begins* to *develop* when a *cell experiences* a *mutation* that *makes* the *cell more likely* to *divide* than it *normally would.* Chemotherapy is the major treatment of cancer. Usually, they induce apoptosis in tumor cells by activating the caspase or inhibition the cell proliferation by inhibiting the protein kinase. Materials and Methods: The cytotoxic activities of a series of benzochromene derivatives against seven cancer cell lines including Nasopharyngeal epidermoid carcinoma (KB), Neuroblastoma (SK-N-MC), Hepatocellular carcinoma (Hep-G2), Prostate cancer (PC3), Breast cancer (MCF-7, MDA-MB-231 and T47-D) were determined by standard MTT assay. Also, we used etoposide as a positive control and different concentration of our compounds to determine the IC50 for each cell line. Results and conclusion: All of compounds showed *significant cytotoxic* activity with IC50 values in low micromolar range (4.6 - 21.5 μM/ml). Anti cancer effects of these compounds in tumor cells indicated that they can be good candidate for further pharmacological studies to discover effective chemotherapeutic for the treatment of human cancer diseases.
Keywords: Anti-Cancer Activities, Benzochromene Compounds, Cancer Cell Lines

157. The Effect of CD137 Costimulation on Human Natural Killer Cells

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Background: CD137 is well known for its costimulatory effect on T cells. Like T cells, NK cells also express CD137 molecule, however, the costimulatory function of this molecule in NK cell activation is still unclear. In this study, we investigated the effect of CD137-CD137L interaction on activation and functionality of human NK cell sub-populations. Materials and Methods: MCF-7 breast cancer cells were infected in advance by non-replicative adenovirus vectors to overexpress 4-1BBL transgene or GFP. Negatively purified peripheral blood NK cells were activated with suboptimal concentrations of IL-2 and IL-15 and cocultured with the infected tumor cells. The cytotoxic function of the NK cells was examined by CD107a mobilization assay, Granzyme-B staining and was visualized on CFSE labeled K562 target cells. Results: CD137 signal at the time of cytokine-mediated activation of NK cells decreased the frequency of $CD107a^{+}GrB^{+}$ NK cells. Although the activation resulted in the downregulation of CD56 on NK cells in all conditions, this effect was augmented in the presence of CD137L. There was an increase in the frequency of $CD107a^{+}GrB^{+}$ cells in the $CD16^{+}CD56^{-}$ subpopulation while decreased in all other subsets. Despites this increase, the percentage of $CFSE^{+}/PI^{+}$ killed target cells decreased from 81.4% to 21.3% when NK cells were costimulated by CD137L expressing MCF7 cells. Conclusion: CD137-CD137L interaction may affect the activity of sub-populations of NK cells differently. This interaction negatively affects the cytotoxic function of some NK cells against K562 and could produce similar effects after exposure to other tumor target cells.
Keywords: CD137, Natural Killer Cells, MCF-7 breast cancer

158. Increased Frequency of $CD8^{+}$ and $CD4^{+}$ Regulatory T cells in Chronic Lymphocytic Leukemia: Association with Disease Progression

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Background: Little is known regarding the frequency and function of regulatory T (Treg) cells in hematopoietic malignancies, particularly in chronic lymphocytic leukemia (CLL). Materials and Methods: The frequencies of $CD4^{+}$ and $CD8^{+}$ Treg cells were analyzed in 40 CLL patients and 15 normal subjects by flow cytometry. Suppressive function of CD4+ Treg cells on the proliferation of leukemic and normal B cells and also on the effector T cells was analyzed by H3-thymidine incorporation proliferation assay. Results: Our results showed that the frequencies of $CD8^{+}CD25^{+}FoxP3^{+}$ and $CD8^{+}FoxP3^{+}$ cells were significantly increased in progressive ($4.3 \pm 0.44\%$ and $7.67 \pm 0.65\%$ of total $CD8^{+}$ T cells) as compared to indolent patients ($1.65 \pm 0.26\%$ and $4.6 \pm 0.81\%$, $p<0.001$ and $p=0.006$) and normal subjects ($0.42 \pm 0.09\%$ and $0.97 \pm 0.22\%$, $p<0.001$ and $p<0.001$), respectively. The frequency of $CD4^{+}CD25^{+}FoxP3^{+}$ cells was also significantly higher in progressive ($11 \pm 0.8\%$ of total $CD4^{+}$ T cells) compared to indolent patients ($5.75 \pm 0.7\%$, $p< 0.001$) and normal subjects ($1.53 \pm 0.36\%$, $p< 0.001$). Enriched $CD4^{+}$ Treg cells induced a similar level of inhibition on polyclonally activated B cells and effector T cells from CLL patients and normal subjects. No association was found between Treg frequency and IGHV mutation or CD38 and ZAP70 expression. Conclusion: Our results suggest that the increase in circulating Treg cells results in down-regulation of the tumor specific immune response leading to tumor expansion and disease progression.
Keywords: Regulatory T cells, chronic lymphocytic leukemia, IGHV mutation, CD38, ZAP70, disease progression

159. Effect of 1, 3-Unsubstituted 1H-Pyrazolo [3,4-b] Quinoline on Cell Cycle And Death of k562 Erythroleukemia Cell Line

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Background: cancer is the most popular agent of mortality in human being, after cardiovascular system disease and accident, so there is great threat on research of cancer in the diagnostic, treatment and prophylactic points of view. In the present study, the apoptotic and necrotic effects of 1,3-unsubstituted 1H-pyrazolo[3,4-b] quinoline compounds on k562 erythroleukemia cell line were studied. Material and Methods: in the first step the effective dose range was determined by MTT test, and then 100,200 and 300μM of compounds were used to determined necrotic as well as apoptotic death of cells, using commercial available Annexin V/PI kit and flow cytometry. Cell cycle analysis was carried out by PI and flow cytometry as well. Results: the results of MTT test showed that the effective dose range of these compound is 25-400μM, the highest cell death was happened in 300μM concentration after 48 hours incubation(83-89%),In comparation to doxorubicin(as standard), these compound killed cells for necrosis rather than apoptosis pathway. The 8-methyl substituted had the highest killing effect among four tested materials. Cell cycle analysis showed these compound cease the cells in GO/G1 phases of cell diviation. Conclusion: we found that these suslituted compound of pyrazoloquinoline had variable but significant effect on killing of k562 erythroleukemia cell line and can be rocced to in vivo studies as well as clinical trials.
Keyword: 1,3-Unsubstituted 1H-Pyrazolo[3,4-b]Quinoline, k562, Apoptosis, Necrosis, cell cycle

160. Variant Toll-like Receptor4 (Asp299Gly and Thr399Ile alleles) and Toll-like Receptor2 (Arg753Gln and Arg677Trp alleles) in Colorectal Cancer

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Background: The innate immune system recognizes the presence of bacterial products through the expression of a family of membrane receptors known as Toll-like receptors (TLRs). Polymorphisms in *TLRs* have been shown to be associated with increased susceptibility to diseases such as inflammatory bowel disease. The aim of this study was to determine whether there was a correlation between polymorphisms of *TLR4* (Asp299Gly; Thr399Ile) and *TLR2* (Arg677Trp; Arg753Gln) gene and risk of colorectal cancer. Materials and Methods:DNA from 60 colorectal carcinoma patients from 3 major races in Malaysia (22 Malays, 20 Chinese and 18 Indians) and blood from 50 apparently healthy individuals were evaluated. Control group were matched to study group by race and age. The polymorphisms were determined by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Results: Genotyping results showed two out of sixty tumors specimens (3.3%) harbored both variant *TLR4* Asp299Gly and Thr399Ile alleles. In contrast, DNA from 50 apparently healthy individuals harbored wild type *TLR4*. In the case of *TLR2* Arg753Gln genotyping, all of the fifty normal and 60 tumors were of the wild type genotype. *TLR2* Arg677Trp genotyping showed a heterozygous pattern in all samples. However, this may not be a true polymorphism of the TLR2 gene as it is likely due to a variation of a duplicated (pseudogene) region. There was only a low incidence (2/60; 3.3%) of *TLR4* polymorphism at the Asp299Gly and Thr399Ile alleles in colorectal cancer patients. All normal and tumor samples harbored the wild type *TLR2* Arg753 allele. Conclusion: Our study suggests that variant *TLR4* (Asp299Gly and Thr399Ile alleles) as well as *TLR2* (Arg753Gln allele) are not associated with risk of colorectal cancer.
Keywords:colorectal cancer, Polymorphisms, Toll-like receptors

161. Mutational Analysis of EGFR Gene by PCR-SSCP

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Background: Many molecules affect cancer process, epidermal growth factor receptor (EGFR) is a transmembrane receptor that contributes many processes involved in cell survival, proliferation and inhibits apoptosis that lead to cancer development.Gastric cancer is one of the most common cancers of digestive system that has low 5-years survival. The aim of this research is study of EGFR tyrosine kinase domain gene polymorphism in gastric cancer. Materials and Methods: In this experimental study, 83 patients with gastric cancer and 40 normal subjects were investigated for EGFR gene polymorphisms. Genomic DNA was extracted by DNA extraction kit according to manufacture s protocol. PCR-SSCP was performed for investigating of exon 20 & 21 of EGFRgene polymorphism, After that DNA sequencing was done for different migrate band , finally the data were statistically analyzed using chi-2 test and SAS (version 9.1) program. Results: SSCP pattern of Exon 21 of EGFR gene didn't show different migrate band neither in patients nor in normal subjects and DNA sequencing confirmed it. Exon 20 of EGFR gene showed multi different migrate bands in SSCP pattern, DNA seguencing showed that 2 mutations in this exon: one mutation was 2587 T>A that changes codon TGC> AGC and cause amino acid change Cys>ser, another mutation was 2607 G>A, a silent mutation with any amino acid change. Conclusion: It seems that screening of EGFR tyrosine kinas gene polymorphism between patients with gastric cancer and also TKi therapy could useful in prevention of disease development and improve of treatment process that lead to better quality of life in this patients.
Keywords: Epidermal growth factor receptor, Gastric cancer, gene mutation

162. Evaluation of Human Leptin Expression in a Liposarcoma Cell Line By Anti Leptin Monoclonal Antibody

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Background: Leptin as an important regulator of food intake and energy expenditure, initially was thought to be expressed exclusively in and secreted by adipocytes. Leptin is a product of the LEP gene which produces a 16 kD non-glycosylated polypeptide of 146 amino acids. Materials and Methods: In the present study, a monoclonal antibody against recombinant human leptin was produced and utilized for detection of leptin on SW872 human liposarcoma cell line. Expression of leptin was studied by Western blot, immunocytochemistry and flow cytometry. Results: The monoclonal antibody was shown to be reactive with leptin in Western blot. It also reacted with protein in its native form in immunocytochemistry as well as in flow cytometry on SW872 cell line. Conclusion: The antibody is a valuable reagent for many experimental systems assessing leptin expression in different cell types and tissues.
Keywords: Leptin, Liposarcoma Cell Line, Anti Leptin Monoclonal Antibody

163. Regulatory B Cell and Cancer

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Cancer escape is an active process that regulates immune responses by employing at least 2 types of suppressive cells, myeloid suppressive cells (MSC) and T-regulatory cells (Treg). In spite of ability to produce Abs, B cells possess additional immune functions, including the production of cytokines and the ability to function as a secondary APC. The B cell population contains functionally distinct subsets capable of performing both pathogenic and regulatory functions. Phenotypical studies have revealed a specific B-cell subset, termed regulatory B (Breg), which are actively generated from normal B cells in response to the direct effects of cancer-produced factors and characterized by CD19+ expression and IL-10+ ; with different groups using various combinations of surface markers, such as IgM, IgD, CD1d, CD5, CD21, CD23, CD24, CD43, and CD93, to describe such cells. Although the production of IL-10 is considered to be involved in the regulatory activity of Breg cells, the exact mechanisms of action of Breg-cell suppression of inflammation remains unknown. Recently, have reported the regulatory role of B cells in antitumor immunity. Cancer cells have been found to directly activate resting B cells to form suppressive regulatory B cells (tumor-evoked Bregs = tBregs) by secreted factors such as TGF-ß and promote the generation tBregs and utilize them to evade immune surveillance and mediate metastasis. tBregs directly inhibit CD4+ and CD8+ T cell activity in a cell contact-dependent manner, induce FoxP3+ T cell activity, and promote Treg-dependent metastasis. Hence, tBregs need to be controlled to efficiently combat cancers, as in the absence of tBregs, cancer cannot metastasize into other organs due to a poor Treg conversion.
Keywords: Regulatory, B Cell, Cancer

164. Molecular profiling and clinical approaches of human breast cancers

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Malignant tumors are resulted from a complicated interactions between environmental elements and human genome. Breast cancer is the most common malignancy among western women with over 220,000 women newly diagnosed breast cancers in 2010. Approximately 45,000 of these breast cancer patients have died. Over twenty different genetic abnormalities have been identified to be associated with human breast cancers. BRCA-1/BRCA-2 and P53 which are known as the tumor suppressor genes consisted of 2000 different genetic mutations which have been previously documented in the scientific literatures. BRCA-1 gene is located on chromosome 17q21 with 100,000 base pairs length which produce a 220 kilo-daltons protein. BRCA-2 gene is located on chromosome 13q12 with 70,000 base pairs length which also produces a 380 kilo-daltons

protein. P53 gene is located on chromosome 17p13.1 with 53000 kilo-daltons protein. All these three genes are considered as tumor suppressor genes with DNA repairing capacities as well as gene transcriptional activities. In addition to these tumor suppressor genes, other mutated genes (i.e. PTEN, STK11, AR, ATM, NOEY2, ERB2/neu, P73, BARD1, RUD50, and Cowden gene) have been discovered to be involved with the formation of breast and ovarian cancers. The new discipline of proteomics technology initiated to complement the physical genomic research to further uncover the biological functions of over 400,000 proteins. The application of microarray technology has enabled us to analyze over 1000 gene expression and proteomics at any given experiment. Thus, protein profiling of any tumor tissue can assist us to understand the protein concentration, conformation and its interactions with other molecules. Using integrated technologies (genomics, proteomics and bioinformatics) to show multiple expressed biological factors (i.e. SNPs, gains or losses, genetic rearrangements, genetic mutations, epigenetic, micro-RNAs) which are associated with disease progression and drug resistance to conventional therapies. One of the most promising areas in breast cancer research is to immuno- radio-chemo-targeting the breast cancer cells without damaging the normal cells. However, the breast tumor heterogeneity has been a challenge to pinpoint the biological basis of this dreadful tumor at the molecular and cellular level. Oncotype diagnostic test can examine specific breast tumor genes behavior in response to designated therapy. In personalized breast cancer management, staging, grading, hormone receptors and chemotherapy play a vital role in final effective treatment. Present chemotherapy only target tumors which are triple negative early stage breast cancers (negative for estrogen, progestrone and Her-2). Pharmacogenetics can also provide a personalized therapy based on the individual genetic constitution with genetic variability that can increase efficacy and minimize adverse effects.

Keywords: Molecular profiling, clinical approaches, breast cancers

165. Foxp3+Treg inCD4 and CD8 T Cell Subsets in Breast Cancer Patients

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Background: Functional immune cells and their cytokine production could be involved in progression of many malignancies. Two subsets of effector TCD4+ cells are categorized; T_H1 and T_H2 which differ in their cytokine profile. T_H1/T_H2 cell balance could shift toward T_H2-type responsiveness in many malignancies. Tregs are a new group of T cells which are indicated to adjust other immune cells including T helper cells. Foxp3 is a lineage-determining transcription factor for Treg cells. Several subsets of $Foxp3^+$regulatory T cells have been identified; $CD4^+Foxp3^+$Treg and $CD8^+Foxp3^+$Treg are the main cell population in circulation, which are the subject for evaluation in this study. Material and methods:Peripheral blood mononuclear cells (PBMC) were obtained from 48 patients with breast cancer (BC) and 21 healthy controls. Monoclonal antibodies including anti-CD4, anti-CD8 and anti-Foxp3 were used and specific staining process was performed. Flow cytometry were applied for evaluation and assessment the markers. Results: The percentage of $CD4^+Foxp3^+$Treg cells was 1.75% ± 0.74% in BC group and 1.25% ± 0.3% in control group (P=0.004). In addition, $CD8^+Foxp3^+$ Treg cells were 0.71% ± 0.17 % in BC group and 0.63% ± 0.16 % in control group (P=0.08). The means of WBC or lymphocyte population in BC group were lower than the control group but it does not reach statistically significant (P>0.05). Conclusion: These data demonstrate that altered frequency of Treg cells might be involved in the prognosis of BC. This may be a contributory factor for the susceptibility to breast cancer and could indicate targeting for evaluation or a novel therapeutic approach in this disease.

Keywords: Breast cancer, Regulatory T cells, Foxp3, CD4, CD8

166. Investigation of Serum Levels of IL-17, TGF-β and IL-6 in Peripheral Blood of Breast Cancer Patients: Implication for a Decreased Response of CD4+IL-17+ T Cells in Early Stages of Breast Cancer

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Background: Despite the important role of Th-17 cells in the pathogenesis of many autoimmune diseases, their prevalence and the mechanisms by which they are generated and regulated in cancer remain unclear. They are the third subset of $CD4^+$ T-helper cells recently been described that express IL-17. In addition to $CD4^+$ T-helper cells, IL-17 is expressed by $CD8^+$ T cells, NK-T cells, γδ T cells, and neutrophils under certain conditions because there are few studies examining IL-17 in cancer, We therefore examined IL-17 expression in human breast cancer and bladder cancer. The aim of this study is investigation of IL-17, TGF-B and IL-6 serum levels in peripheral blood of patients with breast cancer. Materials and Methods: For this reason, peripheral blood was collected from patients with breast cancer case (50 patients) and normal control male and female (50 health people) as control from hospitals of Shiraz University of Medical Sciences. Then serums were isolated and assessed for IL-6, TGF-β and IL-17 using by ELISA (ebiosciences kit). Results: As a result, analysis of cytokine production profiles revealed that IL-17, IL-6 and TGF-β serum levels in patients with breast cancer were lower than control females. Moreover it was not shown any significant difference between noted cytokines expression among patients with different stages and grades. Conclusion: As most of the cases studied in this investigation were in stage I and II, it is concluded that tumor cells by influencing on immune cells can decrease pro-inflammatory responses of CD4+IL-17 T cells and increase anti-inflammatory responses by TGF-β that may progress disease to advanced stages.

Keywords: Breast Cancer, IL-6, TGF-β and IL-17

167. In Vitro Cytotoxicity Study of Some 6-Chloro-2H-Chromene Derivatives on Five Human Cancer Cell Lines

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Background: Cancer is a disease of worldwide importance and its incidence is rising. According to information from the World Health Organization (WHO), more than eleven million people are diagnosed with cancer and, also, more than 13% of overall deaths, are directly caused by cancer every year. Chromene-based compounds have been reported to possess many pharmacological activities, including antibacterial properties and apoptosis inducing. A series of novel 6-chloro-2H-chromene derivatives (1–14) have been synthesized in our laboratory. All the synthesized compounds were evaluated for their in vitro cytotoxicity activities. Materials and Methods: The synthesized compounds (1-14) were tested against a panel of five human tumor cell lines including MCF-7 (breast cancer), MDA-MB-231 (breast cancer), SK-N-MC (neuroblastoma), KB (nasopharyngeal epidermoid carcinoma), Hep-G2 (liver carcinoma). The percentage of growth inhibitory activity was evaluated using the MTT colorimetric assay in comparison with etoposide a well-known anticancer drug. Results: Rapid glance to the obtained results revealed most synthetic compounds possessed potent activity (IC50 < 100 µg/ml) against cell lines. Five compounds showed significant activitywith *IC50* values ranging from 2.47 to 14.39 µg/ml against all tested cell lines. Conclusion: These finding indicate 6-chloro-2H-chromen have high therapeutic potential and should be investigated further.

Keywords: Cytotoxicity, 6-Chloro-2H-Chromene Derivatives, Human Cancer Cell Lines

168. Anticancer Activity of Some Benzylidene Cyclohexanone Derivatives

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Background: Cancers present a serious clinical problem and pose significant social and economic impacts on the healthcare system. Despite improved imaging and molecular diagnostic techniques, the disease still impacts millions of patients worldwide. Therefore, finding of new therapeutic agents for neoplastic diseases is one of the top subjects in this area of research. In this study the benzylidene cyclohexanone derivatives were prepared and examined for their cytotoxicity activity towards several cancer cell lines. Materials and

Methods:The cytotoxic activities of synthesized compounds (A1-11) were evaluated by MTT reduction assay in four different human cancer cell lines including SK-N-MC (human neuroblastoma), HEP-G2 (Human hepatocellular liver carcinoma), MCF-7 and MDA-MB-232 (human breast cancer). The percentage inhibition of viability for each concentration of compound was calculated compared to the control wells and IC_{50} values (concentration of the compound that induces 50% inhibition of cell viability) were calculated by linear regression analysis, expressed in Mean±SD. Results: Generally, the IC50 values of the test compounds A1-11 indicate that all compounds exhibit high activity against human tumor cell lines (IC50<50 µg/ml). In addition, the growth inhibitory activity of most of target compounds in all cell lines is higher than etoposide as a reference drug. Conclusion: Most of the newly synthesized compounds showed more activity than standard drug (etoposide) and can be considered for further structural optimization and development as potential anticancer agents for the treatment of cancer.
Keywords: Benzylidene Cyclohexanone, human cancer cell lines

169. Anti-Cancer Activities of a Series of Chromen Derivatives

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Background:Cancer is a class of diseases characterized by out-of-control cell growth. It has been known that defects in the apoptosis pathways and the ability to evade cell death is one of the hallmarks of cancers, which results in uncontrollable tumor cell growth, as well as tumor resistance to chemotherapeutic agents. Design, synthesis and evaluation of anticancer agents continues to be a major area of activity, because, despite the progress made in chemotherapy of cancer, complete control of malignancies is still a distinct dream. Materials and Methods*:* The growth inhibitory activities of synthesizedhexahydrobenzo chromen-4-one were determined against three cancer cell lines: Breast cancer (MCF-7 and MDA-MB-231 and T47-D) using an *in vitro* cell culture system (MTT assay).To assess the effects of our compounds on the process of apoptosis, we use a double staining with ethidium bromide and acridine orange by fluorescence microscopy. Results and conclusions*:* The cytotoxicity data of tested compounds demonstrate that these compounds had varying degree of toxicity. Analyses of the compounds treated (MCF-7) and (MDA-MB-231) and (T47-D) cells by acridine orange/ethidium bromide double staining showed that the synthetic compounds induces apoptosis in the cells in a time dependent manner.
Keywords: Anti-Cancer Activity, Chromen Derivatives, Breast cancer

170. Concomitant Increase of P-35 and EBI3, Expression with Other Treg Marker in Peripheral Blood Mononuclear Cells of Patients with Different Stages of Breast Cancer

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Background: Today cancer is one of the biggest problem in worldwide which threats human health and its incidence is rising in developed countries. There is a great need to identify prognostic and diagnostic cancer biomarkers which can be applied for vaccine and drug development. Many reports indicated high prevalence of Tregulatory population in peripheral blood of cancer patients. It has been reported that FOXP3+ Treg exert suppression by cell contact-dependent mechanisms and mechanisms mediated by soluble factors such as immunosuppressive cytokines IL-10,TGF-β,IL-35. In this study, IL-10, TGF-β, IL-35, FOXP3 mRNA gene expression are compared in two groups: peripheral blood of breast cancer patients and peripheral blood of normal women as normal healthy controls. Materials and Methods: IL-10, TGF-β, p-35, EBI3, FOXP3 mRNA expression were assessed in peripheral blood of 40 breast cancer patients, 40 normal sex/age matched control, by quantitative real-time PCR (Q-PCR) procedure using Master Mix reaction containing SYBER Green and GAPDH housekeeping gene. Results: Data of this study indicates up-regulation of IL-10, TGF-β, p-35, EBI3 and FOXP3 gene expression in peripheral blood of patients compared to normal healthy controls that was significantly different (p 0.000 for FOXP3, EBI3, IL-10 and p 0.001 and TGF-β and p 0.035 for P35). Conclusions: With regard to up-regulation of IL-10, TGF-β, p-35, EBI3, FOXP3 gene expression in peripheral blood of patients. It can be concluded that immune system is suppressed in the patients, which may belong to elevated of Treg population. These results may useful for diagnostic or therapeutic approach which requires more investigations.
Keyword: Regulatory T cells, IL-10, TGF-β, IL-35, FOXP3, Breast cancer, Real-time PCR, Gene expression

171. Elevated IL-17A Levels in Early Stages of Bladder Cancer Regardless of Smoking Status

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Background: Bladder cancer is one of the five most common cancers among men in Iran. Th17 cells increase in tumoral tissue of bladder cancer; however, nicotine can down-modulate the Th17 cells. Therefore, we investigated the interrelation between nicotine and IL-17A in bladder cancer. Materials and Methods: We recruited 98 smoker, 81 non-smoker patients, 19 patients who smoked water pipe, 30 healthy smokers and 31 healthy non-smokers. The control groups were age/sex matched with the patients. The blood was collected and sera were stored at -20˚C until used. Clinical and pathological data along with demographic characteristics of the patients were recorded at the time of sampling. The level of IL-17A in serum was measured using a commercial ELISA assay. Results: IL-17A levels were elevated in smoker and non-smoker patients compared to smoker and non-smoker controls (p=0.004 and p=0.004, respectively). Interestingly, the level of IL-17A in smoker patients who smoked both water pipe and cigarette was the highest (4.35 ± 8.57 pg/ml) followed by that of patients who only used water pipe (1.33 ± 3.122 pg/ml). The smoker patients who were only exposed to cigarette smoke had the lowest level of IL-17A (0.79 ± 2.26 pg/ml, p=0.001). A statistically significant higher level of IL-17A was observed in lower stages (I and II) compared to higher stages (III and IV) of the disease (p=0.013). Conclusions: IL-17A levels are elevated in a part of bladder cancer patients, especially at lower stages of the disease. This may imply that IL-17A is an important factor in the inflammatory process during the tumor progression. The finding that IL-17A was elevated in bladder cancer patients regardless of smoking status indicates that the inflammatory process associated with cancer is stronger than the modulating effect of nicotine on Th17 cells. However, it cannot be ruled out that tumor cells may have participated in the production of IL-17A in these patients.
Keywords: IL-17A, Bladder Cancer, Smoking Status

172. Serum Levels of Interleukins 7 And 8 in Head And Neck Squamous Cell Carcinoma

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Background: The aims of the current study were to investigate the associations of head and neck squamous cell carcinoma (HNSCC) with serum levels of interleukin 7 (IL-7) and IL-8, the two cytokines whose associations with HNSCC need more clarifications. Material and Methods: Sera were collected from 48 untreated patients and 34 age/sex matched healthy donors. Commercial enzyme-linked immunosorbent assay kits were used for the quantification of the cytokines. Results: Serum IL-8 level was neither significantly different between HNSCC patients and control individuals nor associated with smoking status, gender, age, tumor location, tumor grade and stage of the patients (p>0.05). Regarding to IL-7, every control individual had serum levels below the sensitivity of the kit (3 pg/ml), but seven patients had detectable levels, and that mean serum IL-7 was significantly higher in the patients compared to the controls (p=0.02). Conclusions: Mean serum of IL-8 is not significantly associated with HNSCC. With the sensitivity of the kit we employed, it seems that elevated serum IL-7 levels might be more specific than sensitive in HNSCC. Data from other independent studies using kits with lower detection limit for IL-7 are required for the possible employment of IL-7 as a HNSCC biomarker.

Keywords: Interleukin 7, Interleukin 8, head and neck neoplasms, serum, biomarker

173. Activating KIR2DS5 Receptor Plays an Important Role to Develop Thyroid Cancer Disease

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Background: Natural killer (NK) cells are key members of the innate immune system. A family of fourteen killer cell Immunoglobulin-like receptors (KIR) regulates the development and function of NK cells. The number and type of KIR genes are substantially varied between individuals, populations and species. Specific KIR receptors are associated with certain human diseases including a solid tumor (e.g.,cervical neoplasia). Herein we investigated if a specific *KIR* gene is susceptible to the development of thyroid cancer. Materials and Methods: DNA from 85 patients with thyroid cancer and 248 healthy controls from Fars province were characterized for the presence and absence of 14 KIR genes. Results: Overall, a trend toward more activating KIRs was observed in patients compared to the controls. Particularly, the activating *KIR2DS5* gene was statistically increased in patients compared to the controls (37.6 % vs. 25.4%, p=0.03, odd ratio (OR) = 1.77, 95% confidence interval (CI), 1.05-2.99). Additionally, *KIR3DS1* and *KIR2DS1*, the genes linkage to *KIR2DS5* at telomeric region of KIR complex were predominant in the patients than healthy controls. Interestingly, we have observed similar trend toward an increased activating *KIR* gene profile in other solid tumors, Lung cancer and breast cancer patients compared to the healthy controls. Conclusion: These data suggest that increasing activating *KIR* genes in cancer patients could generate a chronic inflammatory condition resulting in tumor microenvironment that may favors tumor growth.
Keywords: Natural killer, KIR2DS5 Receptor, Thyroid Cancer

174. Development of AIDS Associated and Endemic Kaposi Sarcoma: HHV-8/KSHV Viral Load in Cutaneous and Oral Tumor Cells

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Background: Kaposi's sarcoma (KS) is a highly and abnormally vascularized tumor-like lesion which usually presents as a cutaneous lesion and eventually progresses to systemic disease usually in the gastrointestinal (GI) tract, lungs, oral cavity and lymph nodes. KS is the most common neoplasm of HIV+ patients and included in the staging of disease progression to AIDS. The consistent demonstration of HHV-8 or human herpesvirus-8 in tumor lesions, blood cells, serum and saliva of KS patients validates the pathogenic role of HHV-8 in KS oncogenesis. KS lesions develop from early patch/plaque to late, nodular tumors. The histology is characterized by the appearance of an increasing number of tumor spindle cells (SC). Various studies favour an endothelial origin (CD34+) of the KS SC but whether of vascular (VEC) or lymphatic (LEC) origin is not clear. The HHV-8 latency associated nuclear antigen type 1 (LANA-1) protein is the most expressed viral antigen in infected cells. Materials and Methods: In this study we have used formalin fixed paraffin embedded surgical biopsies from Tanzania (Dar Es Salam) at early and late stages of patch/plaque and nodular AKS / EKS development in, oral and cutaneous lesions. The histopathological studies by triple antibody immunofluorescence showed that: (a) continuous recruitment of non-infected endothelial cell into the KS lesion; (b) the resident LECs represent an early target of primary HHV-8 infection; (d) phenotype switch from LEC to VEC. Results: With quantitative real time PCR on DNA from oral and cutaneous AKS and EKS biopsies, HHV-8 load in oral was higher than in cutaneous AKS concordant with the finding of more LANA+ cells and LANA+ granules per nucleus of SC in oral compared to cutaneous AKS. This may reflect an increased effect of LANA since we observed more proliferating SCs (Ki67+ frequency) in oral than cutaneous AKS. The high viral load in oral KS lesions is consistent with the known high risk of HHV-8 horizontal transmission and highly associated with HIV. Immunohistochemistry showed higher frequency of HHV-8 infected tumor cells in oral vs. cutaneous AKS. Conclusion: The results generally suggest that oral AKS may have a worse clinical and biological prognosis than cutaneous AKS indicating differential susceptibility of various tissues to HHV-8 infection and subsequent KS development.
Keywords: AIDS, Endemic Kaposi Sarcoma, HHV-8/KSHV

175. Evaluation of FoxP3 and IL-10 Gene Expression in Breast Cancer Patients by Using TaqMan RT-PCR

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Background: Breast cancer is a worldwide disease with great social and economical impact on society. The immune cells of the patients have been shown to respond poorly to stimuli and exploring the causes has been a hot area of research in recent decade. Forkhead/winged helix transcription factor (FoxP3) and Interlukin 10 (IL10) are two molecules with crucial roles in development and function of regulatory T (Treg) cells. The aim of this study was to investigate the expression level of Foxp3 and IL-10 genes in peripheral blood mononuclear cells from Iranian patients with breast carcinoma. Materials and Methods: 40 breast cancer patients and 22 age and sex matched healthy individuals with no family history of cancer and autoimmune diseases were enrolled in the study. Total RNA was extracted from whole blood of both patients and controls and cDNA was synthesized. TaqMan Real Time PCR was done using specific primer-probe for Foxp3 and IL-10. β-actin expression was simultaneously checked as internal control to normalize the Ct values from the main genes. Then data were analyzed with Rest 2009 software package. Results: Data analysis indicated Foxp3 mRNA to be up-regulated 2.3 folds in patients compared with control group (p= 0.019). No difference was observed in IL-10 expression between patients and controls (P=0.6). The expression level of FoxP3 and IL10 observed not to be associated with cancer progression factors including tumor type, stage, size, grade and lymph node involvement Conclusion: These data are in consistent with the published data regarding Treg cell elevation in breast cancer patients, emphasizing that one reason for immune suppression in breast cancer patients might be FoxP3 over expression.
Keywords: FoxP3, IL-10, Breast Cancer, TaqMan RT-PCR

176. Association of TAP2 Gene Polymorphism and Gastric Cancer

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Background: Gastric cancer is the fourth most common cancer and second leading cause of cancer death. The 'transporter associated with antigen processing' (TAP) gene products are involved in the processing of endogenous peptides that bind to class I molecules. Mutations and/or polymorphism within these genes could alter the efficacy of the immune response which might be relevant for the development of autoimmune diseases and cancer. Materials and Methods: In this case-control study, 137 patients with gastric cancer (92 males and 45 females) with age range of 28-86 years, and 123 healthy volunteers with age and geographical conditions matched with same patients were recruited. All originating from Mazandaran. TAP2 polymorphic residues at positions 379 and 665 were found using amplification refractory mutation system–polymerase chain reaction (ARMS-PCR). Results: The polymorphism of TAP2 gene was found in 137 patients were as followd: in TAP2-379, 0.7% (AA), 58.7% (A/G), 36.4% (G/G) and in TAP2-665, 4.9% (AA), 87.4% (A/G) and 3.5% (GG). In the 123 controls that was studied, the frequencies of polymorphisms were: 0% (AA), 38.2% (A/G), 55.7% (GG) in 379 allelic position and 0% (AA), 87% (A/G) and 6.9% (GG) in 665 allelic position. Conclusions: Our result showed that there was no association between polymorphisms of TAP-2 and gastric cancer (p>0.05).
Keywords: TAP2, Polymorphism, Gastric Cancer

177. Down regulation of CXCR1 and CXCR2 mRNA expression in peripheral blood mononuclear cells of patients with acute myeloid leukemia

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Background: 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 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 acute myeloid leukemia (AML). Materials and Methods: We evaluated the mRNA expression of CXCR1 and CXCR2 in peripheral blood mononuclear cells of thirty-one patients with newly diagnosed AML and twenty eight healthy controls by Quantitative Real time PCR. Results:Expression of CXCR1 and CXCR2 mRNA in leukemia cells diminished about 11 and 17- fold in leukemia cells compared to control cells (P<0.001 for bothcomparisons). CXCR1 mRNA expression in the peripheral blood mononuclear cells was positively correlated with the CXCR2 mRNA in the patients and the control group (rs= 0.709, p<0.001 and rs= 0.755, p<0.001, respectively). Conclusion: Our data show that AML is associated with a decreased expression of CXCR1and CXCR2 mRNA. Down-regulation of CXCR1 and CXCR2 may be important in AML pathogenesis, but to clarify the role of these receptors in AML, further comprehensive studies with larger sample size are required.
Keywords: CXCR1, CXCR2, Peripheral Blood Mononuclear cells, Acute Myeloid Leukemia

178. GeneExpressionofVEGF-AandVEGF-CinPeripheralBloodMononuclearCellsofIranianPatientswithAcuteMyeloidLeukemia

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Background:Thecrucialroleofangiogenesisinthepathophysiologyofacutemyeloidleukemia(AML)ithasbeenproposed.Oneofthekeyregulatorsofangiogenesisisthevascularendothelialgrowthfactor(VEGF).AmongVEGFfamily,ithasbeenobservedthatVEGF-AandVEGF-CareexpressedbyAMLcellsandmediatedleukemiccellproliferation,survivalandresistancetochemotherapy.Emergingevidencehowever,suggeststhatelevatedlevelsofVEGF,oraproangiogenicphenotype,mayimpede,ratherthanpromote,earlytumordevelopmentandprogression.AsthesignificanceofVEGF-AandVEGF-
ClevelsinthepathogenesisofAMLhasnotbeenclarifiedwell,theaimofthisstudyistoevaluategeneexpressionofthesegrowthfactorsinperipheralbloodmononuclearcellsofIranianpatientswithAML. Materials and Method:WeinvestigatedthemRNAexpressionofVEGF-AandVEGF-CinperipheralbloodmononuclearcellsoftwentysevenpatientswithnewlydiagnosedAMLandtwentyeighthealthycontrolsbyQuantitativeRealtimePCR. Results:ExpressionofVEGF-
CmRNAwassignificantlylowerinAMLpatientsthaninhealthycontrols(p<0.001).However,therewasnosignificantdecrementinexpressionofVEGF-AmRNAofAMLpatientscomparedtocontrolgroup(P=0.861).
Conclusion:Inconclusion,ourdatashowedthatAMLisassociatedwithadecreasedexpressionofVEGF-CmRNA.
Keywords: VEGF-A,VEGF-C,PeripheralBloodMononuclearCells,AcuteMyeloidLeukemia

179. Expression of VEGF-A and VEGF-C mRNA in Bone Marrow of Patients with Acute Myeloid Leukemia

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Background: The pivotal role of angiogenesis it has been suggested in the pathophysiology of acute myelogenous leukemia (AML). One of the most key regulators of angiogenesis is the vascular endothelial growth factor (VEGF). VEGF-A and VEGF-C are expressed by AML blasts and participate in the growth and survival of myeloid leukemic progenitors. Emerging evidence, however, suggests that elevated levels of VEGF acts as a negative regulator of tumor development and progression. The purpose of the present study was to evaluate gene expression of VEGF-A and VEGF-C in bone marrow leukemic cells of AML patients before and after chemotherapy. Materials and Methods: We investigated the mRNA expression of VEGF-A and VEGF-C in bone marrow leukemic cells of thirty-one patients with newly diagnosed AML before and 29 days after initiating the chemotherapy with standard Cytarabine/ Daunorubicin (7 plus 3) protocols by Quantitative Real time PCR. Results: Data analysis showed significant differences in median expression of VEGF-A and VEGF-C mRNA in leukemic cells before treatment compared after completion of treatment. There were increases in mRNA levels of VEGF-A and VEGF-C after cancer therapy (P= 0.002, and P= 0.003 respectively). Conclusion: Our data show that AML is associated with an increased expression of VEGF-A and VEGF-C mRNA after cancer therapy. It seems that VEGF had a tumor inhibitory role and up-regulation of VEGF-A and VEGF-C inhibits the growth and progression of AML leukemic cells through recruitment of tumor inhibitory monocytic cells.
Keywords: VEGF-A, VEGF-C, Bone Marrow, Acute Myeloid Leukemia

180. Gene Expression of CXCL8 in Peripheral Blood Mononuclear Cells of Iranian Patients with Acute Myeloid Leukemia

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Background:Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells that is characterized by accumulation of immature malignant myeloid cells in the bone marrow. 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. In view of the role of IL-8 in angiogenesis in AML, the aim of this study is to evaluate gene expression of CXCL8 and its possible prognostic valuein peripheral blood mononuclear cells of Iranian patients with AML. Materials and Methods:We investigated the mRNA expression of CXCL8 in peripheral blood mononuclear cells of twenty seven patients with newly diagnosed AML and twenty eight healthy controls by Quantitative Real time PCR. Results:Expression of CXCL8 mRNA was significantly higher in AML patients than in controls (p<0.001). However, CXCL8 expression was not able to predict clinical outcome. Conclusion:Our data show that AML is associated with an increased expression of CXCL8 mRNA. Up-regulation of IL-8 probably drives tumor progression through its paracrine actions. Although expression levels display no prognostic significance in our study, CXCL8 may be important in AML pathogenesis.
Keywords: CXCL8, Peripheral Blood Mononuclear Cells, Acute Myeloid Leukemia

181. Toll-like Receptors Expression in Adipose Derived Stem Cells (ASCs) of Breast Cancer Patients and Healthy Volunteers

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Background: Mesenchymal stem cells (MSCs) have the ability to localize in breast carcinoma and modify anti-tumor immune responses through immune modulatory effects. Besides, TLRs, as important immune regulated receptors, play crucial roles in immune responses. Here we investigated the expression of TLRs on breast cancer ASCs in comparison with those isolated from healthy volunteers. Materials and Methods:

ASCs were isolated from breast adipose tissue of breast cancer patients by collagenase. Then the expression of mesenchymal stem cell specific markers was checked using flow cytometry method. Expression of TLR2, TLR3, TLR4, TLR5, TLR9, NAPL and NOD2 were determined by quantitative real-time PCR (qRT-PCR)method. Results: ASCs from breast cancer and normal subjects were appeared with similar morphologic and phenotypic characteristics. Although no significant different was found in the expression of different TLRs between patients and controls, TLR3 and NOD2 showed 2.7- and 3.2-fold higher expression in ASCs of patients. In contrast, NAPL was expressed 6.5-fold higher in normal cases compared to patients. Conclusion: As ASCs are present in tumor microenvironment the differential expression of TLRs may have crucial roles for regulation of immune responses.

Keywords: Toll-like Receptors, ASCs, Breast Cancer

182. Investigation of the Relation between Gene Expressions in Laryngeal Tumor Tissue and Neck Lymph Nodes

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Background: The aim of this study was to investigate the relation between EGFR, HER2, HER3-s, HER3-1, JAG1, WEE1, RANTES, CCR5, SDF-1 and CXCR4 transcripts in laryngeal tumor tissue and neck lymph nodes. This attempt should be made to identify new factors that could be useful to identify molecular targets for immunotherapy of laryngeal squamous cell carcinoma (LSCC) patients. Materials and Methods: The mRNA expressions of 56 tumors of laryngeal cancer and 31 lymph node samples were detected by real-time quantitative RT-PCR. Results:In LSCC tissue samples a statistically significant correlation was found between EGFR and HER-2, HER3-s, HER3-1, JAG1, WEE1, RANTES, CCR5 and CXCR4. However, there was not significant correlation between EGFR and SDF-1. HER3-s and HER3-1 also revealed the similar results. However, there was a significant positive correlation between HER2 and SDF-1 and also other tested markers. Similar result was found for WEE1 andCXCR4. RANTES and CCR5 had a significant correlation with all tested markers except JAG1. In lymph node samples a statistically significant correlation was found between EGFR and HER-2, HER3-s, HER3-1, JAG1, WEE1, RANTES and CCR5. HER3-s revealed a similar result. There was a significant positive correlation between HER2 and other markers excluding JAG1, SDF-1 and CXCR4. Also, HER3-1 had not correlation with SDF-1, CXCR4 and CCR5. WEE1 and RANTES revealed that they had not correlation to JAG1 and SDF-1. CCR5 showed similar pattern except it had not correlated to HER3-1. Interestingly, SDF-1 expression had not correlation to any of tested markers in lymph node samples. Conclusions: Our results show the comparable pattern of gene expression correlation in tumor tissue and lymph node samples. Therefore, the better understanding of the genes association in cancer has led to improve treatments for laryngeal squamous cell carcinoma patients.

Keywords: Laryngeal Tumor Tissue, Neck Lymph Nodes, LSCC

183. Chemokines and Chemokine Receptors Expression in Lymph Nodes of Patients with Breast Cancer

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Background: Lymph nodes (LNs) are among the first sites of tumor metastasis. Expression of chemokines/chemokine receptors in LNs is involved in prognosis of cancer and introduced as a good predictor for tumor progression. The main aim of this study was assessment the expression of important tumor promoting chemokine and chemokine receptors in LNs of breast cancer patients. Materials and Methods: LNs were isolated from twenty breast cancer women and data were compared between positive and negative nods. Expression of chemokine and chemkine receptors in LNs was determined by quantitative real-time PCR (qRT-PCR) and flow cytometry methods. Results: Results of qRT-PCR for LNs showed that all chemokines specially MCP-1, IL-8 and CXCL13 and among chemokine receptors CXCR4 and CCR5 had more mRNA expressions in LN^+ compared to LN^- samples. However, these differences were not statistically significant. Results of flow cytometry analysis showed the higher significant presence of CD69+, CCR5+ and CD3+CCR5+ lymphocytes in LNs of LN^+ compared to LN^- breast cancer patients (P value< 0.05). The expression of MCP-1 was higher in LN^+ patients and P value was near significant (P value= 0.07). IL-8 and CXCR5 were significantly expressed more in LN^+ patients with pathological stage III compared to those with pathological stage II tumors (P value= 0.04). Conclusion: our findings extend more information on the expressions of essential chemokines and chemokine receptors in LNs and their relationships with the important prognostic factors in breast cancer. These findings have important implications in immunotherapeutic interventions for treatment of breast cancer.

Keywords: Chemokines, Chemokine Receptors, Lymph Nodes, Breast Cancer

184. The Decrease of NKG2D+Natural Killer Cells in Peripheral Blood of Patients with Metastatic Colorectal Cancer

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Background: Colorectal cancer is the third most commonly diagnosed cancer in the world, but its prevalence is more in developed countries. As a function, NK cells have a critical role in primary protection versus viruses and tumors such as colorectal cancer. The aim of this study was to investigate NKG2D levels on NK cells as important stimulatory receptor of these cells in the deferent stages of colorectal cancer through both flowcytometric and Real-time PCR methods. Materials and Methods: The percentage of peripheral blood NKG2D+CD3-CD56+ NK cells from patients with different stages of colorectal cancer and normal subjects, as well as the expression intensity of NKG2D on these cells were analyzed by flow cytometry. Also, the mRNA expression of NKG2D was measured by sybr green real-time PCR in patient and control samples. Results: This study indicates that the percentage of NKG2D+NK cells and mean of NKG2D expression in the cells (MFI) by flowcytometric technique reduced with tumor progression, which was significant decrease in metastatic colon cancer patients. These results were confirmed by real-time PCR technique performed on peripheral white blood cells. Conclusion: In humans, NKG2D molecules are one of important receptors on NK cells and cytotoxic T cells (CD8+ & γδT) and their binding to the ligands can activate alone cytotoxicity of these cells. Unlike other receptors, the ligands recognized by NKG2D are 'induced-self' ligands on stressed cells such as colorectal cancer cells. This system requires precise regulation because inappropriate expression of NKG2D ligands might compromise NK cell activation. Thus, in conclusion, it can be considered a double-edged sword role for NKG2D-NKG2D ligands in various stages of colorectal cancer.

Keywords: colorectal cancer, NK cells, NKG2D, NKG2D ligands

185. Evaluation of Angiogenic Factors VEGF, FGF, PDGF in Patients with Glioma and Meningioma and Normal Population in South Iran

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Background: Every tumoral process is in a serious need for formation of blood vessels. Neo-vascularization is inducedvariety of factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF).We decided to measure the serum level of these factors in patients with glial cell tumors and meningiomas in a case-control study. Materials and Methods: This study was a case- control study .Case group was the patients referring to Shiraz Chamran Hospital with diagnosis of either meningioma or glioma.All demographic and clinical data were also obtained and registered. The volume of the tumor and intraoperative bleeding were recorded

.Control group was healthy blood donors. Serum level of VEGF, PDGF and FGF were checked. Results: Ninety-six patients were enrolled in the study, 32 in each group. There was increased level of VEGF in patients with any cranial tumor, either glioma or meningioma.VEGF levels was highest among grade IV tumors,namelyglioblastomamultiforme. VEGF was also higher in larger tumors. There was an increasing trend of serum VEGF levels as glioma grade increased. Highest VEGF levels were seen with parasagittal meningioma. In contrastto VEGF, PDGF was only slightly elevated in glial cell tumors, and it was significantly elevated in patients with meningioma.Higher serum PDGF correlated with a higher amount of intraoperative bleeding,especially in meningioma. Oligodendroglia tumors expressed higher PDGF levels in comparison to other factors,including FGF.Conclusion: We could find an increased serum level of both VEGF and PDGF in CNS tumor patients. A differential role PDGFwas in the pathogenesis ofneovascularization for meningioma,and also for oligodendroglioma .No significant results could be found for FGF.

Keywords: VEGF, FGF, PDGF, Glioma, Meningioma

186. Determination of Serum Concentrations of sCD30 and sCD40L in Patients of Bone Tumors

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Background: Primary malignant bone tumors are heterogeneous groups of malignant neoplasms especially in children and adolescents. The most common types are Osteosarcoma, Ewing sarcoma and chondrosarcoma. The CD30 molecule is a member of the tumor necrosis/nerve growth factor super family. The CD40 ligand (CD40L) is a functional receptor. Th and Tc express CD30 on their surface. The CD40L is a functional receptorElevation of these proteins has been observed in lymphoma, leukemia and autoimmune disorders.We aim to evaluate serum concentrations of sCD30 and sCD40L in patients with primary malignant bone tumors and determine whether these serum markers could help in differentiating the tumor type. Materials and Methods: 54 patients with primary malignant bone tumor. 54 healthy blood donors. Cases with history of prior treatment (surgery, chemotherapy and radiotherapy) were excluded from the study. Blood samples were collected and Serum levels of sCD30 and sCD40L were detected by ELISA. Results: There were 54 patients with malignant bone tumors (31 osteosarcoma, 14 Ewing sarcoma, and 9 chondrosarcoma cases) and 54 healthy controls in this study. Mean serum concentration of sCD30 in Ewing sarcoma was significantly higher than that in the control group and osteosarcoma group ($p < 0.05$), but mean serum concentrations of sCD30 in osteosarcoma and chondrosarcoma groups were not significantly different compared to the control group. Mean serum concentrations of sCD40L in osteosarcoma, Ewing sarcoma and chondrosarcoma groups were significantly higher than the control group ($P < 0.05$). In addition, the mean serum level of sCD40L in chondrosarcoma patients was higher than both Ewing sarcoma and osteosarcoma groups ($p<0.05$). There was no significant difference in serum levels of sCD30 and sCD40L between low grade and high grade osteosarcoma. Conclusion: This study suggests that sCD30 and sCD40L might be useful biomarkers for quick and accurate diagnosis of different bone malignancies.

Keywords: sCD30, sCD40L, Bone Tumors, ELISA

187. The effect of RhoGDIα Transfection on MCF7 (ER+) and MDA-MB-231 (ER-) Breast Cancer Cell Lines

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Background: Rho GDP dissociation inhibitors (RhoGDI) can inhibit cell motility, invasion and metastasis in cancer by inactivation of Rho GTPases. A member of RhoGDI family, called RhoGDIα, has been shown to increase transcriptional activation of estrogen receptor (ER), a receptor that might be inversely correlated with cell motility and invasion in breast cancer. The aim of our study was to investigate the effect of RhoGDIα on the migration and invasion of ER+ MCF7 and ER- MDA-MB-231 breast cancer cell line models. Materials and Methods: RhoGDIα was downregulated using short interfering RNA (siRNA) and lipofectamin and upregulated using GFP-tagged ORF clone of RhoGDIα. Real time PCR and western blotting were employed to confirm down regulation of RhoGDIα, and flow cytometry for confirmation of overexpression. The migration and invasion assays were performed using transwell plates. Results: The results demonstrated that inhibition of RhoGDIα in MCF7 and MDA-MB-231 cells significantly increased the numbers of cells that migrated or invaded from upper champers of transwell plates to the lower champers. Overexpression of RhoGDIα in MCF-7 inhibited migration and invasion, but no significant effect was observed on MDA-MB-231 migration and invasion. Conclusion: Our results indicate that the downregulation of ER interacting RhoGDIα molecule similarly affect the in vitro migration and invasion of non-invasive ER+ MCF7 and invasive ER- MDA-MB-231 cells. However, our assays are differently affected by the upregulation of RhoGDIα . Whether our results are the consequences of differences in ER expression, primary invasive ability and/or other molecules between these two cell line models need more investigation.

Keywords: RhoGDIα, MCF7 (ER+), MDA-MB-231 (ER-), Breast Cancer

188. Increased Number of Circulating $CD4^+CD25^+Foxp3^+$ Regulatory T Cells is Duke Stage Dependent in Colon Cancer Patients

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Background: It has been shown that naturally occurring regulatory T-cells ($CD4^+CD25^+$ $Foxp3^+$ T-cells), have critical roles in tumor invasion and down-regulation of immune response against established tumors. We and others have reported increased frequencies of CD4+CD25highFOXP3+ Treg cells in cancer patients. In this study we examined if increased circulating regulatory T cells is stage dependent in colon cancer patients. Materials and Methods: A twenty-two recently diagnosed patients population with colon cancer were subjected to assessment of circulating regulatory T cells by Flow-cytometry and Semi-quantitative PCR. Twenty healthy individuals served as controls. Results: Circulating CD4+CD25+Foxp3+ regulatory T cell frequencies were significantly increased in colon cancer patients regarding to healthy controls ($p < 0.001$). When patients were subgrouped according to Dukes stages, a linear relationship was observed between Dukes stages and Treg frequencies Patients with low differentiated colon cancer (Duke C and D) were more likely to have significantly higher population of circulating Treg frequencies than Dukes A and B patients when compared to controls. Conclusion: These results suggest that augmented Treg-cell frequencies may be linked to tumor stage, prognosis, and survival, and cancer itself may be able to drive Treg recruitment as a strategy of immune-evasion.

Keywords: $CD4^+CD25^+$ $Foxp3^+$ T-cells, Duke Stage, Colon Cancer Patients

189. Apoptotic Effects of a Specific Single Chain Antibody against Prostate Stem Cell Antigen on Prostate Cancer Cell Lines

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Background: Prostate stem cell antigen (PSCA) is a promising new target for the treatment of PSCA expressing tumors. Over-expression of PSCA has been reported in some cancers including prostate, pancreas and bladder cancer while its expression in normal tissues is predominantly prostate specific. Well-known characteristics of scFv antibodies over the intact antibodies such as small size and fast penetration have offered scFv antibodies as effective alternatives to full-length monoclonal antibodies in cancer immunotherapy. In the present study we intended to evaluate apoptotic effects of a specific single chain antibody against PSCA (selected in our laboratory) on the prostate cancer cell lines. Materials and Methods: The PSCA expressing cells (DU-145) and PSCA negative cells (LNCaP) were treated with 500scFv/cell concentration of an anti-PSCA specific scFv for 24h. The apoptotic effects were evaluated using Annexin-V/PI assay and the data were determined by dual color flow cytometry. Results: The results showed that the selected scFv could significantly induce apoptosis in PSCA expressing cells, DU-145 cells,

(65%) while no apoptotic effect on PSCA negative cell line (LNCaP) was observed. Conclusion: Immunotherapy have shown prominent role in the treatment of a number of cancers. In this study the apoptotic effects of a specific scFv was evaluated. As results show the selected scFv significantly induced apoptosis in the PSCA expressing cells. We have already shown the growth inhibitory effects of the selected anti-PSCA antibody.These results provide further evidence for the efficacy of the isolated anti-PSCA scFv antibody for the treatment of PSCA expressing tumors.

Keywords: Prostate stem cell antigen, scFv antibodies, flow cytometry, Prostate Cancer

190. Effect of Hyperthermia in Expression of UL16 Binding Proteins on Human Cancer Cell Line

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Background: Today hyperthermia was suggeste as a new method in cancer therapy. There are some documents of heat shock increases ULBPs on tumoral cells. This study is acquiring the effect of heat shock on the expression of ULBP1 and ULBP2 in HeLa cell line with epithelial origination and SW-872 cell line with mesancaimal origination and the responses of mentioned cell lines to the heat shock were study. Materials and Methods: In this study two method were used Real time PCR to determine the amount of mRNA expression in cells and method of flowcytometry was employe to measure the expression of ULBP1 and ULBP2 molecules on the cells surface. Results: This research reveals that in HeLa cell line expression of ULBP1 mRNA in 44˚C and 46˚C increased significantly but the expression of ULBP2 did not increased significantly. Expression of mRNA, ULBP1 and ULBP2 in SW-872 cell line did not increase in any of heat shocks. ULBP1 expression on HeLa cell line in 44˚C heat shock after 4 hours increased significantly. ULBP2 expression on HeLa cell line did not change. ULBP1 expression on SW-872 cell line in 42˚C heat shock after 4 hours increased significantly. ULBP2 expression on SW-872 Cell line in 42˚C heat shock after 2 and 4 hours significantly increased. Conclusion: the final conclusion inferred of this study is this that various cell line response differently to heat shock because hyperthermia has various effect on chromatin condensation, transcription factor and DNA replication. We suggest that ULBPs expressions investigate in fever rang (37-40˚C).

Keyword: ULBP, NKG2D, Hyperthermia, RT PCR, Flow cytometry

191. Umbelliprenin from *Ferula szowitsiana* Induces Apoptosis and Cell Cycle Arrest in Human Melanoma Cells

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Background: Melanoma has a bad prognosis mainly due to the development of metastasis. Umbelliprenin is synthesized by *Ferula* species. It has also been found in various plant species consumed as food such as celery and *Citrus limon*. Materials and Methods: In the present study, we assessed the cytotoxic effect of umbelliprenin from *Ferula szowitsiana* (Apiaceae) on human melanoma cell lines including MM200, Mel-RM, Me4405, A375 and human normal melanocytes (HF2FF). Its effect on the growth of cells was assayed by MTT assay and cisplatin was used and positive control. The pattern of cellular death (early apoptosis, late apoptosis and/or necrosis) was evaluated using annexinV-FITC and propidium iodide (PI) staining method by flowcytometry. For establishment of apoptosis and determination of DNA distribution, cells were stained with RNAse and propidium iodide (PI) solution and subsequently analysed by by flowcytometry. Ursolic acid was used as positive control. Expression of cytosolic Bax, Cytochrome C, p21, p53, cyclin and CDK proteins were studied by western blotting. Flowcytometric data were analyzed using FSC Express 3.0 software. Results: Melanoma cell proliferation was dose-dependently inhibited through cell cycle arrest in G1 and induction of apoptosis. The finding that the cytotoxic effect of umbelliprenin is markedly more pronounced in melanoma cells rather than normal melanocytes, suggest a therapeutic margin. Conclusion: Our data suggest that umbelliprenin orally administered and foods and folk medicines containing this coumarin, may afford protection against the development and early recurrence of melanoma.

Keywords: Umbelliprenin, *Ferula szowitsiana*, Apoptosis, Melanoma Cells

192. Membrane Fas Expression and Prognosis in Patients with Acute Lymphoblastic Leukemia

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Background: Fas (APO-1/CD95) is a 45-kDa membrane protein which regulates apoptosis in many lymphoid cell types. The aim of present study was to evaluate the significant and prognostic values of CD95 expression as a marker of apoptosis in B-ALL patients. Materials and Methods: Bone marrow or peripheral blood samples of 52 patients with ALL were assessed for the expression of T lymphoid, B lymphoid, myeloid and non-lineage-associated differentiation antigens by a two-step strategy using panels of monoclonal antibodies and direct flow cytometry. Expression of CD95 in B-ALL group was studied in relation to immunophenotype, clinical and paraclinical findings. Results: CD95 expression was observed in 13.4% (8 out of 52) of ALL patients. The mean expression was obtained as 14.12±15.60. There was a significant negative correlation of CD95 mean expression with Hemoglobin level (P=0.033). However, the mean expression of CD95 molecule in relation to other prognostic factors indicated no significant association with platelet count, white blood cell count, percentage of blasts and the presence of extramedullary involvement Conclusion: Although CD95 expression was conversely related to Hemoglobin level but it shows no correlation with other established prognostic factors.

Keywords: Fas, CD95, Acute Lymphoblastic Leukemia

193. Evaluation of the Serum Prostate Specific Antigen (PSA) and Free PSA Levels in Patients with Probability of Prostatic Pathology

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Background: The prostate is an exocrine walnut-sized gland that forms part of the male reproductive system in most mammals. PSA is a single chain glycoprotein produced by the secretory epithelium of the prostate gland. PSA is normally secreted in to the seminal fluid and only low levels of the PSA are normally present in the blood. Increasing serum concentration of PSA indicates prostatic pathology, including Benign Prostatic Hyperplasia (BPH) and cancer of prostate. Materials and Methods: We studied Correlation between PSA and Free PSA concentration in serum. 176 people with suspected prostatic pathology were investigated with PSA and FPSA Enzyme immunometric assay kit (EIAkit) in the Imam Reza hospital. The PSA EIA is a non-competitive immunoassay based upon the direct sandwich technique. Results: After statistical analysis we observed a meaningful correlation between PSA and FPSA concentration (R=0.84) with a significance P Value (P<1/100000). In this study had been estimated 93.5% of those with PSA higher than normal range also had their Free PSA higher than normal range. Conclusion: We demonstrated that the high concentrations of PSA predict high free PSA levels in serum and amounts of these are correlated. With respect to other achievements, all of the PSA, total PSA, free PSA and free PSA/total PSA ratios are an important indicator for the prostate pathology.

Keywords: PSA, Free PSA Levels, EIA, Prostatic Pathology

194. Study of Vincristine Effect on Normal Proliferating Lymphocytes and Lymphoma Cell Line BCL1

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Background: Proliferation and apoptosis of lymphocytes are essential parts of the immune system. Most anti-cancer chemotherapy drugs such as vincristine target cell cycle and induce apoptosis in cancer cells, furthermore, moredividing cells , undergo more apoptosis , which may also include the normal proliferating lymphocytes responsive to malignanciesas well. Materials and Methods: In this studythe effects of different concentration of vincristine at three differenttime periods onrestingand proliferating spleenlymphocyteswere evaluated and compared with the effect of the drug on mouse lymphoma cell line BCL1.The cytotoxicity of vincristine was determined by MTT assay and IC50 was calculated for all periods. The cells were also stained with double staining acridine orange and ethidium bromide, were observed with fluorescence microscope and the percentage of apoptotic cells were determined. Results: MTT results showed that vincristine at the concentrations of 20 and 10 µg/ml caused cell death in both resting and proliferating lymphocytes but concentrations <5didn't show any significant cytotoxic effectwhile concentration of 5 and 2µg/mlindicated significant cytotoxic effect on BCL1 cells. The percentage of the apoptotic cells which were affected by different concentrations of the drug wasproportional in two methods i.e. (fluorescence microscope and MTT assay). Conclusion: The toxic effect of vincristine on normal cells is highly dependent on time and the activation of the cells.
Keywords: Apoptosis, Proliferation, BCL1 (lymphoma cell line), Vincristine, Spleen Lymphocyte

195. The Evaluation of Colostrum on the Immune Responses in BALB/C Mice against Transplanted Tumor Derived from Breast Tissue

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Background: Colostrum contains antibodies to protect the newborn against disease, as well as being lower in fat and higher in protein than ordinary milk. Colostrum antitumor effect maybe due to the Immunostimulants properties of theseMilk. In present work we have studied the effect of Colostrumon the immune responses of BALB/c mice against transplanted tumor derived from breast tissue. Materials and Methods: 6-8 week-old in-bred BALB/c mice, each weighing 25.30 gr, were used. The mice were divided into two groups each consisted of 9 mice as test and control groups. The mice with a gastric feeding 2 weeks before tumor transplantation and 3 weeks after that. The control micereceived an equal volume of Milk during the study. Results: Results of the present work showed that Colostrum can increase the production of Immunostimulants cytokine IL-12 and decrease the TGF-â which can suppress immune response. Moreover, the growth rate of tumor in group which received Colostrum were decreased and the results of delayed type hypersensitivity (DTH) of this group in 48h were better than control group. Conclusion: The results of our study suggest that daily use of Colostrum can regulate immune response with Th1 dominance and may be helpful for cancer immunotherapy, but further studies are needed to investigate the other mechanisms of this effect.
Keywords: Colostrum, Cellularimmuneresponse, Breastcancer

196. Cloning of Human Interleukin 8 Receptors Alpha Promoter and Its Characterization in Breast Cancer Cells

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Background: IL-8RA, which is also known as CXCR1, is an IL-8 receptor that belongs to chemokine (C-X-C motif) receptor family and mediates signal transduction through G proteins.CXCR1 geneis expressed on a wide variety of cell types including neutrophils, monocytes and is involved in inflammatory pathways and chemotaxis. Recent studies have shownthat in some kinds of tumors, such as breast cancer, CXCR1 expression increases dramatically. Materials and Methods: Herein to understand the mechanisms increasing the expression of CXCR1 in breast cancer, we cloned 5'flanking region and exon 1 of CXCR1 gene from -1016 to +107nucleotides and then inserted into pGL4.14 vector upstream of luciferase reporter gene. We transiently transfected BT474(human breast ductal carcinoma, with the over expression Her2) and MCF10A (normal breast epithelial cell)cell lines by above-mentioned vectors.We used pGL4.50 that have CMV promoter, as a control vector and pEGFP-N1 as a control vector for transfections. Then cells were lysed using a detergent-containing buffer. Cell debris wereremoved by micro-centrifugation and luciferase activity was measured using a luminometer. Results: Our data showed high level of luciferase activity in BT474 cell line while low levelof luciferase activity was detectedin MCF10A cell line, suggesting that the up-regulation of CXCR1 gene expression occurs mainly in the breast cancerous cells. Conclusion: These results suggested that the CXCR1 promoter could be useful for transcriptional targeting of therapeutic genes in breast cancer cells and other CXCR1 positive tumor cells.
Keywords: Cloning, IL-8RA, Breast Cancer

197. Expression of (IP-10) in Different Breast and Colon Cancer Cell Lines

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Background: IP-10 (CXCL-10) is a CXC chemokine which is secreted in response to IFN-γ. This protein can bind to CXCR3 and play crucial roles in chemoattraction of different immune cells such as monocytes, T cells, NK cells and dendritic cells. It has important functions as an anti-angiogenic mediator in different types of cancers. In contrast, IP-10 can mediate the metastasis of tumor cells to the draining lymph nodes. Materials and Methods: In this study we investigated the expression of IP-10 in four different cell lines of breast cancer (MDA-MB-468 , SKBR-3 , MDA-MB-231 and MCF-7) and 3 cell lines of colon carcinoma (LS-180 , SW742 and HT-29/219) by flow cytometry. Results: Expression of IP-10 in breast cancer cell lines including MDA-MB-231, SKBR-3, MDA-MB-468 and MCF-7 were 98.87, 50.9, 29.8 and 25.20 percent respectively. In colon cancer cell lines expression of IP-10 were 99.38% in SW742, 18.91% in HT-29/219 and 11.58% in LS-180 . Conclusion: As IP-10 is produced by tumor cells in tumor microenvironment, it may be introduced as one of the factors regulating the recruitment of immune cells and consequently the anti-tumor immune responses. To shed more light on the true role of IP-10 specially in metastasis of tumor cells more studies are required.
Keywords: IP-10, Breast and Colon Cancer, flow cytometry

198. Analysis of Pattern Recognition Receptors Expression in Human Lung Adenocarcinoma Epithelial Cell Line

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Background: Pattern recognition receptors (PRRs) are the main sensors of pathogen and danger signals in innate immunity. They are mainly expressed by macrophages and dendritic cells of different organs. Toll like receptors (TLRs) are the most studied and best characterized PPRs which are responsible for sensing pathogen associated molecular patterns (PAMP). In the present study, Human lung adenocarcinoma epithelial cell line(A549) was used as a model to investigate the expression of TLR2, TLR4 and CD14 transcript in these cells. Materials and Methods: Total RNA was extracted using the standard Trizol. After treatment with DNase I, RNA was quantified using NanoDrop . Total RNA was used as template for the reverse transcription reaction. cDNA was synthesized using Oligo-dT primers. All samples were reverse transcribed under the same conditions. Touchdown PCR was applied to analysis the expression level of these genes in A549 cell line. Results: According to our results, TLR2 and CD14 transcripts are expressed in A549 cell line. By performing TD-PCR experiment it was appeared that it is possible to simultaneously detect these innate immune receptor genes in human lung adenocarcinoma epithelial cell line(A549).
Keywords: Toll like receptors, Pattern recognition receptors, A549

199. IL-18 Promoter Haplotyes in Iranian Patients with Bone Tumors

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Background: The cytokine Interleukin-18 (IL-18), produced by the immune cells, stromal cells and osteoblasts, inhibits osteoclastogenesis by affecting T lymphocytes release of granulocyte/macrophage colony stimulating factor (GM-CSF). Association of the genetic variations in IL18 gene have already been reported in several types of cancers. In the present study we aimed to investigate the association two single nucleotide polymorphisms (SNPs) in the promoter of IL-18 gene at the positions -137(G/C) [rs187238] and -607(C/A) [rs1946518] in patients with bone tumors. Materials and Methods: Fifty patients with histopathologically confirmed bone tumors from Shiraz hospitals, enrolled during about 4 years, and 150 healthy controls from the same geographic area were served this case-control study. The genotypes of IL-18 gene promoter at both positions were determined by using allele-specific polymerase chain reaction (AS-PCR) methods. Results: Arlequin analysis indicated no deviation of investigated genotypes from Hardy-Weinberg equilibrium neither in the patients nor in the controls. Haplotype frequencies were calculated by using Arlequin software package. The frequencies of GG, GC and CC genotypes at -137 G/C position for the patients and controls were respectively 58.3% vs 51.7%, 35.4% vs 35.6% and 6.3% vs 12.8%. The frequencies of CC, CA and AA genotypes at position -607 C/A for the patients and controls were respectively 36.2% vs 39.3%, 44.7% vs 44% and 19.1% vs 16.7%. Statistical analysis indicated no significant differences in the frequencies of alleles, genotypes, and haplotypes between patient and control groups ($p>0.05$). Conclusion: Present study does not corroborate association of IL-18 gene promoter polymorphisms with susceptibility of Iranians to bone tumors.
Keywords: IL-18, SNPs, Bone Tumors

200. Establishment of a Lymphoma Animal Model in Mice and Flowcytometric Analysis and Confirmation

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Background: Lymphoma is considered as one of the main malignancies in the world.in order to control and manage of this disease, some extensive researches have been performed. In order to develop of new remedies, it seems that using animal models is crucial. The main purpose of our study was establishment and confirmation of an animal model of lymphoma in Balb/c mice. Materials and Methods: 20 Balb/c mice devided into control and test groups. 5×10^6lymphomatic BCL-1 cells was injected through tail vein in model group. In this group 5 mice were killed 2 weeks after BCL-1 injection and 5 remained mice were killed after 4 weeks. Then changes of spleen, peripheral blood, lymph node and liver was investigated in these groups. We investigated affliction of mice with lymphoma by flowcytometric technique (using IgM an CD5 markers). Results: There was a statistical difference in the regard of spleen index between control and test groups; as in injected group ,specially in 4 weeks group, spleen index was greater than controls ($p<0.05$). Percentage of IgM^+CD5^+ cells in spleen of injected groups, particularly in4 weeks group, was greater than control group($p<0.05$). In 4 weeks group, percentage of lymphocytes in peripheral blood had statistical difference as compared with 2 weeks and control groups ($p<0.05$). And there was not any hystological change in lymph nodes and liver. Conclusion: According to considerable increase of cells which were positive for IgM and CD5 markers, and the increase of spleen index in injected mice, we can conclude that cancerous cells deployed in animals and were proliferating.
Keywords: Lymphoma, animal model, BCL-1, flow cytometry, spleen.

CANCER IMMUNOTHERAPY

Oral Presentation

201. Characterization of Novel Murine Monoclonal Antibodies Directed Against the Extracellular Domain of Human HER2 Tyrosine Kinase Receptor

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Background: *HER2* proto-oncogene encodes a transmembrane receptor tyrosine kinase, overexpressed in a variety of solid tumors. Several mouse monoclonal antibodies (MAbs) have been developed that recognize the extracellular part of HER2, of them two MAbs were humanized and employed for targeted immunotherapy. Materials and Methods: In this study we aimed to produce murine MAbs that specifically recognize the extracellular domain of human HER2. BALB/c mice were first primed with *HER2*-transfected NIH-3T3 cells (provisionally designated as 3T3-HER2) and then boosted with recombinant extracellualr part of HER2. Splenocytes from hyperimmunized mice were fused with myeloma cells and growing hybridomas were selected and screened for HER2 reactivity by an indirect ELISA. HER2-specific hybridomas were selected and cloned by limiting dilution assay. Finally, several clones were obtained and further characterized. Results: All clones showed positive reactivity to HER2 with binding affinity ranging from 1.9×10^8 for 4C7 to 5×10^9 for 1F2 MAb. All clones showed no binding competition to HER2 receptor in inhibition ELISA assay, and stained HER2- overexpressing BT-474 breast cancer cell line and 3T3-HER2 cells, but not NIH- 3T3 cells in flow cytometry technique. They also reacted to 185 KD full-length and 95-100 KD truncated forms of HER2 in non-reducing Western blotting, but two MAbs (2A9 and 1B5) failed to recognize HER2 protein in reducing conditions. 1T0 and 2A8 MAbs inhibited the growth of BT-474 cell line in vitro using ^{3}H-thymidine incorporation assay. Different combinations of MAbs demonstrated synergistic and/or antagonistic effects on the growth of BT-474 cells. Conclusion: Our results indicate that based on specific reactivity to human HER2 receptor on cancerous cells and anti-proliferative capacity, some of our MAbs could have potential implication for selective targeting of HER2-expressing malignancies.
Keywords: Monoclonal Antibodies, HER2, BALB/c mice

202. Induction of Tumor-Specific Immunity by Multi-epitope HER2/neu-derived Peptides Encapsulated in LPD Nanoparticles

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Background: The goal of study was first to design multi-epitope peptides from the rat HER2/neu (rHER2/neu) oncogene and then to evaluate the effectiveness of these peptides encapsulated in liposome-polycation-DNA (LPD) nanoparticles for the induction of cytotoxic T lymphocyte (CTL) response in BALB/c mice and also in tumor mice model. Materials and Methods: Four multi-epitope peptides derived from the rHER2/neu were designed by in silico analysis and encapsulated in LPD nanoparticles. To prepare the LPD nanoparticles, liposomes containing peptide were mixed with protamine in dextrose, followed by the addition of CpG solution. Different groups of mice were vaccinated with free peptides or peptides encapsulated in nanoparticles. The mice were euthanized after the last booster dose and their spleens were collected to evaluate cellular

immune responses. ELISpot assays were performed using mouse IFN-γ and IL-4 ELISpot kits. An *in vitro* CTL assay using ex vivo expanded splenocytes was performed by using different ratios of effector to target cells (labeled tumor cells with Calcein AM). The vaccinated mice were challenged by subcutaneous injections using TUBO tumor cells. Tumor size and survival time were monitored for up to 80 days. Results: Two of the four tested peptides (p5 and p435), as well as their combinations with the LPD nanoparticles induced a significantly higher IFN-γ release and more potent CTL responses compared to the control groups. Consequently, these responses led to lower tumor sizes and longer survival time in TUBO tumor mice model. Peptides encapsulated in nanoparticles showed higher and more effective CTL responses in comparison to free peptides. Conclusion: Our results demonstrate that rHER2/neu-peptides (p5 and p435) and their encapsulation can induce an antigen-specific immunity. This study also presents the first attempt to evaluate the effectiveness of natural rHER2/neu-peptides containing CTL multi-epitope and encapsulated in LPD nanoparticles.
Keywords: Tumor-Specific Immunity, rHER2/neu, LPD Nanoparticles

203. Direct Short-term Cytotoxic Effects of BIBR 1532 on Acute Promyelocytic Leukemia CellsThrough Induction of p21 Coupled with Down-Regulation of c-Myc and hTERT Transcription

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Background: PL, an Acute Myeloid Leukemia subtype (AML-M3), is characterized by a specific t(15;17), distinct morphologic picture, and a clinical coagulopathy that contributes to the morbidity and mortality of the disease. Telomerase is consistently activated in nearly all APL patients and telomerase-mediated telomere stabilization is responsible for unlimited replicative potential of the disease. This study was aimed to investigate the effects of the non-peptidic, non-nucleosidic anti-telomerase compound BIBR1532, which represents a potent specific inhibitor of hTERT, on APL cells (NB4). Materials and Methods: Trypan blue dye exclusion test, Microculture Tetrazolume Test, BrdU Cell Proliferation Assay, RQ-TRAP, Caspase activity and quantitative Real-Time RT-PCR were performed to appraise the effects of BIBR1532 on cell viability, metabolic activity, DNA synthesis, telomerase and caspase activity, and quantitative gene expression changes in APL cells. Results: BIBR1532 decreased cell viability; inhibited metabolic activity, DNA synthesis and telomerase activity; and activated caspase. In addition, an expressive decrease in mRNA levels of C-Myc, hTERT and Bcl2 coupled with a significant induction in transcriptional levels of P21 and Bax were observed. Conclusion: In this study, we provide evidence that BIBR 1532 exerts a direct short-term growth suppressive effect in a concentration-depending manner through down-regulation of C-Myc-dependent hTERT expression. Furthermore, we show that overexpression of P21 and subsequent disturbance of Bcl-2/Bax balanced ratio as well as decreased telomerase activity due to further down-regulated hTERT expression may be a rational mechanism leading to selective cytotoxicity of high dose BIBR 1532 against NB4 leukemic cells.
Keywords: BIBR1532, hTERT, APL cells

204. Evaluation of Possibility of Tolerance Breakdown against FoxP3 following Anti-FoxP3 DNA-Protein Prime-Boost Vaccination in Mice

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Background: FoxP3+Regulatory T cells (Tregs) are main obstacle against anti tumor immune responses and depletion of these cells is purpose of many studies in the field of cancer research. In this study we evaluated the breakdown of tolerance against FoxP3 by targeting FoxP3 as most specific marker for Tregs in comparison to CD25 using DNA-protein prime-boost vaccination. Materials and Methods: C57/BL16 mice were primed against FoxP3 by one subcutaneously injection of DNA vaccine coding FoxP3 and boosted by two subcutaneously injections of FoxP3 recombinant protein. Three weeks after last injection, splenocyte separated and proliferation assay was done to compare FoxP3 specific T cells in vaccinated and control groups. Serum of mice was evaluated for detecting anti-FoxP3 total IgG and IgG subclasses including IgG1, IgG2a, IgG2b and IgG3 by ELISA. Results: Our results indicated that FoxP3 specific T cells significantly increased in vaccinated group. Serum level of anti -FoxP3 total IgG in vaccinated group was significantly higher than control group. The analysis of anti-FoxP3 IgG subclasses showed a significant increase in IgG2a and IgG2b levels. Conclusion: In conclusion, this study demonstrates that anti-FoxP3 DNA-protein prime-boost vaccination is possible to breakdown of B-cell and T-cell tolerance against FoxP3. As regards, the specific IgG subclasses pattern induced by a vaccination is indirect evidence for the preferential type 1 or type 2 immune response evoked by the antigen. Elevated levels of IgG2a and IgG2b can be considered as a consequence of a type 1- immune response that is proper for the purpose of this vaccination.
Keywords: FoxP3, Vaccination, DNA-protein prime-boost vaccination, Mice

205. Cross-priming and Immunodominance in Antiviral and Anticancer $CD8^+$ T Cell Responses

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$CD8^+$ T cells play a pivotal role in protection against viral pathogens and in immune surveillance against spontaneously arising neoplastic cells. The $CD8^+$ T cell activation cascade is initiated by professional antigen (Ag) presenting cells (pAPCs), which present 8-11-amino acid residue-long antigenic peptides complexed with major histocompatibility complex (MHC) class I molecules to naïve T cells, each of which bears a T cell receptor (TCR) of unique specificity. This highly specific interaction delivers "signal 1" to the T cell. Signal 1 is necessary but not sufficient for optimal T cell activation, which also requires a second or costimulatory signal typically provided by CD28-B7 interactions. TCR triggering in the absence of costimulatory signaling may lead to T cell anergy or even death by apoptosis. $CD8^+$ T cell activation is achieved by direct priming and/or cross-priming. Direct priming occurs when endogenous proteins provide the substrate for Ag processing. These include viral proteins processed by infected pAPCs and oncoproteins expressed by tumor cells. Importantly, however, many viruses do not infect pAPCs or interfere with the endogenous pathway of Ag processing and presentation. Moreover, many tumor types, especially those of non-hematopoietic origin, lack the requisite costimulatory molecules. Such viruses and tumors are dealt with by cross-primed $CD8^+$ T cells. Cross-priming refers to a process in which a pAPC acquires exogenous antigenic substrates from a donor cell (*e.g.*, a virus-infected cell or a tumor cell), which is not capable of priming naïve T cells on its own. The nature of the cross-priming "material" is still controversial and hotly debated. A hallmark feature of $CD8^+$ T cell responses to a wide array of Ags is immunodominance. This phenomenon dictates that although complex Ags harbor thousands of potentially immunogenic peptides, only few peptides elicit measurable T cell responses. Strikingly, the magnitude of responses to these peptides varies considerably among the responding T cell clones, thus creating a $CD8^+$ T cell hierarchy. Accordingly, immunodominant peptide epitopes induce robust responses, whereas subdominant T cell clones occupy modest ranks in the hierarchy. Many questions still remain regarding how immunodominance hierarchies are established, why $CD8^+$ T cell responses are generated only towards few epitopes and what can be done to increase the breadth of these responses. I will discuss some general features $CD8^+$ T cells including their activation by cross-priming and their immunodominance hierarchies. I will explain our results obtained from an *in vivo* model system in which we study $CD8^+$ T cell responses against a viral oncoprotein called large T Ag. Finally, I will highlight some of our recent observations on modulation of $CD8^+$ T cell cross-priming and immunodominance by regulatory T (T_{reg}) cells and by interfering with the mammalian target of rapamycin (mTOR) pathway. We believe that these findings have important implications for the treatment of cancer, infectious diseases and allotransplant rejection.
Keywords: $CD8^+$ cytotoxic T lymphocytes, antigen processing and presentation, cross-priming, immunodominance, antiviral immunity, anticancer immune responses

206. Synergistic Effect of Toll-like Receptor 4 and 7/8 Agonists is Necessary to Generate Potent Blast-Derived Dendritic Cells in Acute Myeloid Leukemia

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Background: DCs are professional antigen presenting cells recognized as key regulators of the immune system. Active dendritic cell (DC) immunization protocols are quickly obtaining interest as an alternative therapeutic approach in patients with acute myeloid leukemia (AML). Despite apparent progress in DC-based immunotherapy, some discrepancies were reported in generating potent DCs. Leukemic cells of AML patients could be differentiated to DC-like cells possessing the ability of stimulating anti leukemic immune response. Materials and Methods: Here we generated DCs from various subtypes of AML blasts. For DC maturation, combination of TLR agonists was used. Leukemic Blasts from 15 AML patients were differentiated into functional DCs by culturing in the presence of GM-CSF and IL-4 for 6 days. Then, immature AML-DCs were cultured in the presence of maturation factors for an additional day. The morphology, expression of key surface molecules, allostimulatory activity and phagocytic function of resultant DCs were compared with immature DCs and primary blasts. Results: The results showed that concomitant use of TLR4 (LPS) and TLR7/8 (R848) agonists were induced highly efficient DCs with increased IL-12 production and substantial capacity of allogeneic T cell activation. Conclusion: Combination of TLR4 and 7/8 agonist could be regarded as an appropriate maturation cocktail for AML-DC production and its potential use for immunotherapy of AML patients.
Keywords: TLR4 and 7/8, DC, Acute Myeloid Leukemia

207. SS1 Oncolytic Virus as a Novel Cancer Immunotherapy Agent

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Oncolytic viruses are an expanding family of anti-cancer agents which have entered clinical trials. Re-engineering the tropism of viruses is an attractive translational strategy for targeting cancer cells. While direct targeting of cancer cells is achieved by these agents, stimulation of immune system also plays an important role in their anti-tumoral effects. When it comes to oncolytic Herpes simplex Virus (HSV), the interaction with immune system is an essential part of the cytotoxic effects. We have generated the first oncolytic HSV-1 prototype (named as Signal-Smart 1 or SS1 virus) designed to response to Ras pro-oncogenic cell signaling pathway and tested its anti-cancer activity in prostate and pancreatic cancer cells. A significant level of cytotoxicity was observed in targeting cancer cells with elevated levels of Ras signaling pathway. Additionally, metastatic and colony formation capabilities of these cells was markedly decreased upon exposure to SS1. In this presentation we will review our data about the effects of SS1 virus in causing tumor regression in mouse model and the role of immune system in boosting the anti-tumoral effects of this virus.
Keywords: Virus, Cancer, Immunotherapy

208. Co-Transfer of CD3 Molecules Enhances the Anti-tumour Responses of CD8$^+$ TCR-Transduced T cells

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The function of T cell receptor (TCR) gene modified T cells is dependent on efficient surface expression of the introduced TCR a/b heterodimer. We tested whether endogenous CD3 chains are rate-limiting for TCR expression and antigen-specific T cell function. We show that co-transfer of CD3 and TCR genes into primary murine T cells enhanced TCR expression and antigen-specific T cell function *in vitro*. Peptide titration experiments showed that T cells expressing introduced CD3 and TCR genes recognised lower concentration of antigen than T cells expressing TCR only. *In vivo* imaging revealed that TCR+CD3 gene modified T cells infiltrated tumors faster and in larger numbers, which resulted in more rapid tumor elimination compared to T cells modified by TCR only. Following tumor clearance, TCR+CD3 engineered T cells persisted in larger numbers than TCR-only T cells and mounted a more effective memory response when re-challenged with antigen. The data demonstrate that provision of additional CD3 molecules is an effective strategy to enhance the avidity, anti-tumor activity and functional memory formation of TCR gene modified T cells *in vivo*.
Keywords: CD3, CD8$^+$, TCR-Transduced T cells

Poster Discussion Presentation

209. Antitumor Effect of Arteether; Evaluation of Immunoregulation & Regulatory Tcells

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Background: Removing tumor cells by specific methods is one of the major steps in cancer treatment that could increase the success of anti-cancer therapy. This in one hand could destroy cancer cells and on the other hand could increase the immune responses toward tumor. Arteether is an oil-soluble derivative of Artemisinin, which can induce apoptosis in tumor cells, but not normal ones. Materials and Methods: First of all, delayed-type hypersensitivity test and Hemagglutination test were carried out on normal female mice sensitized by sheep RBC in order to specify suitable Arteether dosage, which can be stimulate immune system. Subsequent experiments were carried out on tumor-bearing Balb/c mice to estimate the effects of Arteether on tumor growth and antitumor immune responses. Briefly 6mg/kg/day of Arteether and Arteether diluents were administered for13 consecutive days to group 1and 2 via intraperitoneal (IP) rout, respectively. Tumor sizes were measured using a digital vernier calliper (accuracy of 0.01). Mice were sacrificed and splenocytes harvested for lymphocyte proliferation assay and also the level of IL-4 and IFN-γ cytokines and the percentage of splenic Treg cells were measured. Result: According to the findings, Arteether could increase DTH reaction but it had no effect on hemagglutination antibody production in normal mice. Arteether could decrease the tumour growth rate (p-value<0.05). Proliferation assay did not show any significant difference (p-value>0.05). There were not statistically differences between groups at level of IFN-γ and IL-4(p-value>0.05); finally our result showed that Arteether is effective in the depletion of splenic Treg cells (p-value<0.05). Conclusion: In general these results introduce some antitumor properties of Arteether in vivo that may open up new insights into development of more effective antitumor agents.
Keywords: Arteether, Breast cancer, Immunotherapy.

210. Reduced Efficacy of Repeated Doses of CpG-Matured Dendritic Cell Tumor Vaccine in an Experimental Model

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Background: Dendritic cells (DCs) play a central role in the induction, programming and regulation of tumor-specific immune responses. It is suggested that CpG motifs stimulate the maturation of DCs. Base on our pervious study, a single dose of CpG-matured DCs was not sufficient for complete tumor eradication, so in the current study we hypothesized if CpG-matured DCs administrated more than one dose can augment the antitumor immunity.
Materials and Methods: WEHI164 cells were implanted subcutaneously in the right flank of *BALB/c* mice. During DCs culture, tumor lysate was added to immature DC and after 4-6 hours CpG-ODN or control CpG were added. One, two or three doses of CpG or control CPG-matured DCs

were injected around tumors at 7, 10 and 13 days after tumor implantation, respectively. Estimation of cytotoxic activity, tumor growth rate, survival percent and FoxP3 expression were done.Results: Our result showed that intratumoral injection of three doses of CpG-matured DCs led to considerable decrease lysis percent and significant increase in tumor growth rate in compare to one dose. Significant increased expression of FoxP3 was also seen in tissue samples obtained from mice received three doses. Conclusions: We concluded that administration of CpG-matured DCs more than one dose result in decreased antitumor immunity in mouse model in association with increased expression of FoxP3.
Keywords: Dendritic Cell, CpG; Vaccine, FoxP3, Tumor Immunotherapy

211. Selection of **Single Chain Antibodies** against Six Transmembrane Epithelial Antigen of the Prostate-2 (**STEAP**2)
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Background: Prostate cancer is the most common malignancy and the second leading cause of cancer death in men.Six transmembrane epithelial antigen of the prostate-2 (STEAP-2) is up-regulated in multiple cancer cell lines including prostate, bladder, colon and ovarian, with restricted expression in normal tissues. STEAP family functions as a channel/transporter protein in cell-cell junctions. It has been shown that STEAP-2 has a role in the secretory/endocytic pathways with an electron transfer domain. Antibody engineering has provided production of single chain antibodies which are very helpful in targeted therapy due to properties such as high affinityand small size. In the present study we intended to select specific single chain antibodies against STEAP-2. Materials and Methods: A highly diverse library of scFvs was panned against an immunodominant epitope of STEAP-2. After four rounds of panning, PCR was done on 20 colonies. DNA fingerprinting usingBstN1 restriction enzyme was done on the clones to find the common pattern among them. Results: After panning, 3 predominant patterns were detected. One predominant pattern was obtained with frequency 35%. The other patterns showed frequencies 10% and 5%.The most common pattern (frequency 35%) was selected for further investigation. Conclusions: Immunotherapy has recently been introduced as a novel therapy for the treatment of prostate cancer. Monoclonal antibodies are currently being investigated for the management of prostate cancer. Alternative approaches based on immunotherapy have had advances in identification of tumor-specific and tumors associated antigens and have shown therapeutic promise. In the present study we used panning process to selected specific scFv against prostate cancer antigen (STEAP-2). Successful panning was done and a pattern with 35% frequency was selected which represent the specific scFv against STEAP-2 peptide. More investigations are needed to evaluate the effects of the selected scFv.
Keywords: STEAP-2, single chain antibodies, Prostate cancer

212. Anti- Tumoral Effects of Punica Granatum Var.Spinosa Peels in Inducing Apoptosis in PC3 Cancer Cell Line, Model of Prostate Cancer
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Background: ethnic herb *Punica granatum* var.*spinosa* is one of the drug herbaceous species diversity that has allocated large areas of steppe regions, dry plains and mountainous regions of Golestan province in the Iran due to ecological requirements and resistance to various environmental stresses. Previous studies have demonstrated the anticarcinogenic activity of punicagranatum extracts in a series of human cancer cells. In the present study, we investigated cytotoxic and apoptotic effect of *punica granatum* var.*spinosapeels* extract on pc3 human prostate cancer cells. Materials and Methods: Test MTT for properties of cell cytotoxicity activity and viability on pc3 cell in times of 24, 48 hours in different concentrations of ethanolic extract was done. ELISA method to study apoptosis within 24 hours in different concentrations was also performed. Results: The ethanolic extract showed the plantpunica granatum peels ability to inhibit the growth of pc3 cells at all tow times is listed. Ethanolic extract caused cell morphology changes to be apoptotic cells. Conclusion: By increasing the concentrations and incubation of extract, cell viability is reduced. Also ethanolic extract of *punica granatumpeels* causes apoptosis in this cell line.
Keywords: punica granatum var.spinosa peels, apoptosis, cytotoxic, PC3

213. Immunomodulatory Effect of *Lactobacillus delbrueckii* on Breast Cancer of Inbred BALB/c Mice
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Background: Breast Cancer made 458,503 of women deaths worldwide in 2008. The probiotic therapy was using for the breast cancer prevention and treatment. Probiotics may be helpful and has anti-mutagenic effects. This study was evaluated the *Lactobacillus delbrueckii subsp. Bulgaricus* probiotic effects on immunity shift in inbred BALB/C breast cancer model. Materials and Methods: The MCF-7 model of breast cancer in female homozygous inbred BALB/c mice (2-3 weeks) was used. Bacteria were cultured on MRS-agar (1×10^9 CFU/ml). The animals were divided into 3 groups as probiotic, MCF-7 control and naïve control. Besides normal feeding, bacteria were fed daily by gastric intubation (200 µl of live bacteria) or 200 µl of PBS in last groups. The treatment was started 7 days before and continued until day 50 after cancer inoculation. The final blood serum and cultured stimulated splenocytes supernatant cytokines (IL-2, IL-4 and IFN-γ) were measured by ELISA kits (Bender Medsystem, Astria). In brief, ELISA plates were coated with anti-cytokine antibody (overnight procedure) and the antibody was blocked with PBS/BSA 1%. A proper standards were prepared with known concentrations of cytokines, covering the detection range of 15.62–1000 pg/ml (incubate overnight). The data were analyzed by one-way ANOVA and Tukey's post hoc test. Results: In the probiotic group the level (pg/mL) of blood serum IFN-γ and IL-2 were higher than that of MCF-7 control group but the level of IL-4 was low. The level of supernatant IFN-γ and IL-2 of probiotic group were higher than that of the MCF-7 control group but the level of IL-4 was low. The differences were significant statistically. Conclusion: The probiotic *Lactobacillus delbrueckii subsp. Bulgaricus* has immunomodulatory effect and it can enhance the immune system against tumor cells by shifting the immunity to cellular type in breast cancer.
Keywords: Lactobacillus delbrueckii, Breast Cancer, Probiotic, MCF-7, BALB/c

214. Antitumour activity of Aged Garlic Extract in Reduceing $CD4^+$ $CD25^+$ $FoxP3^+$ *T* reg Cells in Invasive Dactal Carcinoma in Mice by Aflatoxin-B1 (AFB_1)
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Background: AFB_1, a secondary metabolite of *Aspergillus flavus*, is a hepatocarcinogen in human and can invader tumor cells. If human receive little dosage of AFB_1daily in long time, it will been carcinoma. Among all type of cancers, breast cancer has a top position in women's death. The increasing speed of cancer research cannot catch up with breast cancer growth and many people suffer from it, for this reason, cancer control is a major health issue. There are several cancer therapy but all of them has a specific problem,therefore it is necessary to find a new method for cancer therapy that at first kill tumor cells in a specific manner. Garlic have wide range of biological activities that have been verified in vitro and in vivo. Our previous studies demonstrated that Aged garlic have enriched immunostimulator fractions and reduced immunosuppressor fractions. Therefore in this study we used from Aged Garlic Extract (AGE) instead of fresh garlic extract. Materials and Methods: First of all, AFB_1separated of Aspergillus flavus(PTCC 5004) by HPLC and AGE extracted by Mantis method and DTH and Hemagglutination test were carried out on normal female mice sensitized by sheep RBC in order to specify suitable AGE dosage, which can be stimulate immune system. Subsequent experiments were carried out on tumor-bearing Balb/c mice to estimate the effects of AGE and AFB_1 on number Treg cell. Briefly 10µ/kg/day of $AFB_1$and AGE diluents were administered for 4 consecutive days to group 1: AFB_1, 2: control of tumor, 3: AGE + $AFB_1$and 4: AGE via intraperitoneal (IP) rout, respectively. Mice were sacrificed and splenocytes harvested and the percentage of splenic Treg cells was

measured by Flow cytometry Analysis. Result: According to the findings, AGE could increase DTH reaction and decrease the Treg number rate in spleen (p-value<0.05). AFB_1 could increase the Treg number rate in spleen (p-value<0.05). Conclusion: In general these results introduce some antitumor properties of AGE and tumorogenic properties of AFB_1in vivo that may open up new insights into development of more effective antitumor agents.
Keywords: AFB_1, Garlic, cancer, Immunotherapy

215. Polarization of T Lymphocyte Responses Induced by Allogenic Tumor Antigen Pulsed Dendritic Cells
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Background: Dendritic cells (DCs) induce tumor or pathogen-specific T cell responses in humans. Several laboratories have developed culture systems including maturation factors for human DC from peripheral blood monocytes. We comprehensively studied the effect of addition of poly (I-C) to standard maturation stimulus, monocyte-conditioned medium (MCM) and TNF-α on maturation of monocyte derived DCs and their ability to elicit T cell responses. Materials and Methods: A short (4-day) priming of plastic adherent monocytes with granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) followed by allogenic tumor antigen pulsing and addition of MCM and TNF-α with or without poly (I-C) to generate fully mature and stable DCs. Phenotypic and functional analysis were carried out using anti CD14, anti HLA-DR and anti CD83 monoclonal antibodies, mixed lymphocyte reaction (MLR), phagocytic activity and cytokine release by DC stimulated T lymphocytes. Results: We found that fully matured DCs with large amount cytoplasm and copious dendritic projections were visible at the end of culturing period in the presence of MCM and TNF-α with or without rpoly (I-C). Thus, DCs generated with these maturation factors are nonadherent and have typical satellite morphology. Flow cytometric analysis using anti-CD14 (monocyte marker), anti HLA-DR and anti-CD83 (mature DC marker) revealed that addition of poly (I-C) to conventional maturation factors results in increased expression of all three markers in variable amounts. Functionally, MCM and TNF-α with poly (I-C) treated DCs showed a little stronger mixed leukocyte reaction Flow cytometric and microscopic analysis of phagocytic activity showed that addition of poly (I-C) reduced FITC-conjugated bead uptake and increased mean fluorescent intensity (MFI) of phagocytic DCs. Furthermore our results of cytokine release assay revealed that additional treatment of DCs with poly (I-C) results in reduction of IL-12:IL-10 and IFN-γ:IL-4 ratios in DC and DC-primed T cell supernatants respectively. Conclusion: Our results support this idea that use of the MCM, TNF-α and poly (I-C) as maturation factor could gnerates more mature monocyte derived DCs that prime T lymphocytes to TH2 type cytokine release.
Key words: Dendritic cell, Maturation, Monocyte Conditioned Medium (MCM), TNF-α, poly (I-C)

216. New Approaches in Anti-Tumor Effects of Heat Shock Proteins,*Bacillus* Calmette-Guérin, Angiogenesis Inhibitor and Immuno-Modulator Drug on Fibrosarcoma in Mice
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Background: Despite the major advances in conventional forms of treatment (surgery, radiotherapy and chemotherapy), there are still many patients that are resistant to standard treatments. Immunotherapy is one of the best strategies in cancer therapy. Four important current methods include use of heat shock proteins (HSP), *Bacillus* Calmette-Guérin (BCG),angiogenesis inhibitors and Immuno-Modulator Drug (IMOD). Materials and Methods: For this study 30 male Balb/c mice (age, 6-8 weeks) were provided and were divided to 10 triplicate groups.WEHI-164 tumor cells were used for creation of fibrosarcoma tumor. First, 1×10^6 cells\100µl of WEHI -164 cells were injected to mice and after 11 days, tumors were created. Then, surveys were carried out on the study protocol. Results: Nevertheless injection of certain amount of tumor cells to all of mice, tumor was not created in 5 mice and the size of tumors was different. The size of tumors was measured each week in special day and the volume of tumor was yielded by the formula: length×width2×π/6 in mm^3. The survey of tumors was continued until end of 14^{th} week. In some of groups, drugs showed synergistic effects and inhibited tumor growth significantly. Mice in group 8 (IMOD& Thalidomide) had the least lifetime and the group 9 (BCG& HSP & Thalidomide) had the longest lifetimes.
Conclusion: In some groups, drugs showed synergistic effects and inhibited tumor growth significantly. The BCG + HSP + Thalidomide Group showed very good results. Two out of three mice became healthy and one died in early weeks which it cannot be said that was just due to tumor, because the size of tumor wasn't too big. In this group, Thalidomide inhibited angiogenesis and HSP had role in presentation of tumor antigens to immune system and BCG had a role in enhancing of immune system. Therefore, this study shows the inhibition of tumor growth with simultaneous application of drugs.
Keywords: Cancer, Thalidomide, HSP, BCG, IMOD

217. Evaluation of Anti-Tumor potential of Bacillus Calmette-Guerin, Thalidomide angiogenesis inhibitor, Heat shock proteins and Bifidobacterium simultaneousness on Fibrosarcoma Tumor in Balb/c Mice
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Background: Cancer can be treated by surgery, chemotherapy, radiotherapy and immunotherapy. The choice of therapy depends upon location and grade of the tumors, but we decided to find better way to treat cancer and help cancer patients in clinical trials. In this research we examine the efficacy of four important immunologic approaches in mice alone and simultaneousness. Materials and Methods: For this study, male mice, 6-7 weeks old were prepared, and divided to desired groups. Initially, WEHI-164 cells were injected to mice and after 11 days tumors were created. Next, studies were performed according to the following protocol. Results: Taken together, these surveys revealed that, nevertheless injection of certain amount of WEHI-164tumoral cells to all of mice, tumor was not appeared in 3 mice. The size of tumors as compared to all tumors wasn't similar. The size of tumors measured each week in certain day. To determine the volume of tumor we used this formula: lengh×width2×π/6 in mm^3. Measurement of tumors was continued until end of 14^{th} week. The best results inhibition of tumor growth were seen in the group (BCG &HSP &Thalidomide) compared to control group. Conclusion: The aim of this study was to determine anti -tumor effects of BCG, Thalidomide, and Bifidobacterium and HSP simultaneousness on Fibrosarcoma. The group of (BCG, HSP & Thalidomide), significantly, showed synergistic effects and inhibited growth of tumor. In this group, BCG enhanced of immune system, Bifidobacterium can promote immune system, Thalidomide inhibits angiogenesis and HSP presents tumoral antigens to immune system. Thus, concurrently application of drugs helped to immune system for killing tumoral cells.
Keywords: Fibrosarcoma, Tumor, BCG, HSP, Thalidomide and Bifido bacterium

218. The Effect of Thermotherapy on NK Cell Cytotoxicity against a Liposarcoma Cell Line (SW-872)
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Background: Hyperthermia is a promising way to improve cancer treatment. However the molecular mechanisms underlying the clinical effects of hyperthermia are still poorly understood, it is hypothesized that hyperthermia could have both direct effects on tumor cells and indirect effects on lymphocytes and NK cells which may be involved in the control of tumor growth. Therefore, in this study we investigated the effect of hyperthermia on *in vitro* induction of NK cell mediated cytotoxicity against tumor cell lines. Materials and Methods: Tumor cells (SW-872 and HeLa cell lines as target cells) and NK cells (NK92-MI cell line as effector cells) were subjected to heat treatment at 39°C and 42°C for 1h. NK cytotoxicity was determined by LDH release and Annexin-V/7-AAD assays. The expression of NKG2D ligands (MICA/B, ULBP1 and ULBP2) was determined in target cells by quantitative real time PCR and flowcytometry methods. Results: In contrast to HeLa cells, susceptibility of SW-872 cells to NK cell cytotoxicity was significantly increased 12h after heat treatment at 39°C while NK cell cytotoxicity was not increased when

both target cells and tumor cells were subjected to heat treatment. Expression level of NKG2D ligands in target cells showed no changes following the heat treatment.Conclusion: However the susceptibility of tumor cells to NK cell cytotoxicity is somewhat different among various tumor cells, the results of this study demonstrated that heat treatment at 39°C could improve cytolytic activity of NK cells against SW-872 target cells without increasing the NKG2D ligands.

Keywords: Hyperthermia, NK cell cytotoxicity, Annexin v, NKG2D ligands, Liposarcoma.

219. Evaluation of scFv Antibody against Regeneration and Tolerance Factor (RTF) in Glioblastoma Cell Lines

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Background: Glioblastoma is the most common and malignant brain tumor in human. Targeting of glioblastoma-associated antigen such as Regeneration and Tolerance Factor (RTF) can be considered as a novel therapeutic method. Recombination DNA technology allows the production of high affinity and small scFv antibodies. We have already selected specific scFvs against RTF. In this study we describe the assessment of the anti-RTF scFv on glioblastoma cell lines. Materials and Methods: Specific scFv clones against RTF (selected from a phage antibody display library of scFv in our previous study) were cultured and M13KO7 helper phage was used to rescue the scFvs. Phage-ELISA was done to assess the binding of scFv to RTF epitope and measuring the phage antibody reaction against RTF. The anti-proliferative effects of selected scFv on glioblastoma cell lines (U87 MG and A-172) were evaluated using MTT assay. Results: positive phage ELISA was obtained and the average absorbances 0.441, 0.132, 0.142, and 0.136 were obtained for RTF peptide, unrelated peptide, unrelated scFv, and M13KO7 respectively. The baseline reading from the wells with no peptide was 0.075. Following treatment of U87 MG and A-172 cell lines with different concentrations of scFv against RTF no inhibitory effect was observed. Conclusions: the lack of effective therapies for patients with glioblastoma makes the invention of new strategies for glioblastoma treatment necessary. Tumor-associated antigen targeting by scFv antibodies can be a treatment option. Although we have already shown the growth inhibitory effects of the anti-RTF scFv on prostate cancer cell line, the results showed no inhibitory effects of the selected scFv against glioblastoma cells. This might be due to the lack of accessibility of RTF to scFv because of antigen masking phenomenon, the existence of compensatory mechanism (s) or the presence of other isoform(s) of proton pump in brain tumor cells.

Keywords: scFv Antibody, RTF, Glioblastoma Cell Lines

220. Anti-proliferative Effects of Anti-RTF scFv Antibody on Prostate Cancer Cell Lines

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Background: Prostate cancer is the second leading cause of cancer-related death among men. Current therapeutic options for patients with prostate cancer are not successful methods. Therefore, the need for novel therapeutic strategies seems inevitable. Among these strategies scFv antibodies can target prostate-associated antigens such as Regeneration and Tolerance Factor (RTF) which is over-expressed in prostate cancer cells. Recombinant DNA technology has provided the production of single chain antibodies. These antibodies have several advantages in clinical applications. In this study the anti-proliferative effects of scFv to RTF in prostate cancer cell line were assessed. Materials and Methods: one specific clone against RTF antigen (obtained from our previous study) was rescued using M13KO7 helper phage. The anti-proliferative effects of the selected scFv in prostate cancer cell lines (PC-3, Du-145, and LNCaP) were investigated using MTT assay. Results: The percentages of growth inhibition induced by anti-RTF scFv antibody in treated cells with 500 and 1000 scFv/cell after 24 hours were: 45% and 48% in PC-3, 32% and 39% in Du-145, and 25% and 27% in LNCaP. The results of the assay after 48 hours were 37% and 39% in PC-3, 14% and 20% in Du-145, and 18% and 23% in LNCaP compared to the controls ($P < 0.05$). Conclusions: Finding new and effective therapeutic strategies for treatment of prostate cancer is necessary. Recombinant antibodies can be considered as good candidates for immunotherapy. It has been shown that RTF targeting can limit ovarian carcinoma cell growth in vitro. In this study we select scFv antibody to RTF to evaluate their effects on prostate cancer cell lines. Our findings showed that cell growth was significantly inhibited in three selected cell lines treated with anti-RTF scFv. These results suggest anti-RTF scFv as a potential agent for prostate cancer immunotherapy.

Keywords: Anti-proliferative effects, Anti-RTF scFv Antibody, Prostate Cancer Cell Lines

221. Transfection of GL26 Murine Glioma Cell Lines with mIL-27 cDNA for Glioma Immunogenetherapy

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Glioma is associated with a state of immunosuppression, which appears to be partially mediated by an increased secretion of TGF-b from glioma cells.Patients with gliomas exhibit a broad suppression of cell mediated immunity due to shift in cytokine secretion from Th1 to Th2. Some of IL-12 family cytokines have high potential to induce TH0, TH1 shift by means of IFN-gamma secretion. Interleukin 27 is a newly discovered cytokine, which
consists of a heterodimer of p28 and EBI3. It has been shown that IL-27 acts on immune cells directly or indirectly to promote IL-12 and IFN-gamma production. Antitumor and anti-angiogenic effects of IL-27 have been previously reported. Initial studies indicated that IL-27 promotes Th1 type responses and inhibits tumor development. Additionally, IL-27 possesses potent anti-angiogenic activity, which plays an important role in its antitumor and anti-metastatic properties. Research on IL-27 has revealed the antitumor character of this cytokine. Given that IL-27 has much less toxicity compared with cytokines of the IL-12 family, IL-27 may be an attractive candidate for cancer immunotherapy. Since the effect of IL-27 on synegenic mice glioma has not been demonstrated, we have successfully undertaken the task to develop a tumor cell (GL26) transfected with the IL-27 gene. The aim of this study is to transfect GL26 with mIL-27 cDNA, then the characterization and functional analysis for immune gene therapy of synegenic mice glioma will be performed. Expression of the IL-27 protein in the tumor cell line was confirmed. In the next stage of this work we will treat tumors in the synegenic model using the transfected tumor cell.

Keywords: IL-27, Glioma, Cell Culture, Transfection, Brain, Immunogene therapy

222. Evaluation of Synergic Effect of GP96 Rich Lyset and Naloxone for Immunotherapy of Fibrosarcoma Tumor in BALB/C Mice

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Background: Immunotherapy is a strategy for treatment of some tumors. The goal of this study is the using gp96-tumor peptide complex and its combination with naloxone to achieve of cellular immunity against tumors. Gp96 is protein in the membrane of endoplasmic Reticulum .They bind to endogenic peptide like tumor antigen therefore can be up taken by APCs and presented by MHC-1 to cytotoxic T lymphocyte to induce cellular immune responses. Materials and Methods: After culture, cells were lysed and supernatant were collected. 66 KD isoform was purified by affinity chromatography, and confirmed by western blotting. The mice made tumoric by injection of tumor cells and divided to four groups. Control was injected by PBS, test 1 was injected by naloxone, test 2 was injected by gp96-tumor peptide complex and test 3 was injected by combination of naloxone and gp96-tumor peptide complex. After several days, tumor volume was recorded,and the mice were killed . Then splenic cells were extracted and MTT test was done for proliferation study. Supernatant were assayed by ELISA for measuring IL-4 and IFN-gamma. Spleen and tumor tissue were evaluated for CD4+ T cell, CD8+ T cell, CD25+ T cell, Foxp3+ T cell and CTL assay. Results: The purified proteins were analysed by SDS-PAGE and western blotting. There was single band that reacted with specific antibody. Findings showed that the immunized mice in test 3 have significant reduction in tumor size on days 27 and 32. The measurement of the CD4+CD25+Foxp3+ T

lymphocytes indicated that co-administration of gp96 and naloxone significantly decreases the CD4+CD25+Foxp3+ T lymphocytes. Results indicate a significant decrease in tumor-infiltrating CD4+CD25+Foxp3+ T lymphocytes in the test 3 compared with other groups. Conclusion: Results of the present study demonstrate that naloxone is an effective immunoadjuvant. Since naloxone exhibit no (or little) toxicity compared to the otker adjuvants this could have important implications in anti-tumor vaccine design.
Keywords: Affinity chromatography, purification, gp96, Naloxon, gp96-tumor peptide complex, immunotherapy, Fibrosarcoma.

223. Fractionation of Toxoplasma Gondii Protein Extract for Generation of Potent Dendritic Cells

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Background: Dendritic cells (DCs) recognize pathogen-associated molecular patterns (PAMPs) through their toll-like receptors which leads to maturation and production of cytokines such as IL-12, an important licensing cytokine that mediates the polarization of activated CD4+ T cells to a Th1 phenotype such that they provide help for the generation of potent CD8+ CTL responses against cancers. Dendritic cells (DCs) are professional antigen presenting cells (APCs) that both initiate and modulate the immune response. DCs can be induced in vitro to secrete IL-12. Therefore, their use for the active immunotherapy against cancers has been studied with considerable interest. We have investigated the role of Toxoplasma gondii (TG) protein fractions as PAMPS for DC maturation to induce IL-12 production.Materials and Methods: DCs were generated from Balb/c mouse bone marrow in the presence of GM-CSF and IL-4. Different proteins fractions obtain from sonically fragmented cells by precipitation with different percents of ammonium sulfate were added as maturation factors. The morphology and cytokine production of DCs were compared with immature DCs. Results: Our results showed that the total TG protein and some ammonium sulfate fractions induced efficient DCs.Conclusion: The reported fraction can be used in DC cell based immunotherapy protocols to generate competent vaccines for inducing proper T cell responses.
Keywords: Dendritic cell, immunotherapy, Toxoplasma gondii

224. Evaluation of the Effect of MUC1/Y DNA Vaccine on Cellular and Humoral Immune Response in Mice

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Background: Epithelial mucin is a glycoprotein which is expressed by different kinds of epithelial cells and is a suitable target for active immunotherapy. This research has been done to construct a plasmid DNA vaccine containing MUC1/Y gene in order to evaluating the immune system of C57BL/6 mice in response to an isoform lacking variable number of tandem repeats (VNTRs), relying on just the adjuvanticity ability of own plasmid. Materials and Methods: MUC1/Y cDNA from a grade III breast cancer patient (Iran, Tehran) was obtained, amplified by PCR and was cloned into pcDNA3.1(+) vector and was sent for DNA sequencing. After LPS removal from the plasmids, mice were immunized with 100 µg of plasmid containing the gene (test group) and empty plasmid (control group) with weekly intervals, and a week after third immunization, were sacrificed. The immunostimulatory ability of the vaccine was estimated by measurement of the cytotoxic potential of mice splenocytes. One-way ANOVA was used for the comparisons among three vaccination groups and $p<0.05$ were considered significant (SPSS v. 16.0). Detection of anti-MUC1 antibody in mice sera were done by Western blot test. Results: The results showed that the cloning process was successful and the complete MUC1/Y cloned into the plasmid. Calculation of cytotoxicity percentage showed a significant difference between test and control groups: in test group, this amount was approximately 6 times greater than cytotoxicity percentage in control group ($p<0.001$). Although difference between variable ratios of effector and target cells were not significant in groups ($p=0.942$). Anti-MUC1 antibody was not detected in sera of test and control mice. Conclusion: Given the observed significant cellular immune response in the test group, albeit much less than the amounts reported in the literature and failure to detect antibody in these mice, it seems that MUC1/Y DNA vaccine just relying on immunostimulatory property of pcDNA3.1 plasmid, has the potential ability to elicit an effective cellular immune response and this ability may be improved if we use convenient adjuvants in future studies.
Keywords: Gene Cloning, Breast Cancer, Breast Neoplasms, Vaccines, Cytotoxicity Tests, MUC1

Poster Presentation

225. The Possible Role of Bacteriophages in Cancer Therapy

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The immune system is a complex network of specialized cells and organs that defends the human body against attack from foreign pathogens. The innate immune system cells involved in protecting the body against potential infections, also play an important role in combating tumor growth. Bacteriophages are among the most numerous creatures on earth and they are omnipresent. They are thus in constant natural contact with humans and animals. The ability of bacteriophages to reduce reactive oxygen species production by polymorphonuclear leukocytes in the presence of bacteria or their endotoxins is confirmed. Studies show that the high immunogenicity of bacteriophages may also be employed in anti-tumor treatment.
Keywords: Bacteriophages, Cancer, Therapy

226. Induction of Programmed Cell Death (Apoptosis) in Cancer Cell Lines WEHI-164 and PC3 by Metanolic Extract of Chelidonium majus

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Background: Medical plant Chelidonium majus from Papaveraceae family used by indigenous areas of Iran especially north of Iran. Extensive studies on Chelidonium majus compounds indicated anti-microbial and anti-oxidant activities. In the present study, the growth inhibitory effects of methanolic extract of Chelidonium majus and inducing apoptosis on prostate cancer cell (PC3) and fibrosarcoma cell line (WEHI-164) was investigated. Materials and Methods: 3-(4,5-dimethyl thiazol-2y1)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay was used for measuring the cytotoxicity cell and viability at 24 and 36 and 48 hours in 50,100,200,300,400,500µg/ml concentrations of methanolic extracts. Also, ELISA method was used to measure apoptosis and necrosis in different concentrations within 24 hours and also morphologic changes were evaluated. Results: The result showed that the methanolic extract had ability growth inhibitory and cytotoxic effect in PC3 and WEHI-164 cells in all three times and proved program cell death was happened. Conclusion: Increased concentration of extract and time reduced in cell viability. Also, methanol extract induced apoptosis in these cell lines. Generally these effects depends on concentration of methanolic extract of Chelidonium majus $P<0.01$).
Keywords: Chelidonium majus, cytotoxic activity, apoptosis, tumor cell lines

227. Different Strategies in Cancer Immunotherapy

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According to the recent World Health Organization (WHO) report, cancer is the third leading cause of death all over the world. Majority of the basic and clinical research is focused on cancer biology, pathogenesis and treatment. Immunotherapy is a great hope for cancer treatment. Studies on Immunotherapeutic strategies have shown that immunotherapy in early stages of cancer may lead to complete cure and in later stages can increase progression free survival period as well as better response to chemotherapy and radiotherapy. Immunotherapy is generally thought of as conferring either passive or active immunity. Monoclonal antibodies like Her-2 (Trastuzamab for breast cancer) and CD20 (Rituximab for B cell lymphoma) and cytokine therapy (GM-CSF, IL-12…) are examples of passive immunotherapy. Using cell based immunotherapy (autologous T cells and/or dendritic cells which are activated by cytokines or genetic engineering methods), bactrio-Immunotherapy (using of bacterial superantigens as adjuvant), Gene Immunotherapy (DNA vaccines) and viro-Immunotherapy (viral vectors and viral dsRNAs) are some examples of active immunotherapy. Clearly, different strategies demonstrate benefit in different patient populations and different individuals. It seems better results are obtained with vaccines in combination with a variety of antigens and antibodies and /or adjuvant (Combined Therapy). The effect of the above mentioned strategies in combination with traditional cancer therapies is another possibility, as it has been shown that there are some benefit in terms of duration with cytokines and chemotherapy.The ultimate desired goal might be a prolong anti –tumor response that can be maintained over the patient`s lifespan.

Keywords: Strategies, Cancer, Immunotherapy

228. Constraints of the Allogeneic GL26 / BALB/c Mouse Intracranial and subcutaneous Glioma Models: Concepts for Evaluating Immunotherapy

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Background: Glioblastoma Multiforme (GBM) illustrates many challenges amongst the cancer patients survival, despite current therapies, covering surgery, radiation therapy, and salvage chemotherapy. Improvement of glioma patients outcome depend upon correspondent animal models, to studying disease mechanisms and evaluating novel therapeutic strategies, in order to reducingside effects and toxic consequences of new drugs. In immunegenetherapy of glioma, synegeneic models are excellent, but the lack of access to syngeneic model in glioma, in some countries, inspired us to design intracranial and subcutaneous allogeneic models with usage of varied amounts of mouse glioma cell line GL26 cells within female BALB/c mice. Perhaps, we could detect this fundamental inquiry, whether GL26 has sufficient capacity to induce glioma in BALB/c allogeneic system as an alternative in-vivo model. Materials and Methods: We induced the subcutaneous and intracranial tumors, then; tumors were evaluated in terms of macroscopic and microscopic characteristics. Results: Microscopic analysis of the intracranial model, represented only a mild inflammatory reaction. In subcutaneous inoculation, tumors were induced faster within the groups with the higher number of cells inoculation. In macroscopic examination, the tumor was relatively large, thick and entirely full of blood. Moreover, in microscopic examination, cell proliferation, mitosis, abundant vessels and tumor necrosis were observed. Conclusion: Our data demonstrated that the use of GL26 cell line in BALB/c mice does not induce intracranial tumors while subcutaneous tumors are induced via high numbers of cells after an extended time period. It probably, can be considered as a successful model of allogeneic tumor rejection.

Keywords: Glioma , GL26, Intracranial, Subcutaneous, Immunotherapy, BALB/c mouse

229. Using Activated Natural Killer Cells in Tumor Immunotherapy

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Natural killer (NK) cells are a subset of lymphocytes with a distinct morphologic appearance (large granular lymphocytes [LGLs]) and the ability to spontaneously kill virally infected or tumor targets but to spare most normal cells. These effector cells are now known to be able to eliminate tumor cells by mechanisms involving either necrosis or apoptosis or both, and upon activation to produce and secrete a broad spectrum of cytokines. NK cells respond to a variety of biologic agents, including cytokines such as interleukin-2 (IL-2), IL-12 ,orinterferons, by upregulation of cytolytic, secretory, and/or proliferative functions. They represent from 5% to 15% of peripheral blood lymphocytes in humans and account for a substantial but variable proportion of tissue-resident lymphocytes. In rodents, NK cells are mainly found in the spleen, liver, and lung tissues as well as in the blood. Phenotypic characteristics of NK cells are variable, depending on the state of their activation, but surface expression of CD16 (FcγRIII), CD56 (in humans), NKR-P1 (in rats), or NK1.1 (in mice) and the absence of the T-cell receptor complex on the cell surface have been accepted as the markers defining these effector cells. In cancer-bearing hosts, NK cells have been considered to be the major component of antitumor immunity responsible for rapid elimination of blood-borne metastases. More recently, however, it has been realized that NK cells are also responsible for killing of tumor targets that have downregulated expression of major histocompatibility complex (MHC) class I molecules and are not recognized by tumor-specific T cells. So, these effector cells can be useful in immunotherapy of cancer.

Keywords: Natural Killer Cells, Tumor, Immunotherapy, Cancer, Apoptosis

230. The Effect of Temporin-Ra Peptide on the A549 Carcinoma Cell Line

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Background: Antimicrobial peptides are small polypeptides that modulate inflammatory process and innate immunity to inhibit the growth of microbes. The aim of the present study was to examine the effect of of antimicrobial peptide drived from*Rana ridibunda* at various concentrations (5, 10, 20, 30 µg/ml) on A549 cell line. After 6, 12 and 24 h, the proliferation was determined by MTT assay. Materials and Methods: Temporin-Ra 14-amino acid peptide was chemically synthesized (Shanghai, China) and purified using C18 HPLC. Cells from the A549 human lung carcinoma cell line were cultured in RPMI 1640 medium containing 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin.Viability of epithelial cells was determined by MTT assay. Cells were treated with various concentrations(5, 10 , 20 ,30 µg/ml) of peptide for 6,12 and 24h.Cell viability was assessed using 20 µl of a 5 mg/ml MTT solution in PBS, and incubated at 37°C for 4h. After removing the supernatant, 150 µl of dimethyl sulfoxide was added to dissolve the formazan crystals that remained in the wells. The absorbance was determined using ELISA reader at 570nm. Results: In this study the proliferation effect of antimicrobial peptides derived from *Rana ridibunda* were evaluated using MTT assay. According to the MTT assay, 9% proliferation was observed by increasing concentrations from 5to 30 µg/ml and the incubation time from 6h to 24h.
Conclusion: Airway epithelium forms a continuous barrier against potentially harmful inhaled agents. In response to epithelial injury, initiation a repair process comprises subsequent epithelial cell migration, proliferation, and differentiation is essential. Previous studies have shown that neutrophil defensins enhance proliferation of murine fibroblasts and retinal epithelial cells. Jamil Aarbiou et al. (2002) reported that neutrophil defensins enhance proliferation of cells from the airway epithelial cell lines A549 and NCI-H292.

Keywords: Temporin-Ra Peptide, A549 Carcinoma Cell Line

231. A novel Combine Auto-Immunotherapy and General Immunotherapy (G2 Vaccine with Th-1 activating) of Cancers with Th-1 activator adjuvant and Auto-antigen

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Background: There are immune-suppression reaction in all cancers. These suppression correlate with increasing of the size of tumors and also, depend to chemotherapy, radiotherapy and huge surgery. If these methods of treating could not clear of all cancer cell from patient boy completely, either a single cancer cell could grow and or could metastases to other organs and make new cancers. In this time the mentioned methods would low effect on cancers. There are two important immune responses which could kill cancer cells. The first one is NK cells, and second is CTLs. The reaction to among various immunological effecter cells, CTLs have been considered to play a crucial role in tumor rejection in vivo. CTLs can be defined as CD8+ T lymphocytes that kill tumor cells specifically in the MHC class I-restricted manner; however, many research works have indicated that non-specific CD4+ T lymphocytes capable of killing tumor cells with activation of NK cells and also capable of playing an important role in CTL-mediated tumor cell killing.The pre-clinical trial of immunotherapy for different cancers including, breast cancer, pituitary adenomas, hepatoma, glyblastoma, stomach cancer, esophagous caners, colorectal cancers, pituitary tumors and others, were done by the Th-1 cell activator adjuvants which have approved with ethical committee of the Tehran University of Medical Sciences as clinical trail of Th-1 activators in different diseases like allergic asthma and chronic urticaria and auto-antigens which have been prepared from patients. Materials and Methods: In this novel method more than 30 patients have got 24 up to 40 times s.c. injections of half ml of 20 ug/ml of G2 adjuvant as a Th-1 activator plus the same concentration of auto-antigens one, two or three times per week the same time. Auto-Antigens were prepared from fluid execration of patients with high speed centrifugation and dialyzing with saline for 24hrs at 4' C and concentrated up to 20 ug/ml of proteins and striled with 0.22 um filtrations. Results: The results of this method of immunotherapy could comparable with chemotherapy and radiotherapy depending the stage and grade of patients. Results of therapy were different from control to complete healing of cancers. The most positive results have seen in the intermediate stages of cancer, specifically in the breast cancer. Conclusion: This method may useful for treating of many other diseases.

Keywords: Auto-Immunotherapy, General Immunotherapy, Cancers, Th-1 activator adjuvant, Auto-antigen

DENDRITIC CELLS

Oral Presentation

232. Generation of Large Number of Highly Purified Dendritic Cells from Combined Cells of a Syngeneic Murine Spleen and Bone Marrow

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Background: Dendritic cells (DCs) are called the sentinels of the body because after their exposure to antigens they can present the processed peptides as the most effective APCs to $CD4^+$T cells to cause a protective immune response. In many experiments using DCs, they need more DCs to transfect viruses to them from the early days after bone marrow (BM) isolation. Here we report the novel finding that dendritic cells can be produced in large amount and highly purity from culturing BM and spleen cells (BMSP cells) with each other. We call this combined DC DC^{TME}. The rationale of this high generation of DCs is on three principles: first the existence of two sources of DC precursors in both BM and spleen tissues, second extreme cell-cell contact between the cells of BM and spleen in culture, and third GM-CSF and stromal cell dependent DC culture which shows the need of DC^{TME} to GM-CSF as a growth factor.Materials and Methods: In our study we have cultured BM and spleen precursors near each other in bacteriological petri dishes which may efficiently synergize DC generation. Of particular interest, we demonstrate the lowest need of BMSP cells to changing media because of the reparative role of the splenic stromal cells in which only renewing immature DCs need fresh complete media supplemented with GM-CSF every 2 to 3 days to support sooner DC differentiation. Results: DC^{TME} has a myeloid origin with lineage surface markers of $CD11c^{hi}CD11b^{hi}$ $CD8\alpha^-$ $F4/80^{LO}$ and also immature DC phenotypes with molecules of $MHCII^+$ $CD86^+$ $CD80^+$and $CD40^{LO}$. They express about 13% (±3%) more CD11c and produce lower IL-10 than bone marrow dendritic cells (BMDCs). Conclusion: This novel and economical method, which by using fewer mice shows an importance to animal ethics, dominates DC^{TME} cells as more effective cells in DC immunotherapy and vaccination.

Keywords: Dendritic Cells, Syngeneic Murine, Spleen, Bone Marrow

233. The Synergistic Effect of Toll-Like Receptor Agonists on Maturation and Activation of Dendritic Cells

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Background: Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system, with the potential to either stimulate or inhibit immune responses, based on their maturation state. DCs recognize pathogen-associated molecular patterns (PAMPs) through their toll-like receptors which leads to maturation and production of cytokines (such as IL-12p70). As pathogens may contain several TLR agonists, we sought to determine whether different TLRs cooperate in DC activation.Materials and Methods: DCs were generated from peripheral blood monocytes of 20 healthy donors in the presence of GM-CSF and IL-4. Different TLR ligand cocktails were added as maturation factors. The morphology and cytokine production of DCs were compared with immature DCs. Results: Our results showed that the combination of TLR4 (LPS) and TLR7/8 (R848) agonists together with TLR3 agonist [poly (I:C)] or TLR2/6 agonist (FSL-1), induced efficient DCs. Conclusion: The reported cocktails can be used in dendritic cell based immunotherapy protocols to generate competent vaccines for inducing proper T cell responses.

Keywords: Dendritic cell, Immunotherapy, Toll-like Receptor

234. Study of the Effect of Zinc on the Process of Autophagy in BCG Infected Macrophages

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Background:Eukaryotic cells can degrade their own components, cytosolic proteins and organelles, using dedicated hydrolases contained within the acidic interior of their lysosomes. This degradative process, called autophagy. The effective stimulus autophagy is starvation and the presence or absence of certain elements in this process is effective. In this study the effect of different concentration zinc of autophagy in peritoneal Balb/c mice macrophage was investigate. In this study the effect of different concentrations of the zinc on the process of peritoneal macrophages autophagy in Balb/c mice with in the face BCG was evaluated. Methods: Peritoneal macrophages was 6 and 9 Balb/c mice was harvested and divided to four group and infected with BCG as stimulus of autophagy. Different concentrations of zinc (10%, 50%, 100%) was added to each well of macrophage culture. after 5 days macrophage of each well were cultured stained and observed fluorescent microscopy followed electron microscopy was used as golden standard to confirm result .Results: Four groups considered here were studied in the first group that includes cell and BCG were completely degenerated into had died in the second group that includes cell and BCG and 10% zinc cells is completely died and the third group were included BCG, and 50% zinc, about 58% cells were alive. The fourth group consists of cells and BCG, 100% zinc, 98% of the cells were alive. Conclusion: The result shown that concentration zero and ten percent of zinc concentration does not any effect on autophagy and 50 and 100 percent of the increase autophagy. It could be concluded that higher concentration of zinc stimulate autophagy and protect cells against BCG ($p \leq 0/05$).

Keywords: Autophagy, Macrophage, BCG, Zinc

Poster Discussion Presentation

235. The Effects of Nanoparticles on Maturation of Dendritic Cells

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Background: The Dendritic cells are major antigen processing cells to naive T cells. Prepared nanoparticles were used for enhancement of the antigen capture and transfection of vector in dendritic cells. Concentration and surface charge of nanoparticles has different effects on dendritic cells maturation. Hence investigation of these effects on dendritic cell maturation is necessary.
Materials and Methods: Dendritic cells were separated from mouse spleen with magnetic bead method. Dendritic cell was cultured overnight in RPMI-1640 and 0.5% mouse serum and pulsed with different concentration of nanoparticles. Dendritic cells maturation was studied with using cell surface markers include CD40, CD86 and HLA-DR by flowcytometry assay. Results: The results will be presented in Congress. Conclusion: Nanoparticles can be the effect in dendritic cells maturation. Therefore, selection of optimize concentration of nanoparticles in antigen pulsing and vector transduction in dendritic cell is necessary.
Keyword: Dendritic cells, Nanoparticles, Maturation

236. Investigation of Maturation State of Spleen-Derived DC Before and After an Overnight Culture

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Background: after encounter with danger signal, the expression levels of CD86 and MHCII are considered among the markers of DC maturation. Interestingly, the expression of CD86 and MHCII are increased after an overnight culture of spleen – derived DCs In the absence of any stimulation. Therefore the aim of the present study was to investigate the maturation state of splenic DCs. Materials and Methods: In this study we have investigated MHCII and CD86 expression levels and dextran-FITC phagocytosis (as markers of maturation) in freshly isolated spleen dendritic cells before and after an overnight incubation. DCs were separated by centrifugation on the nycodenz gradient. Results: The results showed that expression levels of MHCII and CD86 were increased significantly after an overnight culture of spleen-derived DC ($p<0.02$). Conclusion: Mouse spleen DCs, are immature DCs and their isolation by nycodenz gradient has not any effects on DC maturation. However, culture affects DC maturation state. In this respect, cell-cell contacts and cell culture dish contact may be important for DC maturation.
Keywords: Maturation, Spleen-Derived DC

237. A Study on Ex vivo Generation of Dendritic Cells

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Background: Dendritic cells are the most potent antigen presenting cells. They capture, process and present antigens to T cells and secrete various soluble factors to initiate adaptive immune responses. Dendritic cells are also important in induction of immunological tolerance to self antigens. Their crucial role in immune responses during infections, cancers, transplantations, allergies, and autoimmune diseases has made them as an important target for many biological and clinical studies. Usually large number of dendritic cells is required for these studies; however, isolation of dendritic cells from blood or tissues is difficult. Therefore, ex vivo generation of these cells is useful for research and clinical applications. So far, various methods have been used for generation of dendritic cells from peripheral blood mononuclear cells or bone marrow cells in the presence of different cytokines/growth factors and various incubation periods. The aim of this study was evaluation of the most popular methods that has been utilized for generation of dendritic cells and examining the effects of GM-CSF, IL-4, TNF-α and duration of incubation period on properties of dendritic cells. Materials and Methods: Bone marrow-hematopoietic progenitor cells, without depletion of any cell population, were cultured in medium in the presence of GM-CSF and IL-4. TNF-α was added at the last day of culture period. Morphology and immunophenotype of the cells were analyzed at various time points. Results: Generation of dendritic cells was observed from day 2. however, the number of cells that posses the morphological characteristics and typical surface markers (CD11c, MHC-II, CD80, CD86, CD40) of dendritic cells was elevated by increasing the culture duration, and at day 9; 80% of cells had dendritic cell properties. The results also showed that both GM-CSF and IL-4 are important for generation of dendritic cells. Conclusion: This study presents a simple and efficient method for generation of dendritic cells from bone marrow precursor cells.
Keywords: Ex vivo, Generation, Dendritic cells

238. Phenotypically Comparison of IgG-differentiated Immature Dendritic Cell with GM-CSF & GM-CSF/IL-4 Derived Immature Dendritic Cell from Human Blood Monocyte Precursors

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Background: Immune complexes causes activation of the innate immune system and subsequent induction of host inflammatory responses. In particular, the binding of IgG immune complexes to FcγR on monocytes triggers differentiation into immature dendritic cells (iDC). Materials and Methods: In this study, we phenotypically compared the IgG-derived iDC with GM-CSF & GM-CSF/IL-4 iDC. CD14+ monocytes isolated from PBMC of healthy individuals. Isolated monocytes was added to different concentration (25,50,100µg/ml) of plate bound human IgG. monocytes were also cultured with GM-CSF & GM-CSF/IL-4. After 2 & 5 days, immature dendritic cellswere harvested and the expression of the DC markers (CD1a, CD1b, CD86, CD83) was measured by flow cytometry. Results: Our results showed that On day 2, IgG-derived iDC in comparison with GM-CSF iDC expressed lower levels of CD14 (5% vs 50%) , CD1b (7% vs 74%) , CD1a (0.1% vs 0.6%) , CD83(0.4% vs 0.6%). Immobilized IgG induced iDC in a dose-dependent manner. So On day 5 IgG-derived iDC in dose 100µg/ml human IgG vs 50 ,25 expressed higher levels of CD1a (10% vs 3.5% ,1.6%) , CD1b (24% vs 10% ,7%) , CD83 (8% vs 5% ,1.5%) & expressed lower levels of CD14 (3% vs 9% ,24%) & in comparison with GM-CSF iDC expressed lower levels of CD14(3% vs 60%), CD1b (24% vs 75%) & higher levels of CD1a(10% vs 0.8%), CD83 (8% vs 0.7%) & in comparison with GM-CSF/IL-4 expressed higher levels of CD83 (8% vs 3%), lower levels of CD14(3% vs 11.6%), CD1b (24% vs 75%), CD1a(10% vs 58%). Conclusion:Our data suggested that, the iDC induced by FcγR vs GM-CSF and GM-CSF/IL-4 were phenotypically and probably functionally distinct from each other.
Keywords: IgG-differentiated Immature Dendritic Cell, GM-CSF, GM-CSF/IL-4

Poster Presentation

239. Design and Development of an In-Vitro Cell Based Assay for Determination the Biological Activity of Rhu G-CSF

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Background: G-CSF is a pleiotropic cytokine best known for its specific effects on the proliferation, differentiation, and activation of hematopoietic cells of the neutrophilic granulocyte lineage. It is produced mainly by monocytes and macrophages upon activation by endotoxin, TNFα and IFNγ. In this study the proliferative activity of two recombinant human G-CSF (Neupogen and Neukine) was tested in culture using a mouse myeloblastic cell line NFS-60 cells. Materials and Methods: The potency is determined by comparison of the dilutions of test preparation

with the dilutions of the international satandard (NIBSC) by starting at a concentration of about 800 IU/ml, plus a series of 10 twofold dilutions in a 96-well microtitre plate.
50 µl of a NFS-60 cell suspension containing 3×10^7 cells/ml was added to above plate containing serial dilutions of samples and standard. After incubatin the plate at 37 °C for 72 h in a humidified incubator using 5 percent CO_2 , 20 µl of a 5mg/ml sterile solution of tetrazolium bromide (MTT) was added to each well and reincubated for more extra 5 h. 100 µl of a 240 g/l solution of sodium dodecyl sulphate previously adjusted to pH 2.7 with hydrochloric acid was added to each well for solubilizing the formazan product.The absorbance of each well was measured using a 96-well microtitre plate reader at 570 nm. The potency of samples were calculated using a suitable statistical method, for example the parallel line assay.
Results and Conclusion: Biological activity of Neupogen and Neukine were 3.34×10^7 and 2.5×10^7 IU/ml respectively. The estimated biological activities were not less than 80 per cent and not more than 125 percent of the stated (3×10^7 IU/ml) potency. The confidence limits (P= 0.95) of the estimated potency are not less than 74 percent and not more than 136 percent of the stated potency.
Key words: G-CSF, biological activity, proliferative assay.

240. Different Source Antigens For Induction Dendritic Cells In ImmunologicalLaboratory Technique

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Back ground: Dendritic cells (DCs) are professional antigen presenting cells. They can to induce a primary immune response in resting naïve T lymphocytes by capturing, processing and presentation of antigens on their cell surface along with appropriate costimulation molecules. Functions of dendritic cells are T cell activation,Immune tolerance andB cell stimulation. Our aim is search of presenting Sources of Antigens for Induction Dendritic cells in immunologicallaboratory Techniques. Material & Methods: Different Sources of Antigens for Induction Dendritic cells are searched and many articles are studied and classified. Thus their applications in immunological laboratory Technique are remembered.Results & Conclusion: Source of Antigens for Induction Dendritic cells classify in 8 groups that include: Peptides, Exosomes, Dead or Dying Tumor Cells, Recombinant Viruses, DNA Transfection, RNA Transfection, Cell Hybrids, In Vivo Targeting of DCs. Everyone have its Function and application for many immunologicallaboratory Technique example for Vaccine preparation for tumors, activation cells (T & B Lymphocyte) in culture and so on.
Keyword: Antigen, Dendritic cells, DC.

241. Effect of Adding Natural Oils to Culture Medium of *Malassezia Furfur* on IL-10 and IFN-Γ Levels in Co-Incubation of the Yeast with PBMC from Normal Volunteers

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Background: *Malasseziafurfur* is a lipophilic fungus, which lives as a saprophytic organism on human skin, and causes some skin diseases. The aim of this study is to evaluate the level of some cytokines in co-incubation of peripheral blood mononuclear cells (PBMCs) from healthy individuals with *M.furfur* grew in the presence of some different types of natural oils. Materials and Methods: PBMCs were obtained from blood samples of four normal volunteers, and using the RPMI, the PBMCs reached the concentration of 10^6 cell/ml. Then, *M. furfur* was cultured in specific groups on culture media containing almond oil (almond oil group- AOG), fish oil (fish oil group- FOG), walnut oil (walnut oil group- WOG), full-fat milk (full-fat milk group- FFMG), and fat-free (fat free group- FFG); and the yeasts grew were used for co-incubation with PBMCs *in vitro*. The IFN-γ, IL-10, and IL-12P70 levels in the supernatant were measured using ELISA method at different hours. Results: The IFN-γ and IL-10 levels in co-incubation of yeasts in WOG and FOG were higher than those in AOG and FFMG. However, in some groups the difference was not statistically significant ($P > 0.05$). Although the IL-12P70 was higher in some groups such as AOG, FOG, and WOG; the increase was not statistically significant. Conclusion: The results obtained demonstrated that the type of fat used by *M. furfur* can influence the immune system response and *in vitro* production of IFN-γ and IL-10.
Keywords: Natural Oils, *Malassezia Furfur*, IL-10, IFN-γ

242. Role of Tolerogenic Factors in Maintenance of Hematopoietic Stem Cell Therapy

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Background: Hematopoietic stem cell therapy (HSCT) as a promising therapy was used in many life-threatening Immune system disorders especially auto immune diseases. Allogenic HSCT is a procedure in normalization of bone marrow functions by reconstitution of patient immune system with safe donor HSCs. The main problem of allogenic HSCT could be graft versus host disease (GVHD). Materials and Methods: Pubmed and ISI data bases were searched using HSCT, T regulatory cells (Treg), Facilitating cell (FC), Toll like receptors (TLRs),Interleukin (IL) 7 and 15 as keywords. Results: Prosperity of HSCT is related to insight and augmentation of effective factors in maintenance of immune system tolerance after therapy. In this regard, (Treg) mainly host Tregs are sound the most important operator at persistence of HSCT predominantly at first term of transplantation. In case of increasing Treg in successful HSCT several factors could be involve: 1) Ablation of immune system and reduction of T cell repertoire coincidence with release of so many self antigen. 2) Generation of more potent signals because of cytokine storm that induce production of Treg more than conventional T cell, like IL-15 and IL-7. 3) Three groups of CD8α+ cells that celled facilitating cell (FC) which stimulate generation of TCD4+ CD25+ FOXP3+ from CD4+ CD25- in host spleen, mainly via TLR9 activation. Conclusion: HSCT could be a curative therapy for autoimmune diseases if the signals that affect it, were identified and reinforced. We categorized and reviewed how immune system impress and support HSCT.
Keywords: Tolerogenic Factors, Hematopoietic Stem Cell Therapy (HSCT)

243. Impact of Sodium Arsenite on Gene Expression of *GSTO2* in Jurkat Cell Line

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Background: Inorganic arsenic compounds are one of the most abundant components of environmental pollution specially in foods and drinking water which are well-known as carcinogenic and clastogenic elements that notably affects aboriginals whom exposed to this contaminated elements. Instantly after ingestion of contaminated foods or water, in firing line one of vulnerable cells that influenced with arsenicals are blood cells, specially lymphocytes which can reduce the potency of cellular and humoral immune responses. Although some gene products are nominated as factors for metabolize and detoxify arsenic compounds, such as *GSTO1*, *GSTO2*, *As3MT* and *PNP*. In the recent study we attempt to recognize the effect of sodium arsenite (iAs(III)) on gene expression of *GSTO2* at the low-dose. Materials and Methods: We used Jurkat cell line for treatment with iAs(III) and utilized MTT assay for recognition of dose that have minimum effect on cell inhibition. Real-time PCR is used for determination of mRNA level.*GAPDH* gene was used as normalizer. Results: According to MTT assay, 1[µM] of iAs(III) was chosen as concentration that has minimum effects on cell inhibition. With regard to gene expression of *GSTO2* in negative control the gene expression was reduced till 36.75% ($t= -7.68$, $df: 3$, $p= 0.005$). Conclusion: In view of *GSTO2* function for metabolizing and reducing toxicity of iAs(III) by binding of glutathione to iAs(III), reduction of *GSTO2* expression can increased the genotoxicity of iAs(III) through the production of reactive oxygen species that probably result to cell death or carcinogenesis.
Keywords: Sodium Arsenite, *GSTO2*, Jurkat Cell Line

IMMUNOBIOTECHNOLOGY & NANOTECHNOLOGY

Oral Presentation

244. Designing and Evaluating Highly Efficient Sirnas for Silencing RORC2 in Human TH17 Cells

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Background: A new powerful tool for studying gene functions is RNAi. Retinoic acid-related orphan nuclear receptor-C2 (RORC2) is the key transcription factor orchestrating Th17 cells differentiation, the cells which are known as the pathogenic elements in various autoimmune diseases. The aim of this study was to design efficient siRNAs specific for RORC2 and to evaluate different criteria affecting their functionality. Materials and Methods: Using a combination of different bioinformatics criteria proposed by various studies, three siRNA duplexes specific for RORC2 mRNA were designed. Cultured naïve $CD4^+$ T cells isolated from cord blood samples were treated with IL-6 plus IL-23 in order to polarize to Th17 cells. Then, the T cells were transfected with the siRNAs against RORC2 and expression of RORC2 and IL-17 genes was measured using quantitative RT- PCR. Results: Different levels of knocking down of RORC2 expression was observed using three siRNAs designed in current study. In comparison, the less efficient siRNA with 46.6% silencing activity had the most number (eight) of deviations from bioinformatics criteria while the most effective one (91.1% inhibition) met the most criteria having only 3 deviations. Conclusions: Our data indicate that, although all recommended criteria are important for designing siRNA but, not all proposed sequence preferences are of equal importance and could be categorized to three groups: criteria with low, medium and high importance. However, it seems that these categories are not essentially public for all genes and may be interchangeable for any given gene.
Keywords: RNAi, RORC2, siRNA, Th17

245. *In vitro* Leishmanicidal Activity of Recombinant Human α-defensin-1 (rHNP1) in Comparison with Commercial HNP1

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Background: α-Defensins belong to a family of antimicrobial peptides contributing to innate immunity. *Leishmania* is the causative agent of leishmaniasis and chemotherapy is the only treatment available, but its efficiency is threatened by the growing incidence of resistant parasites, so therapy based on rational use of such peptides is a feasible alternative. In this study, recombinant production of human α-defensin-1 (HNP1) in prokaryotic system and its parasiticidal activity are investigated. Material and Method: PMNs' total RNA reverse-transcribed into cDNA. Following PCR and insertion of expected product into pGEM-II (transformed into *E. coli* DH5α), colonies containing pGEM-HNP1 were identified. HNP-1 gene was ligated into pQE-30 (transformed into *E. coli* M15). After peptide expression, SDS-PAGE and western blotting analysis were carried out. After purification of rHNP1 by FPLC, its molecular weight was determined by mass spectrometry. Peptide folding procedure was conducted in $CuSO_4$ solution and its proper folding was determined through antibacterial activity assay against *E. coli*. The parasiticidal activities of rHNP-1 and commercial HNP1 were evaluated against GFP-transfected *L. major* promastigotes. After incubation with defensins, peptide-treated parasites were incubated with PI and analyzed by flow cytometry to determine changes in parasites' GFP intensity and the percentage of PI-stained cells. Results: SDS-PAGE and Western blotting analysis confirmed expression of HNP1. Mass spectrometry result showed the expected purity and molecular weight. rHNP1 showed desirable antimicrobial and parasiticidal activity for *E. coli* and *L. major* respectivelyin comparison with commercial HNP1. Conclusion: HNP1 has attracted attention due its strong antibacterial activity. Owing to the difficulty of extracting and purifying HNP1 from neutrophils and the high cost of chemical synthesis, we employed prokaryotic system as a simple and cost effective way to acquire HNP1. The activity of folded rHNP1 was confirmed through antibacterial activity assay. Commercial HNP1 showed acceptable parasiticidal activity against *L. major* promastigotes both at logarithmic as well as metacyclic stages.
Keywords: α-Defensins, Leishmanicidal Activity, rHNP1

Poster Discussion Presentation

246. Cloning, Expression and Characterization of Recombinant Human Fc Receptor Like (FCRL) 1, 2 and 4 Molecules

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Background: The Fc receptor like (FCRL) molecules belong to the immunoglobulin (Ig) superfamily with potentially immunoregulatory function. Among the FCRL family FCRL2 and 4 are predominantly expressed on memory B cells and FCRL1 is a pan- B cell marker. In the current study the human FCRL1, 2 and 4 proteins were expressed in prokaryotic system, purified and characterized. Materials and Methods: The extracellular part of human FCRL1, 2 and 4 were subcloned into prokaryotic expression vectors pET-28b(+) and transformed into BL21-DE3. Protein expression was optimized by fine adjustment of the induction time, incubation temperature, expression hosts and prokaryotic expression vectors. Recombinant FCRL proteins were purified by metal affinity chromatography using Ni-NTA resin. Purified FCRL proteins were further characterized by SDS-PAGE and immunoblotting using His-tag and FCRL specific polyclonal antibodies. Results: Our results demonstrated that FCRL1, 2 and 4 were successfully expressed in pET-28b(+) vector. Optimization of the expression procedure resulted in the highest expression levels of FCRL proteins ranging from approximately 15% (FCRL1) to 25% (FCRL2 and 4) of the total bacterial lysate proteins. The results of PAGE and immunoblotting confirmed the identity and purity of the purified recombinant FCRL proteins. Conclusion: The human FCRL proteins expression and purification were successfully optimized in prokaryotic expression system. These purified recombinant proteins are potentially a valuable tool for investigating the immunoregulatory function of FCRL molecules and production of specific monoclonal antibodies for immunotherapeutic interventions.
Keywords: Cloning, FCRL, prokaryotic system

247. Encapsulation of pEGFP-ferroportin Vector into Chitosan/alginate Nanoparticles for Gene Therapy in *Leshmania major* Infected BalbL/c

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Background: Leishmaniasis is a serious infectious disease. Certain strains such as BALB/c mice fail to control *L. major* infection and anemia is considered to be one of the reasons of their death. Ferroportin (Fpn) is a conserved membrane protein that exports iron across the duodenal enterocytes, macrophages and hepatocytes into the blood circulation. Fpn has also influence on survival of microorganisms whose growth are dependent upon iron, thus preparation of Fpn is needed to study the role of iron in immune responses. Materials and Methods: Total RNA was

extracted from Indian zebrafish duodenum and used to synthesize cDNA by RT-PCR. PCR product was first cloned in Topo TA vector and then subcloned into pEGFP–N1 expression vector. The resulted plasmid (pEGFP-ZFpn) was used for expression of FPN-EGFP protein in Hek 293T cells. Recombinant Fpn was further characterized by TMHMM V2.0 prediction server. Nanoparticles comprising chitosan- alginate polymers were formed through pregel preparation method to deliver pEGFP-ZFpn plasmid to entrocyte cells. BALB/c mice were divided to three groups. The first and second groups were fed with chitosan/alginate nanoparticles containing the pEGFP-ZFpnandpEGFP plasmid and the third group (control) didn't get any nanoparticles. Hematocryte, iron level, footpad thickness and cytokine concentrations were analysed. Results: The resulting nanoparticles had an average size of 188 nm as confirmed by Scattering Particle Analyzer. The results of pEGFP-ZFpn fed mice showed higher hematocryte, iron level, more limited increase of footpad thickness and significant reduction of viable parasites in lymph node in comparisen to other groups. pEGFP-ZFpn fed mice showed lower levels of IL-4 and IL-10 cytokines and higher levels of IFN-γ/IL-4 and IFN-γ/IL-10 ratios than that in other groups. Conclusion: These data strongly suggests the *in vivo* administration of chitosan/alginate nanoparticles containing pEGFP-ZFpn suppress Th2 response and may be used to control the leishmaniasis.
Keywords: iron homeostasis, ferroportin, cloning, expression, topology

248. Isolationtion and Characterization of Anti-TNF-α ScFv through Phage Display Technique

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Background:The world of monoclonal antibody was revolutionized by the advent of antibody phage display technology which obviates the limitations of traditional hybridoma technology. TNF-α has become increasingly interesting for many researchers and is an important candidate for anti-cytokine therapy. Among several strategies, use of monoclonal antibody (mAb) technology is a major method to negate the deleterious effects of TNF-α in disease states. Materials and Methods: For production of single chain fragment antibodies against TNF-α, five panning rounds were performed. The assessment of selected clones was conducted through monoclonal phage ELISA, PCR, RFLP and western blotting. Results:The results showed that the specific clones to TNF-α were successfully enriched from the in vitro selection. All plasmids contained two insertions. Conclusion:Furthermore, most of the variations seen in hot regions could affect protein function.
Keywords: Phage display, Human TNF-α, Monoclonal antibody, ScFv

249. Recombinant *Leishmania tarentolae* Expressing ImmunodominantParasite Antigens as an Effective Vaccine against Visceral Leishmaniasis in Mouse Model

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Background: Visceral leishmaniasis (VL) is the most severe systemic disease among the three main clinical manifestations of leishmaniasis. No effective vaccine currently exists for human use. Several studies suggest that using live attenuated parasites hold the most promise for an anti-leishmanial vaccine. We currently focused on generation of a novel, non-pathogenic live recombinant *L. tarentolae* harboring selected immunogenic components of the *L. infantum* including the A2 gene combined with cysteine proteinase type I and II genes as a tri-fusion. Here, the comparison of protective effects between the two main DNA/live and live/live vaccination strategies against VL in BALB/c are performed. Materials and Methods: For production of live stable *L. tarentolae* expressing tri-fusion, the promastigotes were transfected with linearized pLEXSY-A2-CPA-CPB^{-CTE}-GFP by electroporation. Confirmation of the A2-CPA-CPB^{-CTE}-GFP fusion gene expression in transgenic *L. tarentolae* was done at DNA, RNA and protein levels. Then immunological potency assessment of two different vaccination modalities DNA/Live and Live/Live in BALB/c was carried out via cytokine assay, parasite burden and antibody response. Results: The linearized pLEXSY-A2-CPA-CPB^{-CTE}-GFP was integrated into the chromosomal *ssu* locus of *L. tarentolae* and transcription of tri-fusion gene was confirmed through RT-PCR. Western blotting, fluorescence microscopy and flow cytometry analysis indicated that A2-CPA-CPB^{-CTE}-GFP is successfully expressed.Our results showed that a prime and boosting administration of A2-CPA-CPB^{-CTE}-GFP recombinant *L. tarentolae* strain protects BALB/c mice against *L.infantum* challenge and it is associated with high levels of IFN-γ production pre and post challenge and reduced levels of IL-10 after challenge, leading to a potent Th1 immune response. Also this protective immunity manifested by a dramatic decrease in parasite burdens in the liver and spleen of immunized mice. Characterization of antibody response also corroborated the cytokine assay findings. Conclusion: In our previous results recombinant *L. tarentolae* expressing A2 protein could stimulate effective immune responses and protect BALB/c mice against infectious challenge. In present study, the results presented that A2-CPA-CPB^{-CTE}-GFP expressing recombinant *L.tarentolae* shows a promising protective live vaccine against *L.infantum* infection in BALB/c. Finally, we are pretend to use this effective vaccine in outbreed Hamsters as an appropriate animal model against VL.
Keywords: *Leishmania tarentolae*, ImmunodominantParasite Antigens, Vaccine, Leishmaniasis, Mouse Model

250. Construction and Expression of a Fluobody Containing Enhanced Green Fluorescent Protein Fused to Single Chain Variable Fragment of Anti-human CD4 Antibody

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Background: Antibodies, chemically conjugated with the fluorochromes have been used extensively in techniques such as immunofluorescence and flow cytometry for various applications including immunophenotyping and cell sorting. Since conjugation of antibodies with chemical fluorescent labels is often problematic in terms of conjugation level and loss of activity, new alternative fluorochromes have been developed. Green fluorescent protein (GFP) has been genetically fused to many proteins in various species to produce stable chimeras, apparently retain both of original biological activity and fluorescent properties of native GFP. In the present study, a vector was designed for efficient production of a single recombinant protein fluorescent antibody in *Escherichia coli* by fusing EGFP to a single-chain variable fragment (scFv) antibody specific for human CD4. Materials and Methods: EGFP CDS was inserted at the N-terminus of scFv by PCR and digestion with appropriate enzymes. The cloning authenticity was confirmed by colony-PCR and enzymatic digestion. Moreover, sequencing performed in order to ensure that no mutation was occurred in structure of fusion protein with a correct reading frame. Finally, the expression of fluobody in *E. coli* was checked by dot and western blotting using anti-EGFP. Results: The sequencing result showed that recombinant plasmid was constructed successfully expressing EGFP fused with ScFv in a correct reading frame. The fluobody expressed by *E. coli* HB2151 had a molecular size of about 59 kDa on a SDS-PAGE gel as expected. Conclusion: There are many reports describing the co-expression of GFP and a specific antibody or cytokine gene, with the fusion protein possessing both the fluorescent activity and biological activity of the original protein. To our knowledge, there is no report describing co-expression of GFP and anti-human CD4 scFv. The produced fluobody can provide an alternative approach than chemical coupling of antibodies for immunofluorescence approaches such as phenotyping by flow cytometry, cell sorting, HIV reseach and cellular-based fluorescence assays.
Keywords: Green Fluorescent Protein, Anti-human CD4 Antibody, scFv

251. Investigation of Biological Characteristics of Recombinant Cholera Toxin B Subunit and Its Immunological Effects In rabbit

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Background: Cholera toxin (CT) is the key virulence factor of *Vibrio cholerae*, which is encoded by the *ctxAB* operon, which resides in the genome of a filamentous bacteriophage (CTX) that specifically infects *V. cholerae*. The symptoms of cholera are mainly caused by cholera toxin (CT), B subunit of which binds to the GM1 ganglioside and promote the endocytosis of CT. The aim of this study was to clone and express ctxB using pAE as an efficient expression vector, purify the recombinant protein with chromatogeraghic column and investigate the immunological of this recombinant CTB in rabbit model. Materials & Methods: The recombinant pAE-CTB was transformed to the competent *E.coli* BL21 to express CTB protein. The system was induced by IPTG after which cells were harvested from LB medium by centrifugation and analyzed by 15% SDS-PAGE. Western blotting performed using cholera toxin-specific antibody. Recombinant CTB was expressed in this system with 6XHis tag at N-terminus and was purified through Ni2+-charged column chromatography. Concentration of protein measured with Bradford assay. The functionality of the CTB pentamers was assessed by GM1-ELISA assay. Purified rCTB inoculated into rabbits through intestinal rout singly and in combination with inactivated whole-cell *V. cholerae* strains. Finally the titer of anti-CTB IgG in sera of immunized rabbits was obtained with GM_1-ELISA assay. Results: SDS-PAGE analysis showed the expression of rCTB in the system and western blot analysis confirmed the presence of recombinant CTB in blotting membranes. Recombinant CTB was able to bind GM1 in a dose-dependent manner. Some part of rCTB may be expressed in the inclusion bodies so we also lubricated the inclusion bodies. The anti-CTB IgG titer showed that rCTB which produced in this study has an immunological effect and can enhance the secretion of anti-CTB IgG in rabbits which immunized with WC.rCTB. In addition to rCTB without whole cell *V. cholerae* strains can also stimulate the mucosal immune response when inoculated into rabbit intestine. The immunized rabbits were protected against challenge *V. cholerae* starins. Conclusion: Our results confirmed that although expressed in the inclusion bodies, 6XHis-tagged rCTB was properly refolded, easily purified, and as expected was free of possible CTA contaminants. We studied CTB immunological properties, oral tolerance, its use as mucosal adjuvant or in vaccine development and it was proved that rCTB which produced in this system can be used as a good immunogen protein to enhance the immunity against *V. cholerae* strains and can be use for developing vaccine against cholera.
Keywords: *Vibrio cholerae*, recombinant CTB, cholera toxin, immunological effects

252. Fc Fusion Recombinant Protein: A Useful Tool for Making Stable Pharmaceutical Agent; KSHV vOX2:Fcγ1 as an Example

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Background: Recombinant fusion proteins containing the constant (Fc) domain of immunoglobulin represent a growing class of human therapeutics. The Fc domain prolongs the serum half-life of proteins, moreover in many cases improves the biophysical properties of its fusion partner such as the solubility and stability. Other advantages are high expression, secretion to cell culture medium, and protein-A affinity purification. One of the most challenging tasks in the development of pharmaceutical proteins is to deal with physical and chemical instabilities of proteins. vOX2 which is encoded by KSHV open reading frame K14 is an immunoregulatory protein. vox2:Fcγ1 as a humanized viral recombinant protein is the subject of an international patent for its anti-inflammatory effects is the target of this study for its stability as a Fc-fusion proteins. Materials and Methods: The extracellular domain of the vOX2 gene was cloned into a eukaryotic expression vector to create an N-terminal fusion with human IgG1 Fc. Culture supernatants of stable CHO transfectants were analyzed by western blot with anti-human IgG-HRP, and afterwards purified by affinity and size exclusion chromatography. Then the stability of the recombinant vOX2: Fc protein was evaluated at room temperature, 4-8˚C, -20˚C and -70˚C with Agilent bioanalyzer and western blotting using anti human IgG-HRP or anti vOX2 monoclonal antibodies which we raised. Results: At room temperature and 4-8˚C, vOX2:FCγ1 didn't show remarkable stability however, after adding protease inhibitor it showed more stability. The best stability of this recombinant protein seems to be at -20˚C and -70 ˚C. Also the bioinformatics analyses are consistent with our experimental findings. For vox2 as an unstable protein, FCγ1 didn't help as we expected. Conclusion: For producing humanized microbial agent which has pharmaceutical benefits, Fcγ1 has two advantages; firstly, Fc fragment makes the protein bivalent and more efficient, and secondly, prolongs its biological half-life. However, in the case of vOX2 this method did not work properly.
Keywords: Fc, Pharmaceutical Agent, KSHV vOX2:Fcγ1

253. A Phagmide Library Containing Nanobody against Bap Antigen of *Acinetobacter baumanni*

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Background: *A. baumannii*, an important nosocomial pathogen,causes various human infections, such as meningitis, bacteremia, pneumonia, and urinary tract infections. Its remarkable resistance to a wide range of antibiotics and also its high mortality rate have made the treatment of the infections very difficult. High ability of *A. baumannii* to form biofilm, and correlation of biofilm with multiple drug resistance was demonstrated recently. A specific cell surface protein named Biofilm-associated protein (Bap) was defined in *A. baumannii* isolates. Bioinformatic tools have proven a core domain of seven repeat modules namely A–G of which D, B, C and A are more predominant. Scientists obtained high antibody titers in mice using a conserved region of Bap construct-B consisting of 371 amino acids. In this study, the phagmide library, containing nanobody derived from camel heavy chain antibodies (HcAb), was produced against a Bap construct antigen of *A. baumanni* by phase display technique. Materials and Methods: For this purpose, the recombinant Bap-Con was injected to a camel and polyclonal antibody production was confirmed by Elisa. cDNA was prepared from isolated blood lymphocytes and VHH(variable fragment of HcAb) coding fragments were then amplified via a nested PCR. The PCR products were digested and ligated to pComb3x phagmide vector. Results: Recombinant vectors were transferred to *E. coli* TG-1 via electroporation and their release was induced by infecting the bacteria with M13K07 helper phase. Conclusion: Since the antibodies are used in treatment and diagnosis, this library can be used to produce antigen-specific nanobodies.
Keywords: *Acinetobacter baumanni*, Bap Antigen, Phagmide Library

254. Construction and Expression of Candidate Vaccine fimH/fliC against *Escherichia coli* Urinary Tract Infection

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Background: Urinary tract infection (UTI) is one of the most common infections in the world.Uropathogenic *Escherichia coli* (UPEC) are the most frequent cause of cystitis and pyelonephritis. Type1 pili by having the adhesion FimH and flagella by having flagellin (FliC) are the important virulence factors of UPEC. To date, any ideal vaccine against UTI has not been approved for human use and thus we need to test new target antigens to develop an ideal and safe vaccine against UTI. In this study, we constructed recombinant fusion fimH/fliC of UPEC as a novel candidate vaccine against UTI. The immunological properties of the fusion protein is in progress. Materials and Methods: Uropathogenic *Escherichia coli* were isolated from the UTI patients. The *fimH* and *fliC* genes were amplified by PCR. Construction of fimH-fliC fusion protein was performed by overlap PCR with fusion primers and the genes were cloned in pET28a vector. The confirmation of expression of the proteins was done by SDS-PAGE and Western blot. Results: The *fliC* and *fimH* genes were amplified in all of the UPEC strains tested.The fimH and fliC sequences showed significant homology with the sequences in Genbank. We generated a fusion protein consisting of the fimH protein linked to the N-terminal end of fliC. Sequencing of the fusion fimH-fliC by internal and universal primers showed that fusion was constructed precisely. SDS-PAGE and western blot confirmed the expression of the proteins. Conclusion: Urinary tract infections (UTI) are the second most common

infection. Some of the virulence factors of UPEC have been tested as vaccine targets against UTI that have limited success. Recombinant fusion protein strategies offer a significant advantage in inducing enhanced antigen specific cellular and humoral responses.
Keywords: fimH/fliC, *Escherichia coli* Urinary Tract Infection

Poster Presentation

255. Seaweeds: Some of Pharmaco-Immunological Effects

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Seaweeds are usually found on the beaches during the ebb tide of sea. Several species of seaweeds are commonly used as food or food additives in many countries. Since last decades, some species, especially brown seaweeds, used as an traditional medicine. Several ethnopharmacological studies have been demonstrated their effectiveness in the treatment of allergic, cancers, arthritis, inflammatory, and brain diseases. Therefore, compounds from seaweed extract containing polyphenols and phlorotannin with antihyaluronidase activity and stabilization of mast cell, focoidan by inhibition of IgE production are useful in the treatment of allegic diseases. In addition, β-glucan isolated from seaweeds, biologic ingredient as an immunostimulants and anti-angiogenic against cancers and also, phenolic compounds of seaweeds with inhibition of chronic inflammation could be used in therapy of several cancers and chronic inflammatory diseases.
Keywords: Seaweed, Allergy, Cancer, Inflammation, Disease, Immunostimulant

256. Isolation and Detection of T Lymphocyte by Conjugated Anti-Human CD4 Monoclonal Antibody to Magnetic Nanoparticles

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Background: Detection and separation of specific classes of cell through their surface markers by Monoclonalantibodies are very valuable for research and clinical diagnosis. Nanoparticlescaneffectivelybindtomonoclonalantibodiesto identifytheirtargets indifferentpopulationsof cells. In the present study,anti-human CD4 antibody was covalently immobilized on the surface of core-shell Fe_3O_4/SiO_2 nanoparticles to analyze CD4 T lymphocyte in human blood. Materials and Methods: In this study, first Fe_3o_4 nanoparticles were synthesized using a chemical precipitation method then these particles coated with Tetraethylorthosilicate. Silica coated nanoparticles where chemically modified in order to react with amino (−NH2) groupsof antibody. Attachment of antibody to nanoparticles is confirmed by various spectroscopic and biological methods. The amount of immobilized antibody was estimated by Bradford method.After conjugation, the binding ability of antibody to its receptor was investigated. An agglutinationtest was performed to prove the presence of fixed antibodies. The presence ofnanoparticles on the lymphocytes is confirmed by iron staining andfluorescencemethods.Results: Microscopic images were takenfrom theculturelymphocytesclearlyshowedthe presence ofnanoparticlesat thecellular level. Moreover, aftereachstep of synthesis andantibodyconjugation withnanoparticles, spectrumof thenanoparticlesconfirmed the successful conjugation offunctionalgroups.The resultsshow that functional antibodies conjugated to nanoparticles successfully.Conclusions: Anti human CD4 monoclonal antibody could be conjugated to chemically modified nanoparticles efficiently. The conjugated antibody have biological activity and could be used in different application such as detection and separation of CD4 T lymphocytes. Comparedwith the conventional fluorescent antibody, the conjugated antibody was simple, rapid and stable method for detection and isolation of human CD4 T cells.
Keywords: T Lymphocyte, Anti-Human CD4, Magnetic Nanoparticles

257. Assessment of Monoclonality in B Cell Non Hodgkin's Lymphoma

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Background: Most B-cell malignancies are diagnosed based on morphologic and immunohistochemical criteria. In some cases, however still present a challenge for the pathologist to discriminate between reactive hyperplasia and neoplasic disorders. In such cases, molecular techniques can be used as a helpful diagnostic tool. In this study, we assessed the value of polymerase chain reaction (PCR) technique in determination of the clonality of immunoglobulin heavy chain gene rearrangements for diagnosis of B-cell non Hodgkin's Lymphoma in in paraffin embedded tissue specimens. Materials and Methods: DNA was extracted from paraffin embedded tissue of 31 diffuse B-cell lymphoma specimens. Framework 3 to joining regions (FR3/JH) of variable segment of immunoglobulin heavy chain genes were amplified using in house designed degenerate primers. PCR products were analyzed on 15% polyacrylamide gels following AgNo3 staining. Result: Monoclonal rearrangements were identified in 22 of 31 cases (71%) of B-cell lymphoma specimens using FR3/JH primers. Conclusion: PCR analysis, using degenerate primers can be used as sensitive, reliable and valuable diagnostic adjunct to conventional morphological and immunocytochemical evaluation of lympho- proliferative disorders particularly in cases with limitation in quantity and type of diagnostic material like needle aspirates and cellular fluids.
Keywords: Monoclonality, B Cell, non Hodgkin's lymphoma

258. Controlling and Inhibition of Rheumatoid Arthritis Progresses by Injection of Nanobodies (VHH) against Proinflammatory Cytokines at Sites Involving in Diseases

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Background: Rheumatoid arthritis is a chronic, inflammatory, multisystem, autoimmune disorder causes the attraction of immune system to joints. RA affects about 1% of the population of world and can lead to disability and reduced quality of life. It is found that proinflammatory cytokines (IL1 -α, *IL*-6 and TNF-α) have role in increasing risk for developing RA. The field of recombinant antibody technology has rapidly progressed, mainly because of the interest in their human therapeutic use. Single-domain antibody fragments (nanobodies, VHH) possess structural features, such as a small dimension, an elevated stability, the singularity of recognizing epitopes non-accessible for conventional antibodies and with high homology to human immunoglobulin VH domains that make them interesting for several therapeutic applications. Materials and Methods: After camel immunization with human interleukin-1 (IL-1-α), interleukin-6 (IL-2) tumor necrosis factor α (TNFα) and VHH phage display library preparing from a peripheral blood stream and successfully employing in the isolation of VHHs that bind and neutralize these cytokines. At end, nanobodies will inject to patient at joints that most severely affected and are in acute phase of disease. Results & Conclusion: The aim of this article is to investigate the potential of nanobodies to controlling and inhibition of Rheumatoid arthritis progresses and also to evaluate the applicabilityof these Nanobodies as alternative IL1-α, *IL*-6 and TNF- α blocking agents(like monoclonal antibody, MAb), drugs that are holds an enormous cost, side effects and also these drugs unavailable to many patients. Combination therapy with nanobodies maybe more effective than drugs therapy alone and the disease symptoms of the patients will reduce. We suggest starting combination therapy for the patients with early RA, when the diagnosis has been established.
Keywords: Arthrid rheumatoid (RA), Nanobodies (VHH), Camel, IL-1, IL-6, TNF- α

259. Purpose of Using Of Nanobody Technology as Complement Therapy in Order to Control Human Ornithosis (Parrot Fever)

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Background: Ornithosis occurs worldwide and is mainly associated with occupational exposure to birds (such as poultry farming). Clinical signs include headache, myalgia, cough, increased respiratory rate, secondary purulent lung infection, and a faint macular rash. Camelids produce functional antibodies devoid of light chains of which the single N-terminal domain is fully capable of antigen binding. These single domain antibody fragments (V_{HH}) have several advantages for biotechnologicaland therapeutic applications. V_{HH} is easily produced as recombinant proteins, designated single domain antibodies or nanobodies. They have a high stability and solubility, small size, refolding capacity, and good tissue penetration. Materials and Methods: We designed a strategy to control and inhibit Ornithosis in patients by producing nanobodies. The cystine-rich major outer membrane protein (MOMP) is an immunodominant protein and has a protective role in immunity to chlamydial infection. After immunization of camel by this protein, specific lymphocytes that produce antibodies against MOMP will be harvested by phage display method and then single domain antibodies (also called nanobodies) by the aid of genetic engineering techniques will be express in *E. coli*. At least these nanobodies will be checked in animal model. Results and Conclusion: This hypothesis indicate that synthetic camel anti-MoMP single domain antibodies would be an excellent tool for research purposes on this diseases, as complementary therapy in patient with ornithosis and for diagnosing infections in laboratory.

Keywords: Ornithosis , Single domain antibodies, nanobody, complement therapy

260. Investigation of GBSSI Promoter for Human Calcitonin Expression in Transgenic Potato Plants

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Background: Cells of the thyroid gland. It plays an important pharmaceutical role in treatment of osteoporosis, hypercalcemia and Paget's disease. The main physiological function of hCT is Human Calcitonin (hCT) is a peptide hormone which is secreted by the parafollicular the lowering of the calcium level in blood. So far, hCT for therapeutic use has been industrially produced by completely chemical synthesis. Molecular farming is a new and promising industry involving plant biotechnology for the production of pharmaceutically.Because of the lack of contamination with viral or bacterial materials, mammalianpathogens, low cost and industrial storage of recombinant protein in specific plant organ or organelle, host plants that produce recombinant protein are important in modern technology. Potato is a globally important crop producing high yields of nutritionally valuable food crop and as a potentially significant source of interest compounds. Materials and Methods: In this study two construct were made, one for the hCT expression of a GBSS I transit peptide – hCT fusion protein in potato plant, one for the expression of a hCT without potato by direct method with the recombinant binary vector prepared from E.coli clone.Potato tuber discs were transformed using these Agrobacterium tumefaciens, GBSS I transit peptide. Granule bound starch synthase I (GBSS I) was previously implicated as the enzyme for amylose synthesis in tuber storage starch and was found completely within the granule matrix. Agrobacterium strain LBA4404 cells were transformed transgenic plants were selected on medium containing kanamycin (Km). The presence of the hCT gene in the transgenic plant genome was detected by the PCR technique. Results:Expression of the hCT gene under the GBSS I promoter –Tp result in accumulation of hCT in potato microtuber 3 times higher concentration compared to its amount in potato microtuber expressing hCT gene controlled by GBSS I promote.

Keywords: GBSSI Promoter, hCT, Transgenic Potato Plants

261. PerformanceEvaluation of Salting OutDNA ExtractionMethod, onBlood SampleswithDifferentStorage Conditions

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Background: Todays in most areas of basic sciences, molecular tests are being used as the dominant techniques. Meanwhile, The DNA and RNA Extraction process is the first and most important part of these Techniques. So choosing the appropriate techniques to extract DNAs and RNAs is necessary. the aim of our study was to evaluate the performance of non-commercial Salting out DNA extraction method, on blood samples with different storage conditions. *Materials and Methods:* In this study for evaluating the non-commercial Salting out DNA Extraction method, a 500µl of total 184 blood samples containing EDTA anticoagulant were used. Out of these samples, 114 blood samples stored for 2 years at a temperature of minus 20 degrees and 70 fresh samples were being used. All solutions used in this technique were set up and prepared manually in lab condition. Then, the extracted DNAs were evaluated with the NanoDrop apparatus in wavelengths 230, 260 and 280 nm. Finally, the results between the two groups of frozen and fresh blood samples were calculated and compared with SPSS18. Results: The average concentration of extracted DNA for 184 samples was 647 ± 260 ng / µl per 500µl blood. The average concentrations of extracted DNAs from two sample groups of frozen and fresh samples were 620±258 ng/µl and 691±258 ng/µl, respectively. Between the OD 260/280 and the average concentrations of extracted DNAs from frozen samples, there was a significant reverse correlation. (P. Value <0.05, Beta: -0.204). Conclusion: Our results show that the non-commercial Salting out DNA Extraction method is highly efficient in DNA extracting of blood samples in different storage conditions. Due to the high concentration and quality of extractive products and its cost-effectiveness, this method can be used instead of common commercial DNA extraction kits.

Keywords: Salting out,DNA extraction, blood

262. Inosine Monophosphate Dehydrogenase Redundance Lead to Mycophenolic Acid Resistance

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Background: Mycophenolic acid (MPA) is a secondary metabolite of *Penicillium brevicompactum.* MPA and its derivatives such as mycophenolate mofetile (MMF) are immunosuppressive agents which have been approved by FDA and used for decreasing the incidence of graft rejection in organ transplant patients. Since MPA has special function on B and T lymphocytes and low toxicity for other cells in human body, in comparison to other similar drugs such as cyclosporine A, it goes to be the main alternative of all of them. MPA has inhibitory effect on inosine monophosphate dehydrogenase "IMPDH", the rate-limiting enzyme of GMP synthesis. So, it can stop the biosynthesis of DNA and RNA and cell reproductivity. Despite abundant application of MPA, chemical synthesis is the most available route of production. But, the structural complexity of polyketides usually precludes chemical synthesis. Moreover, natural producers have some limitations to use in industrial scale. Therefore, a generic heterologous host for high-level production is desirable. Materials and Materials and Methods: According to antimicrobial effect of MPA, the first step in heterologous production is development of a resistant host cell. Therefore, in this work, IMPDH coding sequence from MPA synthesis gene cluster of *P.brevicompactum* (Accession number ADY00133.1) was subcloned into pPICZB vector. Presence of the insert was confirmed by colony PCR, restriction digest and sequencing. The recombinant plasmid was used to transform *Pichia pastoris* GS115. At the end, MPA resistance of recombinant strains was studied. Result and Conclusion: One strain with 5-fold more MPA resistance was obtained. Here we demonstrate that MPA resistance in *Pichia pastoris* can be enhanced by introducing further copies of the Inosine 5´-monophosphate dehydrogenase.

Keywords: Mycophenolic acid, *Penicillium brevicompactum*, Organ transplant rejection, Inosine monophosphate dehydrogenase

263. Evaluation of HCV core immunogenicity in Balb/c mice "Preparation of T-Cell Clone of Hepatitis C Virus Core Protein Supported by Studies with Elispot Study

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Background: There are more than 170 million individuals suffering from hepatitis C worldwide (approximately 3% of the world's population). Despite ongoing research, there is currently no vaccine to prevent hepatitis C virus infection. Core protein is one of the most conservative proteins among different Hepatitis C virus genotypes and a proper candidate for preventive applications. Materials and Methods: Core gene was cloned into pETDuet-1 vector .The Core recombinant protein, purified and the antigen with Freund's adjuvant injected to Balb/c mice.14 days after the last booster injection, the proliferation and cytokine secretion of spleen, inguinal and popliteal lymph nodes lymphocytes in-vitro and ex-vivo conditions were analyzed by MTT and ELISPOT (Enzyme-linked immunosorbent spot) techniques. Results: In immunized mice with recombinant core protein in comparison to the control group, an increased amount of proliferation in spleenocytes, inguinal and popliteal Lymph node lymphocytes were observed; among these three cell sources, the popliteal lymph node contains the highest population of HCV-Core specific lymphocytes and highest cytokines secretions. Conclusion: The protection ability of HCV Core protein were evaluated for vaccine designing and suggested HCV core specific T-cell colons as candidates for T-cell therapy.
Keywords: HCV; Core; Cytokines; MTT; ELISPOT

264. Nano Structure Surface Layer in Some of Pathogen Bacteria and Pathogenesis

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Background: An Nano structure of S-layer (surface layer) is a part of the cell envelope commonly found in bacteria, as well as among *Archaea* .It consists of a monomolecular layer composed of identical proteins or glycoproteins. As for many bacteria the nano structure of S-layer represents the outermost interaction zone with their respective environment, its functions are very diverse and vary from species to species. The subject of this study was study role of surface layer in increase of pathogenesis in pathogen Bacteria. Materials and Methods: Relater papers to role of surface Layer in increase of bacterial pathogenesis were extracted of articles in PubMed, Elsevier Science from 1995 to 2011 years. For this study key words which were search include surface layer, pathogenesis, and pathogen bacteria. Results: About the surface layer in all of similar original and review articles, there is consensus that the existence of this surface structure in bacteria lead to increased pathogenesity in bacteria. Conclusion: Nano structure of S-layer in pathogen bacteria protection against bacteriophages and phagocytosis, resistance against low pH, adhesion, stabilization of the membrane and provide adhesion sites for exoproteins due to more pathogenesis, infection resistant and antibiotic resistant in bacteria. Result this search show prevalence nano structure of S-layer in pathogen bacteria and important determine nano structure of S-layer producer strains in laboratory.
Keywords: Nano Structure Surface Layer, Pathogen Bacteria, Archaea, Pathogenesis

265. Co-Utilization of TLR5 Agonist and Nano-Particle in the Improvement of HIV Vaccine Candidate: A Preliminary Study

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Background: The transport properties of nanoparticles and adjuvant properties of bacterial flagellum to stimulation of TLRs are two immunostimulant for immune responses. The aim of this study was to evaluate humoral and cellular immune responses against HIV P24Nef vaccine candidate in the presence of PLGA nanoparticles and FLiC as agonists of TLR-5 in BALB/c mice. Materials and methods: Six-to eight-week-old In-bred BALB/c mice were divided into 11 groups. Mice immunized intradermally with vaccines HIV-p24-Nef/FLiC/PLGA, HIV-p24-Nef/PLGA, FLiC/PLGA, PLGA and PBS with two doses consisting of 20 and 5 µg. Three weeks after the last booster injection proliferation assay was done using Brdu/ELISA based method and splenocytes cytokine secretion (IL-4 & IFN-γ) and also antibody titers with IgG subtyping were assayed by commercial ELISA kit. Results: Immunization of mice with HIV-1 p24-Nef –FLiC fusion peptide formulated in PLGA nanoparticles significantly increased cellular immune responses and enhanced lymphoproliferative activity of splenocytes in the lower doses of candidate vaccine. Conclusion: Co-utilization of TLR-5 agonist and nano particle in vaccine strategy improves vaccine immunogenicity and decrease immunogenic dose of vaccine candidate.
Keywords: TLR5 Agonist, Nano-Particle, HIV Vaccine

266. Transduction of Dendritic Cells by Adenoviral Vectors, Evaluation of Maturation States and Optimization the Procedures

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Background: Dendritic cells (DCs) remain as the most important model for in vitro assay of viral protein interaction with immunologic system. Adenoviral vectors expressing viral proteins due to their efficiency in DC transduction employed for this purpose. The results achieved by this method are usually controversial owing to some parameters like differences in procedures used for Adenovirus preparation, transduction and other variables. Here, we report an experiment in order to evaluate the suitable MOI of the Adenoviral vectors for better DC transduction. Materials and Methods: The adenoviruses expressing GFP or Luciferase had been prepared and finally titrated by commercially available quantification methods. After that, Adeno-GFP was employed to assess it's infectivity at different MOIs from 50-1000. The MOIs of 250 and 1000 were selected based on the results of GFP detection and viability test. Dendritic cells status, maturation markers and function were evaluated by MLR assay, IL-12 and TNF-α cytokine production method as well Flowcytometery of surface markers (CD40, CD86, MHC-II). Adeno-Luciferase was also employed as another control virus. Results: Virus titer in 1000 MOI was more effective in DC transduction than 250 MOI albeit more toxicity that led to cell death among more than half of the DCs population. Both adenoviral vectors (Adeno-GFP or Adeno-Luc) induced DCs maturation by increasing the expression of surface markers and secretion of IL-12 and TNF-α in comparison to control groups. The DCs transduced by adenoviral vectors (GFP or Luciferase) also induced more proliferation of spleen lymphocytes in MLR assay. Conclusions: Adenoviral vectors, even in low titer, infect DCs and infectivity is dose dependent in all experiments. At high MOI, due to toxicity, a large part of population was not viable. Treatment of DCs with 2 different Adenoviruses leads to their maturation which observed by changes in CD40, CD86 and MHC-II surface expression markers and function. Conclusively, for study of effect of virus protein on DCs, to avoiding the saturated maturation of DCs following Adenovirus transduction, it is important to perform various controls and titration studies prior to starting a research. Here we explain simple points to establish an informative comparative basic study on DCs by Adenoviruse expressing viral proteins in order to distinguish even fine differences among viral proteins.
Keywords: Adenoviral vector, Dendritic cells, DC Transduction

267. Inhibition Effect of Variable Domain of Heavy Chain Antibody (Nanobody) Against Urease Activity of *Helicobacter Pylori*

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Background: Antibody administration against urease has been studied as a new effective therapeutic strategy. *Helicobacter pylori* infection is associated with gastritis and in some cases with gastric and duodenal ulcers or even adenocarcinoma. Urease is an enzyme that helps *H.pylori* to colonize the epithelium in the acidic environment of stomach. This enzyme release NH_3 by hydrolyzing urea. Produced ammonia can neutralize acidic environment and enable bacteria to survive in stomach. Neutralizing this enzyme unable the bacterium to colonization in the stomach acidic environment and will ultimately results in its death. In the present study, production of variable domain of heavy chain antibody (nanobody) against of UreC recombinant protein and evaluation of inhibitory effect was aimed. Materials and methods: A nanobody library derived from cDNA pool of dromedary was generated and an anti-UreC nanobody successfully was selected. After purification of nanobody, urease inhibitory of nanobody was measured. For assessment of inhibition effect of nanobody, 10^9*H. pylori* bacteria mixed with different concentrations of nanobody and were incubated in microplate wells for 16 hour at 4°C. 100 µl PBS containing 500 mM urea and 0.2 g/l phenol red were added to wells and incubated for 3 hour at 37°C. Three controls including the culture media, *H. pylori* bacteria and nanodody were used. Colorimetric measurement was done every 30 min during 3 hours in OD_{550} nm. The inhibition percentage was calculated as follow, Inhibition percentage = [(the enzymatic activity of bacteria without nanobody–the enzymatic activity of bacteria with nanobody)/the enzymatic activity of bacteria without nanobody] × 100%. Results: *H. pylori* was treated with nanobody and the optical density of the mixture was determined at 550 nm by the indicator of phenol red that shows 35% inhibition percentage. Conclusions: The result of urease inhibitory test showed that extracted nanobody against UreC can successfully inhibit the surface urease activity of *H.pylori* and could be an alternative to antibiotic treatment.
Keywords: Nanobody, Urease Activity, *Helicobacter Pylori*

268. Cross-Reactions against Recombinant InvH Protein with Sera from Mice Infected with Different Strains of Salmonella
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Background: Salmonella is one of the most prevalent food-borne diseases over the world. Vaccination is generally accepted as the most practical measure in that is easy to apply and the most economic, however, present vaccines have limited efficacy. Except for *Salmonella arizonae*, *invH* is present in all *Salmonella* strains. No homologous sequences were detected in *Yersinia, Shigelia, Proteus*, and several strains of enteroinvasive and enteropathogenic *E. coli*. InvH protein was present in the peptidoglycan and role of this protein for efficient bacterial adherence and entry into epithelial cells is inevitable. This research suggests that Invh could be a candidate for a new generation vaccine and diagnostic measures against prevalent serotypes of *salmonella enterica.* Materials and Methods: Six groups of mice were infected with *S.enteritidis, S. typhi, S.paratyphi* serovars A, B, C and *E.coli*. One group of mice was immunized with recombinant InvH protein. Five micrograms per well of the recombinant protein was used to coat the surface of a 96-well microtiter plates. Serial dilutions of mice sera against *S.enteritidis, S. typhi, S.paratyphi* serovars A, B, C, E.Coli and Immunized mice were added to the wells. Results: Antibody response with sera from immunized mice or inoculated with S*almonella* strains were significantly higher than *invH* deficient bacteria such as *E.coli* and control group. Conclusion: Cross reaction with sera of *Salmonella* strains inoculated mice is indicative of possessing by *Salmonella* strains of the surface protein, InvH, that can be employed in both prophylactic and diagnostic measures against *Salmonella enterica*.
Keywords: Cross-Reactions, InvH Protein, Sera, Salmonella

269. Immunization against *Salmonella enterica* Serovar Entritidis with Recombinant InvH Protein
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Background: *Salmonellaenteritidis*, is one of the main causes of food-borne illness. It grows under natural conditions. Epidemic of human infections mediated by *Salmonella enterica* serovar Enteritidis was witnessed by last two decades of the 20th century. Poultry and poultry products such as fowl, shell eggs are the most prevalent sources of infection of *SalmonellaEnteritidis* in humans. Acellular vaccines containing bacterial immunodominant components such as surface proteins may be potent alternatives to live attenuated vaccines in order to reduce salmonellosis risk to human health. *invH* gene, an important part of needle complex in type three secretion system (TTSS) plays important role in efficient bacterial adherence and entry into epithelial cells.
Materials and Methods: The *invH* gene was amplified and cloned in *Escherichia coli BL21,* the protein was expressed. The recombinant protein purified by nickel–nitrilotriacetic acid (Ni–NTA) affinity chromatography was injected into BALB/C mice to induce immunity. The sera collected after second and third immunizations were assessed for specific IgG by ELISA. The immunized and control mice were orally challenged with various doses of *Salmonella Enteritidis*. Results: The purified InvH provoked significant rise of IgG in mice. Active protection induced by immunization with InvH against variable doses of Salmonella enteritidis, indicated that the immunized mice were completely protected against challenge with 10^4 LD_{50}. Conclusion: RecombinantInvH protein can induce production of antibody in mice. Immunization with InvH protein can develop protection against infection with *Salmonella Enteritidis*.
Keywords: Immunization, *Salmonella enterica*, Recombinant InvH Protein

270. Short Exposure to Collagenase and Co-Culture with Mice Embryonic Pancreas Improve the Human Dermal Fibroblast Culture. Introducing a New andSimple Co-Culture System
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Background: Fibroblast, the mother of connective tissue and the collagen producing cell, also produces a dozen of growth factors and cytokines including fibroblast growth factor, IFN-β (fibroblast interferon) and TGF-β. For these reasons this cell is considered as a cell type that plays a variety of roles in wound healing, immune regulation and homeostasis. Indeed fibroblast could have a cross talk with immune system Isolating dermal fibroblasts is an appropriate way to expand these fast growing cells in vitro. Although using dissociated fibroblast culture method is more convenient than skin explants culture, its enzymatic digestion is critical, because large number of cells can be lost over prolonged exposure to collagenase. This study was performed to increase the number of viable cells after digestion of fresh human foreskin of donors aged from 1 to 3 months with collagenase and also to design a co-culture system for resuscitation of the injured fibroblast.Our results demonstrate that we can maximize cell yield while maintaining the cell viability by cutting the specimens into very small pieces (1-2 mm^3) after removing the epidermal layer with dispase II and also collecting released cells every 20 minutes subsequent to digesting the dermal layer with Collagenase. Moreover our data strongly indicated that co-culturing of isolated fibroblasts with embryonic pancreas explants can enhance the rate of proliferation in cultured fibroblasts.
Keywords: Dermal fibroblasts; foreskin; collagenase; enzymatic digestion; co-culturing; pancreas explants

271. Analysis of Secreted Proteins from Human Immortalized Fibroblast MRC-5 Cell Line Using Two-Dimensional Electrophoresis in Combination with Mass-Spectrometry
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Background: Secreted proteins are namely the complex set of proteins, which released in the cell medium from living cells in classical and non classical pathways. These proteins play potential roles in vital processing cells. Recently, the study of secreted proteins in culture conditions, have been proposed as a valuable means for identification of different biomarkers. Analysis of component secreted proteins in condition media of normal cell lines is necessary for variability reduction in profiles of proteins in cell culture model system studies. The aims of the current study

were the identification of secreted proteins from human MRC-5 cell line. Materials and Methods: We used two- dimensional polyacrylamid gel electrophoresis in combination with liquid chromatography tandem-mass spectrometry to identify the secretom of human immortalized fibroblast cell lines. Results: A total 17 distinct protein species were identified. According to evidence existing, of these identified proteins, 14 founded in serum, different body fluids, membrane compartment or condition media of other cell lines. Some of the identified proteins previously reported in the human adult lung fibroblast reference map, such as cofilin, transgelin and gluthathion s- transferase- p. Conclusion: these identified proteins might be used in investigation studies, especially changes discovery in protein profile expression in response physiological agents between normal and human lung cancer cell lines.

Keywords: MRC-5 Cell Line, Two-Dimensional Electrophoresis, Mass-Spectrometry

272. Super Monoclone Related to Large Scale Production of Anti-Human IgG in Peritoneum of Balb/c Mice

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Background: Monoclonal antibodies and related conjugates are key reagents that are used in biomedical researches and diagnosis of infectious and non- infectious diseases. The aim of this study is large scale production of anti-human IgG in peritoneum of Balb/c mice using super monoclone. Materials and Methods: For large scale production of monoclonal antibody, super monoclone related hybridoma cells were injected into the peritoneum of the Balb/c mice, which have previously been primed with 0.5ml pristane. After 7-10 days, approximately 5ml ascitic fluid was harvested from the peritoneum of each mouse. Ascitic fluid purified with protein a coupled affinity chromatography column, and conjugated with HRP. The subclass of Mab determined with Isotyping kit. Results: The titer of conjugate was 64000 and didn t show cross reactivity with IgM&IgA. The subclass of antibody was IgG_1 and its light chain was kappa. Conclusion: The conjugated monoclonal antibody could have application in diagnosis of infectious diseases like Toxoplasmosis, Rubella, H.Pylori and IgG class of other infectious and non- infectious diseases.

Keywords: monoclonal antibody, ascitic fluid, human IgG

273. Super Anti-human IgG Monoclone with Optical Density over than 3

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Background: Monoclonal antibodies have many applications in diagnosis, treatment and purification. The conjugated monoclonal anti human IgG is as a key reagent in most diagnostic kits. The aim of this study is large scale and semi-industrial production and standardization of this product towards self-sufficiency of the country.
Materials and Methods: Balb/c mice were immunized with purified human IgG and then spleen cells of the most immune mouse were fused with sp2/0 in the presence of Poly Ethylene Glycol (PEG). Supernatant of hybridoma cells was screened for detection of antibody by ELISA method. Cloning of selective high absorbance wells were done with limiting dilution method. Then, the supernatant of suitable monoclones was assessed for cross-reactivity with IgM and IgA by ELISA and confirmed by immunobloting. The subclasses of the selected monoclonal antibodies were determined and the clones were frozen and kept in liquid nitrogen. Results: In the present study, over than 50 clones were obtained that 1 clone had optical density over than 3. We named this clone as supermonoclone and was selected for limiting dilution. The yield of limiting dilution was many clones with absorbance over than 3 and about 2 at 0.01 dilutions which did not show any cross-reactivity with IgM and IgA. Conclusion: The subclass of supermonoclone was IgG1with kappa light chain which selected for reproducing in FCS free RPMI1640 in order to purify with Affinity Chromatography for obtaining supermonoclone as unique monoclone.

Keywords: Monoclonal antibody, human IgG

274. Generation of Random Mutant Library against ureC Subunit of Urease from *Helicobacter Pylori* by Error-prone PCR

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Background: Introduction of antibody molecules and their fragments in research, diagnosis, and therapy has prompted the development of methods to improve their affinity and stability. Single-domain antibodies (VHHs) from naive libraries have dissociation constants (KDs) in the low range and thus, for most antibody applications, their intrinsic affinities need to be improved significantly. The process of somatic hypermutation can be mimicked by *in vitro* random mutagenesis. The most commonly used random mutagenesis method is error-prone PCR, which introduces random mutations during PCR by reducing the fidelity of DNA polymerase. Materials and Methods: Error-prone PCR was performed on the VHH gene obtained against ureC subunit of Urease from *Helicobacter pylori*.Higher concentrations of $MgCl_2$ (7 m*M*) and $MnCl_2$ (0.1, 0.3 mM) along with dITP (200Mm) was added to the reaction mixture in order to increase the error rate. To know the rate of mutation, PCR product were sequenced and compared with original VHH gene used as a template in PCR reaction. The PCR products as a mutant VHH library is then displayed on pComb3x phagmid. Result: Nucleotide sequence analysis compared with original VHH gene revealed that there is a direct correlation between rate of mutation and concentration of Mn,dITP. The rate of mutation observed in this research was 1-2 mutations per 400 bp. Conclusion: Error prone PCR has several advantages in improving the enrichment of the library. The method is rapid and the rate of mutation can be varied with controlling the content of the reaction mixture.

Keywords: Random Mutant Library, ureC Subunit, *Helicobacter Pylori*, Error-prone PCR

275. Cloning and Expression of an Inherent Inhibitory Peptide for TGF-Beta Signaling

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Background: Smurf2 is a member of the E3-ubiquitin ligase family of proteins. Its role is in inhibition of TGF-beta signaling cascade. A tryptophan rich sequence by the name of WW2/WW3 within the molecule is accountable for the binding of the molecule to Smad7. This final complex is the active form which can practically play its part in the inhibition. As depicted, it is clear that WW2\WW3 sequence is an essential component to take part in the negative regulation of the signaling, since, it provides a bridge for the assembly of the two molecules into one single active complex. Materials and Methods: The total RNA was extracted from human PBMC and was *reverse* transcribed into *cDNA*. The sequence of interest encoding the peptide was amplified using PCR primers containing TAT sequence at the 5'-end of forward primer (Because the final aim of this research project is to explore the impact of ww2\ww3 on TGF-beta signaling in living cells, it must enter into cells and for this purpose, tat peptide provides the means) and then the amplified region was inserted into expression vector PGEX-2. Recombinant vector was transformed into BL-21 codon plus for expression. After expression, the bacteria were lysed and the solution dialyzed. Finally, the peptide under investigation was purified by GST-tag chromatography and analyzed with SDS-PAGE and western blotting. Results: WW2/WW3-tat peptide was successfully expressed in codon plus bacteria. The peptide WW2\WW3 along with the GST-tag has a molecular weight of 36 KD which corresponds to the position of the peptide band in SDS-PAGE. Additional confirmation was carried out by western blotting against tat peptide with positive results. Conclusion: Ww2\ww3 peptide can be cloned and expressed in codon plus prokaryotic cells and can be separated using GST-tag chromatography so that it can be later examined for its perturbation on the signaling cascade.

Keywords: Cloning, Inherent Inhibitory Peptide, TGF-Beta

276. Computational Prediction for the Binding Affinity of Interleukins 3 and 5 and GM-CSF to Cell SurfaceReceptors on Human Eosinophils

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein with molecular weight of 14.477 kD comprise 144 amino acids resdiues. The encoding gene of this glycoprotein is located on chromosome 5 in human. This protein stimulates proliferation and differentiation of macrophages. N-terminally seventeen amino acids are serving as a signal peptide and the rest of 127 amino acids are known as molgramostim. Data have revealed a high affinity of this protein for binding to a heterodimer receptor on surface of the cell. The respective receptor includes α and β chains which the β chain is similar to interleukins 3 and 5 receptors. Due to this similarity, interleukins 3 and 5 are able to compete with GM-CSF in binding to the receptor. In the present study, to compare binding affinity of interleukins 3 and 5 and GM-CSF to the related receptor, a computational prediction study carried out using Modeller, Hex and Molegro softwares. According to the results, interleukin 3 with -517.09 kJ/mole, interleukin 5 with -538.05 kJ/mole and GM-CSF with -606.17 kJ/mole energy bind to the α and β chains of receptor. In the next step the two chains of the receptor were separated and the affinity of each protein to this chains were studied. Based on the results the binding affinity of all three considered proteins to α chain of the protein was weaker than the binding to β chain. The binding energy of interleukin 3, interleukin 5 and GM-CSF to β chain of receptors was -620.37 kJ/mole,-663.80 kJ/mole and -696.07 kJ/mole respectively. According to the results, interleukin 3 and interleukin 5 strongly compete with GM-CSF in binding to Cell SurfaceReceptors on Human Eosinophils.
Keywords: GM-CSF, bioinformatics, docking, interleukin, Receptor, immunology

277. Cloning, Expression, Purification and Production of Antibody against Major Subunit of Coli Surface Antigen 3 as a Candidate Vaccine against Enterotoxigenic *Escherichia coli*

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Background: Enterotoxigenic *Escherichia coli* (ETEC) are the major cause of diarrhea. Colonization in the small intestine is thought to be an essential virulence factor of ETEC. Adhesion is mediated by colonization factor antigens. CS3 fimbriae and is one of the most prevalent fimbria1 antigens found in clinical isolates. Gene cluster responsible for CS3 biosynthesis include cstA-cstH genes. It has been shown that cstH encodes the major fimbrial subunit and cstA-G encodes the assembly cassette. Therefore, it seems that this subunit has a critical role in attachment of bacteria to epithelial cells of small intestine and this protein is a putative candidate for vaccine development. In the present study, we designed a recombinant protein that could be a suitable vaccine candidate against this pathogen. Materials and Methods: The sequence of CStH encoding gene obtained from Gene bank and omptimized by bioinformatic software. Synthetic gene in pUC57 was sub cloned into pET28a expression vector. Cloning was conformed with restriction digestion analysis. Expression of recombinant protein was performed in E.coli Bl21DE3 and verified with western blots using anti-His antibodies. Purified protein was injected to raise antibody in Balb/c mice. ELISA test carried out on mice sera for determination of antibody production. Results: immunological analyses showed production of high titer of specific antibody in immunized mice. Anti CStH Antibody could bind to CS3 fimbriae and inhibit bacterial attachment to epithelial cells. Coclusion: The recombinant CStH protein can be taken into account as one of the most important components of vaccines candidate against ETEC.
Keywords: Enterotoxigenic *Escherichia coli*, CStH, Vaccine candidate

278. *In Silico* Study and Expression of Truncated Forms of fliC Gene of Enteroaggregative *Escherichia Coli* (EAEC)

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Background: Enteroaggregative *Escherichia coli* (EAEC) is an emerging cause of acute diarrhea worldwide. It has been shown that flagellin (FliC-EAEC), a major bacterial surface protein of EAEC, activates the innate immunity system via (IL-8) release from several epithelial cell lines. This activation is mediated by Toll-like receptor 5 (TLR-5), which signals through nuclear factor kappa B (NF-κB) which induces transcription of pro-inflammatory cytokines. Based on the ability of FliC-EAEC to activate innate immunity, the flagellin can be considered as a potent adjuvant in designing new vaccines. Materials and Methods: Truncated forms of FliC-EAEC were designed based on its interaction site with TLR-5. The truncated forms were docked to TLR-5 using *Hex* docking server. Depending on the energy values and the pose of their interactions we have chosen the best truncated forms and then, various GST-tag fusions of the truncated forms were investigated for their interaction with TLR-5. Finally, the most appropriate forms were PCR amplified, cloned to pGEX-5X-1 plasmid and expressed by Top10 strain of *E. coli*. Results: Two different amino acid sequences with the most suitable interaction with TLR-5 were obtained following *in silico* analysis of various FliC truncated forms. Cloning of the fragments, fused to the GST-tag of the pGEX-5X-1 was confirmed by DNA sequencing. Finally, expression of the desired fragments were observed on SDS-PAGE and confirmed by western blotting using anti-GST tag specific antibodies. Conclusion: According to our *in silico* results, two truncated forms of the FliC could effectively interact with the TLR-5 receptor. These forms were cloned and expressed in GST fusion forms. The constructs should be purified and further verified by *in vitro* studies. This finding can lead us toward design of a flagellin based adjuvant. These truncated forms can be capable of inducing immune responses and also they can be easily cloned and expressed in the form of a fusion protein.
Keywords: *In Silico*, fliC gene, Enteroaggregative *Escherichia Coli*

279. Fragment F(ab')$_2$ Produced by Digestion of Rabbit Immunoglobin G with Pepsin

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Background: Immunoglobulin (Ig) fragments can be advantageous for a number of experimental methods. F(ab')$_2$ is a bivalent antibody fragment which is currently used for both diagnosis and treatment. F(ab')$_2$ fragments are smaller than whole Igs but maintain antigen binding function. The smaller size results in better tissue penetration and less steric hindrance leading to more sensitive antigen detection. IgG are a class of large proteins of approximately 150 kDa, made of two identical heavy chains (50 kDa) and two identical light chains (25 kDa). Each IgG has two antigen binding sites that can bind a single antigen molecule independently. Fragments of IgG molecules composed of only antigen binding portions can be obtained through enzymatic degradation. The enzyme pepsin cleaves the Fc portion of an IgG into small subfragments leaving a F(ab')$_2$ fragment with two antigen binding sites connected by disulfide bonds. In this study we used whole proteins of *Mycobacterium Avium Paratuberculosis* (causative agent of Johne's disease) as a antigens to produce polyclonal antibodies and produced F(ab')$_2$ through pepsin digestion of rabbit IgG. Materials and Methods: Polyclonal antibodies prepared by immunization of New Zealand white rabbit against *Mycobacterium Avium Paratuberculosis* (MAP) proteins. Rabbit was bleeded and serum was sedimented and Immunoglobulins were obtained. IgG was purified by ion exchange chromatography (DEAE-celloluse) and IgG solution were digested by pepsin for isolated the fragment F(ab')$_2$. Results: Purification was carried out by ion exchange chromatography on DEAE-celloluse that we collected seven fractions composed of IgG and result to one peak. SDS-PAGE of Digested IgG by pepsin gave a single band. Conclusion: In conclusion, we produced a rabbit anti-MAP polyclonal antibodies by immunization and The F(ab')$_2$ fragments are produced by digestion of the whole antibodies with pepsin. We checked the separated F(ab')$_2$ fragments by SDS-PAGE and we obtained high purity of F(ab')$_2$ fragments.
Keywords:Fragment F(ab')$_2$, Rabbit Immunoglobin G, Pepsin

280. Selection of Recombinant Antibodies to Human Epidermal Growth Factor Receptor 4 (HER4)

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Background: The human epidermal growth factor receptor (HER) family comprises four homologous members: EGFR (ErbB1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4). Alterations and disruptions in the function of the HER-kinase axis can lead to malignancy. Among HER family members, EGFR and HER2 are the most studied. However, data provide evidence for the significance of HER3 and HER4 alterations in cancers especially breast carcinogenesis. The heterodimerization of HER2 with HER3 and HER4 lead to tumor cell proliferation. Recombinat single chain antibodies (scFv) have been introduced as the most desire agent for cancer immunotherapy due to their human origin, fast penetrating and high affinity properties. In this study scFv to HER4 antigen was selected. Materials and Methods: A phage antibody display library of scFv was panned against immunodominant epitope of HER4. The peptide was coated in Nunc tube, after adding the phage antibody (10^9 PFU/ml), elution was done using log phase Ecoli TG1. The clones were PCR amplified and DNA fingerprinted to select the specific clones against the epitope. Results: Results represented 2 predominant patterns with frequencies 25% and 30%. The other patterns showed frequencies 5%. Conclusion: Recombinant antibodies are the most ideal form of cancer immunotherapy. Trastuzumab, a humanized monoclonal antibody, targets cancer cells that overexpress HER2. Since it is not a full humanized antibody, it produces HAMA (human anti-mouse antibody response). Human scFvs not only are able to overcome this problem but also are able to penetrate to tumor tissue effectively. We have already selected scFvs against HER2 and HER3 epitopes and showed their inhibitory effects on breast cancer cell lines. In order to inhibit the HER2 heterodimerization more effectively, in this study anti-HER4 scFvs were selected. Panning results demonstrated 2 specific clones against HER4 epitope. Further investigations are needed to show the effects of the selected single chain antibody.
Keywords: Recombinant Antibodies, HER4

281. Expression of Camelid-derived Heavy Chain Antibody (Nanobody) against ureC Subunit of Urease from *H.pylori* in pichia pastoris

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Background: The methylotrophic yeast *Pichia pastoris* has become a cost affecting system for production of a variety of proteins particularly those require specific folding such as antibody fragments. The increasing popularity of this expression system is due to several factors such as: the capacity to perform many eukaryotic post-translational modifications, such as glycosylation, disulfide bond formation and proteolytic processing. In the present work we have cloned the gene coding for the camelid- derived heavy-chain antibody (VHH) against ureC subunit of urease from H.pylori in the pPink-HC vector and expressed in yeast *Pichia pastoris*.Since this protein is already expressed in E.coli, therefore our aim is to compare the function of the protein expressed in two different systems .Any change in their function will be subject to their folding. Materials and Methods: The VHH gene fragment was subcloned into the pPink-HC vector. The construct was transferred to *E. coli* top10. After multiplication and purification, the pPink-HC vector was linearzed by cutting at a unique site in order to promote integration into the pichia pastoris genome. The vector was transferred in to competent cells. The cells were spread on MD agar selection plates and white colonies were selected as positive clones and further confirmed with PCR. The colonies were expressed in the BMGY and BMMY mediums.The results of the expression was analysed on SDS PAGE. Results: The expression of a 17-kDa protein was observed in the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Conclusion :The affinity of the antibody to its specific antigen will be investigated after purification.
Keywords: Nanobody, ureC, Urease, *H.pylori*

282. Replicating Nonviral Minicircles as a Novel Tool in Gene Therapy: Construction and Transfection Efficiency Evaluation

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Background: Current delivery methods used in gene therapy and vaccination can be subdivided into viral vectors with higher cell transduction efficiency, and nonviral methods with less toxicity without DNA insert size limitations. Bacterial backbone sequences are dispensable for gene transfer application which reduce the efficiency of gene expression. Therefore, an important goal in nonviral vector development is to produce supercoiled pDNA lacking bacterial backbone. Thus, producing episomal minicircle vectors, devoid bacterial backbone was developed. These extra-chromosomal DNA vectors showed persistent and high level of transgene expression. New generation of these vehicles carrying Scaffold/matrix attachment region (S/MAR) elements causes long-term expression of transgene in the absence of selection. Presence of S/MAR elements in minicircle DNA can exploit the cellular replication machinery for episomal replication. Construction of replicating non-viral minicircle can be achieved by site specific recombination in parental plasmid between two copies of recombinase ΦC31 recognition sites in bacterial cells. This study was designed for constructing an efficient vehicle containing S/MAR elements creating minicircle DNAs in purpose of long term expression of target genes in cell lines. Materials and Methods: At the first step, a DNA fragment containing EGFP CDS, S/MAR elements and SV40 promoter was amplified using pGL268 pEpi-FGM18F plasmid as a template, and inserted into pTZ57R/T. Next this fragment was treated with *Nhe*I and inserted into the parental plasmid originated from pBAD/gIII A plasmid, between two ΦC31 recognition sites. Eventually after an induction of parental plasmid to produce integrase, minicircle DNA formation was resulted and checked in CHO cell line by measuring EGFP expression. Results and Conclusion: Data indicated that both parental plasmid and minicircle DNA carrying EGFP-S/MAR were constructed successfully. Moreover, generated minicircle retains functionally to produce EGFP in different passages and generations for at least 2 months. Thus construction of an efficient parental plasmid with S/MAR elements is applicable for long-term transfection in different cell lines in episomal state.
Keywords: Nonviral Minicircles, Gene Therapy, Construction, Transfection, Efficiency

283. Expression of Camelid-derived Heavy Chain Antibody (Nanobody) against *Clostridium botulinum* Neurotoxin E in Pichia pastoris

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Background: The methylotrophic yeast *Pichia pastoris* is one of the standard tools used in molecular biology for the generation of recombinant protein.As yeast, *P.pastoris* is a single-celled microorganism that can be easy manipulated and cultured. Being a eukaryote, Pichia pastoris is capable doing many of the post-translational modifications performed by higher eukaryotic cells such as proteolytic processing, proper folding, disulfide bond formation, and glycosylation. In the present work we have expressed and purified the camelid-derived heavy-chain antibody (nanobody) against Clostridium botulinum neurotoxin type E in Pichia pastoris. Consequently the structure and function of the protein so produced will be compared to that of previously E.coli expressed antibody particularly from affinity point of view . Materials and Methods: VHH gene was cloned into a high copy number vector, pPink-Hc and transferred first to *E. coli* top10. PichiaPink™ vector containing the VHH was linearized by cutting at a unique site to promote integration into the *Pichia pastoris* genome and transfered into competent cells. The transformed cell mixtures was spread on MD agar selection plates and incubated at 28°C for 6 days until colonies were formed. Recombinant VHH was expressed in pichia pastoris. BMGY and BMMY were used for expression of the protein of interest. Results: Recombinant VHH was expressed

in pichia pastoris. Protein Expression was analyzed by SDS-PAGE. Conclusion: Many proteins that end up as inactive inclusion bodies in bacterial expression systems are produced as biologically active molecules in *P. pastoris.*
Keywords: Nanobody, *Clostridium botulinum*, Neurotoxin E, Pichia pastoris

284. Selection of Nanobodies form Naïve VHH Phage Library Derived from Camelied Heavy Chain Antibodies by Whole-cell Panning
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Background: Cholera is one of the major health threats in developing countries. Recent epidemics showed that effective diagnostic and treatment for *V.cholerea* is imperative. Antibodies are being used as tools for such diagnostic and therapeutic measures. They can specifically recognize and neutralize their target. Camels are able to produce antibodies that lack the light chains. These heavy chain antibodies have full capacity of antigen binding through three CDRs within their variable domain. Variable domains (VHH) or nanobodies can be easily mass produced in microorganism and are compatible with display technologies such as phage, ribosome and yeast display. The aim of this study was to select high affinity nanobodies from naïve library using phage display technology and cell panning. Materials and Methods: Lymphocytes were isolated from peripheral blood and total RNA was extracted and converted to cDNA. VHHs were amplified by two sets of primers using nested PCR. pComb3x phagmid and VHHs were digested using *SfiI* enzyme. After ligation recombinant phagmids were transferred into *E.coli* TG1 bacteria. VHH coding phage particles were produced by infecting transformed bacteria with M13K07 helper phages. 10^7 *V.cholerae* bacterium was coated in 96-well ELISA plate. Phage particles were added and high affinity VHH coding phages were isolated and propagated through five rounds of cell panning. Improvement of binding affinity was studied by polyclonal phage ELISA using *V.cholerae* bacterium as an antigen. Results: RNA extraction was resulted in 28s and 18s rRNA on 1% agarose gel. The 600, 700 and 900 bp amplicons were obtained from first PCR. The second PCR resulted in 400 bp VHHs. Ligation was confirmed with PCR on colonies and phagmids. Polycolonal phage ELISA showed increasing affinity after each panning and the highest affinity was obtained on fifth panning. Conclusion: VHHs selected by whole-cell panning from naïve library showed high affinity toward *V.cholerea* bacterium.
Keywords: Nanobodies, VHH Phage Library, Camelied Heavy Chain Antibodies, Whole-cell Panning

285. The Effect of Induced Hyperglycemia on the Expression Levels of TLR2 and TLR4 Genes in the Hippocampus of Male Wistar Rats **during a Time Course Induction of Diabetes Type 1**
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Background: Type 1 diabetes (T1D) is an organ-specific autoimmune disease that results from T cell-mediated destruction of insulin-producing pancreatic beta cells in genetically predisposed individuals. Numerous studies have shown that the inflammation induced by hyperglycemia is the main mechanism of the pathogenesis of diabetic neuropathy. The relationship between inflammation and diabetic neuropathy progression included the processes and complex molecular networks. There is compelling evidence that the innate immune system plays a key role in early mechanisms triggering diabetes. Toll like receptors (TLRs) are key molecules recognizing foreign and endogenous danger signals, activating and regulating innate immunity and inflammation, and finally inducing adaptive immunity. TLRs have been shown to play essential roles in infections, inflammatory diseases and cancer. A number of studies demonstrated that TLRs mediated innate immune responses could contribute to the induction of diabetes. Abundant evidence also suggests that TLRs are important players in neurodegenerative diseases, which involve many inflammatory components. Although many studies have shown that these genes are induced in diabetes, it is not clear whether these genes are involved in the development of diabetes.The role of inflammation in neurological diseases has been recently confirmed. Materials and Methods: In this study the time course expression of the TLR2 and TLR4genes in hippocampal brain tissue of diabetic male Wistar rats were studied. Hyperglycemia was induced in male wistar rats with intraperitoneal (I.P.) injection of Streptozotocin. In different time points (4, 6, 8 and 20 weeks) post diabetes type 1 induction, rats were euthanized and hippocampal brain tissues was removed for further analysis. RNA was extracted from hippocampal brain tissues samples followed by cDNA synthesization using oligo-dT primers. Exon specific TLR2 and TLR4 primers were used to amplify rat TLR2 and TLR4 cDNA. After performing semiquantitative RT-PCR, the expression level of TLR4 mRNA was quantified by real time quatitative PCR (qPCR). Results: Up-regulation of TLR2 and TLR4 transcripts during the time course after diabetes induction as compared to the control group was shown. Conclusion: Our results demonstrate that the expression of TLRs may play a decisive role in the pathogenesis and expansion of diabetes. It is possible that the expression of TLRs can eventually lead to neurodegenerative disease such as Alzheimer. Therefore, studies on the precise role of TLRs in neurodegenerative disease may yieldpotential molecular targets for developing therapeutics for controland prevention of diabetic neurodegenerative disorders.
Keywords: Hyperglycemia, TLR2, TLR4, Wistar Rats, Diabetes Type 1

286. The Inflammatory Properties of Single Walled Carbon Nanotubes Functionalized with Polyethylene Glycol (PEG-SWNT) in Human Monocytic THP-1 Cells
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Background: The use of new nano materials in biology and medicine has been the focus of interest in recent years. The unique physico-chemical properties of carbon nanotubes have made them an excellent candidate in biology and medicine. One of the key advantages of carbon nanotubes in biomedical applications is that they can be easily internalized by cells, and therefore can act as delivery vehicles for a variety of molecules relevant to therapy and diagnosis. Carbon nanotubes can be single-walled or multi-walled and are now produced in substantial quantities for a variety of commercial applications. The clinical applications of using carbon nanotubes in medicine will depend on the outcomes of efficacy and Immunotoxicological studies, which will provide the necessary risk-to-benefit assessments for carbon-nanotube based materials. Materials and Methods: The objective of the present study was to evaluate the *in vitro* inflammatory properties of single walled carbon nanotubes functionalized with polyethylene glycol (PEG-SWNT). Human monocytic cell line THP-1 was cultured with various concentrations of PEG-SWNT in different time points and the expression of several innate immunity receptor genes was analyzed by real time quantitative PCR method. In this study the expression of several pattern recognition receptors (PRRs) including TLR2, TLR4, CD14 and adaptor protein MyD88 was analyzed in the PEG-SWNT treated THP-1 cells. Results: According to our results a significant up-regulation of TLR2, TLR4, CD14 and MyD88 was observed when THP-1 cells were treated with various concentrations (10 µg/ml, 50 µg/ml and 100 µg/ml) of PEG-SWNT. However, when the cells were treated with 200µg/ml PEG-SWNT, the expression of PRRs was down-regulated which might suggest that, higher concentration of PEG-SWNT is toxic for the cells. Conclusion: the results presented in this study shows that PEG-SWNT could trigger cellular inflammatory responses as was shown by the expression analysis of genes involved in innate immunity and inflammation.
Keywords: Single Walled Carbon Nanotubes, Polyethylene Glycol PEG-SWNT, THP-1 Cells

287. Gene Expression Analysis of Toll-Like Receptor 2 And 4 (TLR2 And TLR4) In Peripheral Blood Mononuclear Cells (PBMCs) of Mycobacterium Avium Subsp. Paratuberculosis Infected Cows with Quantitative Real-Time PCR (qPCR)
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Background: Mycobacterium avium subsp. paratuberculosis (M. ptb) is the causative agent of Johne's disease, a chronic granulomatous enteritis in ruminants, characterized by thickening of the intestinal wall and progressive wasting leading to death of the animal. Current estimates indicate that Johne's disease is one of the most costly infectious diseases of livestock in many countries including Iran. The immune response to mycobacterial infection involves phagocytosis of organisms by mononuclear phagocytes and sequestration within phagosomes. The capacity of the organism to prevent macrophage activation and phagosome maturation and to attenuate induction of a Th1 immune response appears to largely determine its pathogenicity. Our current understanding of the biology of M. ptb infection has been hindered by limited knowledge of the host factors involved in the immune response to the organism and the lack of appropriate molecular tools to dissect the host–pathogen interaction. Recognition by the host of molecular patterns displayed by microbes is essential to development of an effective immune response. Pattern recognition receptors (PRRs) are the main sensors of pathogen and danger signals in innate immunity. They are mainly expressed by macrophages and dendritic cells of different organs. Toll like receptors (TLRs) are the most studied and best characterized PPRs which are responsible for sensing pathogen associated molecular patterns (PAMPs). Recent studies have focused on the role of the TLRs family of cell membrane receptors in initiating cell signaling associated with mycobacterial infections. Activation of TLRs has also been shown to coordinate the adaptive immune response to microorganisms. Materials and Methods: In this study, we aimed on the expression levels of bovine TLR2 and TLR4 transcripts in the peripheral blood mononuclear cells (PBMC) of cows infected with M.ptb by Real-time quantitative PCR (qPCR). Blood samples were taken from five cows which were positive in two consecutive ELISA test for paratuberculosis. As for the control, blood samples of five ELISA negative cows were also taken. After isolation of peripheral blood mononuclear cells (PBMC), total RNA was isolated and cDNA was synthesized using Oligo dT primers. Then, the primer pairs for TLR2, TLR4 as target genes and GAPDH and β-actin as housekeeping and calibrator genes were optimized in a gradient RT-PCR experiment. Subsequently, qPCR analysis was set up to quantify and compare the relative expression levels of TLR2 and TLR4 transcripts in PBMC of paratuberculosis positive and negative cows. Results: Statistical analysis using one way T-test showed a highly significant up-regulation of TLR2 and TLR4 expression in PBMC of paratuberculosis infected cows as compared to the negative control group ($p<0.001$). Conclusion: the results presented in this study, can shed more lights to the insight mechanisms behind the molecular immuopathogenesis of paratubeculosis which might eventually pave the way for designing novel and more effective therapeutic as well as preventive strategies.
Keywords: TLR2, TLR4, PBMCs, Mycobacterium Avium Subsp, qPCR

288. Characterization of Recombinant Structural Subunit of Colonization Factor6as a Candidate Vaccine against Enterotoxigenic *Escherichia Coli*

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Background: Enterotoxigenic *Escherichia coli* (ETEC) is recognized as one of the common agent of diarrhea in children under 5 years in developing countries and an important agent for traveler's diarrhea. Enterotoxin and colonization factors(CFs) are the major virulence mechanisms in ETEC .CFs are surface proteins that mediate adherence to host small intestinal. CFs highly immunogenic and appear to be protective antigens in human. One of the most prevalent of the ETEC CF is CS6. CS6 consist of two structural subunit (CssA, CssB) that are appeared to be necessary for attachment to intestinal cells. In the present research, the CssB protein is expressed in *E. coli* and the purified protein was investigated to its immunological effect as a vaccine candidate. Materials and Methods: Information about CssB gene was obtained from gene bank. A sequence encoding the CssB gene was designed using E.coli codon bias. The optimized gene was synthetized and cloned into expression vector, pET28a .The CssB gene was expressed in *E. coli* BL21 (DE3) cells. The recombinant protein was confirmed with Western blot. The protein was purified with Ni-NTA affinity chromatography. The immunization studies were conducted in mice with purified CssB. ELISA test was performed on mice sera for determination of antibody production. Results: ELISA results showed high titer of antibody production in mice. Pretreatment of the ETEC cells with immunized mice antisera remarkably decreased their adhesion properties and blocked their binding to Caco-2 cells. Conclusion: Recombinant protein CssB is an immunogen protein molecule and one of the important components in vaccine strategies against ETEC.
Keywords: Enterotoxigenic *Escherichia coli*, Colonization factor, Recombinant protein, Vaccine candidate

289. Nanobody: Nanotechnology in Nature

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In all cameldis found two types of antibodies: The classical antibody of 2 H and 2 L chains and the HCAbs (unique for camelids). A H-chain of a HCAb lacks one of the constant domain of a H-chain of a classical antibody. The N-terminal domain of the HCAb, the Variable domain contains a few amino acid substitutions compared to the variable domain of a classical antibody, therefore named it a 'VHH'. This two chains antibody called heavy chains antibody (HCAb). The antigen binding site of HCAb so called nanobody. The recombinant, monoclonal Nanobody is well produced in bacteria, is very stable and highly soluble and highly yield and it binds the antigen with high affinity and specificity. Very often the Nanobody recognizes an epitope that is difficult to target with human or mouse antibodies. the largest advantage of Nanobodies comes from their strict monomeric behaviour and the ease to tailor them into larger pluripotent constructs. Now, nanobody applied for many application for example in tumor targeting for Search maximal tumor load and fastest blood clearance (invivo imaging) and in therapy in Ab dependent enzyme prodrug therapy (Nbs against African trypanosomes). There are more than 25 programmes in research and development on nanobody production, and there were five Nanobodies in clinical development. Two Nanobodies are on track to reach potential clinical proof-of-concept during 2011(TNFα, vWF, RANKL, IL-6R ,CXCR4, RSV,…).
Keywords: Nanobody, Nanotechnology, Nature

290. Production of Nanobodies from Camel-derived Heavy Chain Antibodies against Vascular Endothelial Growth Factor (VEGF)

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Background: Vascular Endothelial Growth Factor (VEGF) is a molecule which has important roles in blood vessels in tumors and therefore is an attractive target for anti-cancer therapy. Heavy chain antibodies (HcAbs) are a special kind of antibodies produced in the Camelidae family and have unique features in medical applications. In this research Camelid heavy chain antibodies have been produced against recombinant VEGF and a phage library was constructed. Materials and Methods: A 110 bp sequence of the VEGF protein was synthesized and subcloned into the pet32 vector. The recombinant protein was expressed in *E. coli* and purified by Ni-NTA column and was then injected to a camel. Lymphocytes were extracted from the blood and RNA purification was performed. cDNAs were constructed from RNAs by RT-PCR. Amplification of the cDNA is performed using Nested PCR. and VHH fragments were constructed from the cDNA. The VHH fragments were cloned into phagemid PComb3x and a library was constructed via phage display technique. Results and Conclusion: The 35 kDa VEGF peptide was expressed successfully. After injection, immunization of the camel was confirmed by ELISA. Lymphocytes were extracted successfully from the blood. Total RNA extraction was confirmed with the presence of 28s and 18s RNAs in the Agar gel and cDNA construction was accomplished

successfully. The 600 and 700 bp bands were observed in the gel from the first PCR, and 400 bp from the second PCR. Cloning of the VHH domain was done in PComb3x and the titer of the library was high.
Keywords: VEGF, Heavy chain antibody, VHH domain , phage display

291. Tumour Inhibitory Effects of EGFR-Mimotope Bacteriophage Based Vaccine

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Background: Epidermal growth factor receptor is one of many tyrosine kinase receptors with oncogenic potential. Its over-expression is usually associated with cancer progression, invasion and metastasis. It is over-expressed in a wide variety of human malignancies. Several small molecule inhibitors and monoclonal-antibodies have been used to treat EGFR over-expressing tumours. One example of such antibodies is ICR-62 produced in rat which showed promise in preclinical studies. Previously in our lab, a peptide mimotope of EGFR corresponding to the binding region of ICR62 was isolated from a phage display library and was displayed on P-VIII of M-13 phage and used to immunize mice, the antibody response was evaluated. In this study we assessed the tumour inhibitory potential of the EGFR-mimotope-bacteriophage based vaccine *in vivo*. Both prophylactic and therapeutic effects of EGFR vaccine were evaluated. Materials and Methods: In prophylactic group of animals, the EGFR-momotopre-phage vaccine was used to immunize mice. Helper-phage and PBS injected groups of mice were used as negative control. Two weeks after the last immunization, mice were challenged sc in the right flank by LL/2 cells and tumour volumes were measured for four weeks. In therapeutic group the animals were injected sc in the right flank with LL/2 cells. After tumour formation, the mice were immunized by EGFR-mimotope-phage vaccine, helper phage and PBS as negative control. Tumour volumes were measured for four weeks. Results and Conclusion: In prophylactic group of animals there was no significant difference in tumour growth rate of animals immunized by EGFR-mimotope phage vaccine in comparison with helper phage immunized and non-immune mice.In therapeutic group of animals, both EGFR-mimotope vaccine and helper phage significantly reduced tumour growth in comparison with non-immune mice. However there were no significant difference between EGFR mimotope phage vaccine immunized and helper phage immunized mice. Thus we are yet to decide whether this vaccination strategy is successful.
Keywords: EGFR-Mimotope-bacteriophage, Vaccine, *in vivo*

292. *Erwinia Chrysanthemi* L-Asparaginase: Epitope Mapping and Production ofAntigenically Modified Enzymes

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This study shows that the antigenicity of *Erwinia chrysanthemi* Lasparaginase can be reduced by site-directed mutagenesis. Interest in L-asparaginase (EC 3.5.1.1) has grown considerably since this enzyme was found to have anti-tumour activity. The bacterial L-asparaginases from *Erwinia chrysanthemi* and *Escherichia coli* are effective in treating acute lymphoblastic leukaemia and lymphosarcoma, but their use against other forms of leukaemia or solid tumours is limited, since remissions are invariably of short duration .L-Asparaginase catalyses the hydrolysis of Lasparagine. Some tumour cells are deficient in L-asparagine synthetase and cannot synthesize sufficient L-asparagine. These cells are dependent on extracellular sources of the amino acid in order to complete protein synthesis. They can therefore be destroyed by starving them of L-asparagine by the administration of L-asparaginase.
Ten B cell epitopes of the enzyme were identified using synthetic hexapeptides and polyclonal antisera from rabbits and mice. The region 282GIVPPDEELPG292 near the C-terminus was an immunodominant epitope. Binding of two hexapeptides (2831VPPDE288 and 287DEELPG292) to the antibodies was dependent on Pro285, and Pro286, since their replacement by almost any other amino acid resulted in reduced binding. The other residues were less important for binding the antibodies, as binding was relatively unaffected by amino acid substitutions. Three site-directed mutant enzymes, P285T (proline-285 -. Threonine etc.), P286Q and E288A, were expressed in Escherichia coli. The pI values of P285T, P286Q and the wild-type enzymes were 8.6, and that for the mutant E288A was 9.2. The kcat and Km values for the mutants P286Q and E288A with L-asparagine and L-glutamine were comparable with those of the wild-type enzyme. The Km values for the mutant P285T with both substrates was similar to that of the wild-type enzyme, whereas the kcat was reduced by 2-fold with L-asparagine and by 4-fold with L-glutamine. The change proline -+ threonine reduced the antigenicity of the enzyme by 8-fold, as shown in sandwich using monoclonal antibodies raised against the wild-type enzyme.
Keywords: Epitope Mapping, Antigenically , Enzymes

293. Construction of an Efficient Expression Vector Encoding Soluble Form of Human Hyaluronidase Type PH20 for Purpose MS CompoundTherapy

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Background: The hyaluronidases are the enzymes hydrolyze β-1, 4 glycosidic linkage of hyaluronan. Hyaluronan is a polymer consisting of a repeating disaccharide unit found in cumulus ovuforus complex, semen liquid and other tissue. Addition to hydrolyzing the hyaluronan , hyaluronidase can penetrate through the cumulus cells layer that surrounds the oocyte, thus it terms spreading factor. Moreover, it is used to increase the absorption and dispersion of injected drugs. Hyaluronidase triggers the re-myelination of the affected axons through degradation of hyaluronan accumulated in inflammatory demyelinating lesions in the CNS. Accumulation of hyaluronan has been found to prevent the maturation of oligodendrocyte progenitors into myelinating cells in demyelinating lesions in MS. Materials and Methods: At first step, total mRNA was extracted from testis tissue and cDNA was synthesized. Ph20 coding sequence deleted GPI anchor was amplified by means of specific primers designed based on ph20 special CDS and also contained additional regionsencoding His tag and distinctive sequence recognized by entrokinase enzyme. Then, an amplified fragment was inserted into pTZ57R and treated by appropriate restriction enzyme to sub clone into pBudCE4.1. In this recombinant expression vector, attB region was added for insertion of this construct into genome by phiC31 integrase produced by another vector termed as pCMV-Int. Results: The constructed expression vector was confirmed successfully as verified by sequence analysis. After transfection, culture media was extracted and tested on Granulosa cells. The cell mass was separated effectively that indicate this protein is active. Conclusion: In this study, we produced an appropriate vehicle encoding recombinant hyaluronidase for therapeutic approach and recombinant protein for MS and infertility therapy.
Keywords: Hyaluronidase Type PH20, MS compound therapy

294. Engineering of Recombinant Nanobody against *Clostridium botulinum* Neurotoxin Type E by Error-prone PCR

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Background: Botulinum is one of the most toxic substrate acting on the peripheral nerve system causing acute flaccid paralysis. Rapid diagnosis and treatment of botulinum neurotoxins are crucial. Antibody products are being used for diagnosis as well as for the treatment of adults.VHHs are a new class of single-domain antigen binding fragments derived from heavy chain antibodies, found within sera of the camelidae. These antibodies have several characteristics like persistent to acid and alkaline pH, temperature, high solubility and ability to cross the stomach without loss of biological activity. In the present research in order to enrich the VHH library against binding domain of BONT/E, we introduced random mutations to the previously constructed gene using error- prone PCR. Materials and Methods: Error-prone PCR technique was applied with taq DNA polymerase which have no detectable 3′ to 5′ exonuclease proofreading activity and low-fidelity reaction. In order to increase the

frequencies of mutation, non-standard PCR reaction conditions such as addition of Mn^{2+}, introduction of dITP, unbalanced dNTP levels, raising Mg^{2+} and taq DNA polymerase concentration and reduced template concentration were implemented. Results: DNA fragments obtained from PCR products with different reaction conditions were analyzed for their nucleotide sequences . The results showed varied rates of mutation (3%-48%). Mutagenic effect of $Mn^{2+,}$ dITP and high concentration ofMg^{2+} on different PCR products were significant. Conclusion: In this study, we employed error-prone PCR to generate broad diversity in anti-toxin E nanobody gene. Our aim is to construct various random libraries for this nanobody gene. These libraries could be valuable sources for selection of high affinity anti-toxin E nanobody.
Keywords: Recombinant Nanobody, *Clostridium botulinum*, Neurotoxin Type E, Error-prone PCR

295. Preparation of Infusible Platelet Membrane Microvesicles and Evaluation of Sonication Effects on it

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Background: Platelets are the components of blood necessary for hemostasis and wound healing. At the most of blood transfusion centers, the shelf-life of platelet units are only 5 days. Due to short half-life of platelets, there have been a number of attempts to develop substitutes for platelets. One of the strategies for extending the storage time of platelets is preparation of platelet fragments to synthesizing artificial platelets from purified phospholipids and proteins. The aim of this study is to prepare Infusible Platelet Membrane (IPM) microvesicles and evaluation of sonication effects on it by measuring its particles size. Materials and Methods: Eight units of outdated platelets are collected from Tehran Blood Transfusion Center and the units were pooled and centrifuged at 1000 RPM, 15 min for removing WBC and RBC cells. Supernatant was centrifuged at 2500 RPM, 30 min to discard platelet poor plasma. For fragmentation of platelets, precipitate was resuspended in 25 ml of normal saline and freeze-thaw procedure was repeated three times at -80°C and 22°C respectively. The product was centrifuged at 2500 RPM, 30 min and precipitate was resuspended in 45 ml of normal saline and for viral inactivation, pasteurization method for 20 h at 60°C was applied. Particles size was measured before and after sonication by using Zetasizer, Malvern instrument. Results: The results showed that, before sonication of IPM, %66.5 of particles had 231 diameter (nm) and %33.5 of particles 1020 diameter (nm), meanwhile after sonication of IPM, %94.4 of particles had 232 diameter (nm) and %5.3 of particles 37.3 diameter (nm). Conclusion: With regard to previous studies, Cyplex company have been produced IPM microvesicles with average size of 700 nm. The reason of this selection of particle size was pharmaceutical effectiveness of IPM which is used in the treatment of bleeding due to thrombocytopenia. The results of this study shows that method of preparation of IPM without sonication is closer to 700 nm , in other words our method indicates that sonication causes more fragmentation of IPM microvesicles and so application of freeze-thaw procedure is sufficient to achieve optimal size particles.
Keywords: Infusible Platelet, Microvesicles, Sonication Effects

296. Homology Modelling and Comparison of A Recombinant Anti-Human CD4 Single-Chain Variable-Fragment Antibody with Native Antibody

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Background: Antibodies have diagnostics and therapeutics applications. Full size antibodies are more immunogenic and more difficult to manipulate compared to Single-chain variable fragments (scFv) of antibodies. scFv is a fusion protein of the variable regions of the heavy (VH) and light (VL) chains of immunoglobulin, connected with a short linker peptide. Materials and Methods: The scFv of a monoclonal mouse antibody against human CD4 was cloned from hybridoma cells using the phage display technique and was produced in recombinant form in *Escherichia coli*. Expression, production, and purification of anti-CD4 scFv was tested using SDS-PAGE, Western blotting, and the specificity of scFv for human CD4 molecule was examined using ELISA and flow cytometry. Results: A 31 kDa recombinant anti-CD4 scFv was expressed and produced successfully in bacteria. Sequence analysis proved the scFv structure of the construct. Recombinant scFv t was able to bind to CD4 with the affinity comparable to native original hybridomal anti-CD4 antibody as assessed by ELISA. The binding site of the antibody was domain 3 of the human CD4 molecule. The canonical structure of anti-CD4 scFv antibody was obtained using the SWISS_MODEL bioinformatics tool and compared to scFv general structure and native hybridomal anti-CD4 antibody. Conclusion: Recombinant scFv is able to bind to the CD4 molecule with affinity comparable to native antibody. Engineered anti-CD4 scFv could be used in immunological studies, including fluorochrome conjugation, bispecific antibody production, bifunctional protein synthesis, and other genetic engineering manipulations. Since the binding site of our product is domain 3 (D3) of the CD4 molecule which is different from the HIV binding domain of CD4 molecule (D1), further studies are needed to evaluate the anti-CD4 scFv potential for diagnostic and therapeutic applications.
Keywords: Recombinant Anti-Human CD4, Native Antibody, scFv

297. Luciferase-based Nanobioreporter, a Useful Tool for Targeting IgGs

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Background: Renilla luciferase, a monomeric 36kDa protein from Renilla reniformis, catalyzes coelenterazine oxidation to produce light. Post-translational modification is not required for its activity and the enzyme expresses functionally both in prokaryotic and eukaryotic cells. Renilla Luciferase is widely used to determine biological process, because the enzymatic luminescence assay is highly sensitive, rapid, and nonradioactive and can be quantified in a noninvasive manner. Recently, Renilla luciferase has been interested in clinical diagnosis based on in vivo and cell imaging. Here we report a new generation of IgGs targeting nanobioreporter which is containingRenilla luciferase and Fc binding peptide. Materials and Methods: DNA sequence encoding the luciferase-based nanobioreporter was amplified using specific primers. The PCR reaction was carried out by 32 cycles of 94 ºC for 1 min, 60 ºC for 1 min and 72 ºC for 1 min and the final extension was performed at 72 ºC for 10 min. Amplified products were separated on 1% agarose gel, and ~1kb long fragment were purified from the gel using the gel extraction kit (vivantis). cDNA fragments were then digested by Nhe I/HindIII and inserted into the digested pET21a. Competent cells of *E. coli* XL1-Blue were transformed by pET21-nanoRLuc construct using chemical method. Positive colonies were screened for Nono-probe, by PCR and sequencing. Expression by IPTG and purification by Ni-NTA Sepharose column was designed to achieve according to standard protocols and the purified probe was designed for binding to Fc IgGs. Finally, Bioluminescence signals of the nanoprobe from renilla luciferase were planned to measure with a luminometer apparatus. Results: Sequence encoding the RLuciferase-based nanobioreporter including the binding peptide for Fc reign of IgGs was amplified by PCR. From the sequence designed for the new probe, the nanobioreporter amplicon is about 1000 bp long and has an open reading frame of 330 amino acid residues. The construct obtained from the manipulated sequence is able to express an IgG-binding nonoprobe after expression in *E.coli* BL21. Moreover the probe, which can purify by IMAC method, shows the ability to bind the Fc region of IgG and produce a detectable signal in luminometer device. Conclusion: In this research a new reporter based on the Nanolight-technology was designed, assembled by PCR and developed for binding to IgGs. It seems that it is a useful tool for clinical diagnosis approaches based on IgG-technology. Moreover it seems that the new probe is a useful tool for future analysis in the field of in vivo imaging.
Keyword: IgG, Renilla luciferase, nanobioreporter, Imaging

298. Production of ScFv Antibody Fragments Specific to CCK-BR/gastrin Receptor from a Semi-Synthetic Phage Antibody Library

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Background: It has been found that phage display technology is a powerful and widespread approach for production of monoclonal antibodies (mAbs) with therapeutic, diagnostic and targeting purposes. Cholecystokinin-B receptor (CCK-BR)/gastrin receptor, a member of GPCR superfamily, plays a crucial role in pathogenesis of several malignancies particularly gastric cancers and it has been confirmed that targeting CCK-BR with monoclonal antibody (mAb) seems to be a promising modality for gastric cancer therapy. The aim of this study was production and characterization of CCK-BR specific scFv (single-chain fragment variable) antibodies using a phage antibody library and solution-phase biopanning. Materials and Methods: A semi-synthetic phage scFv library was panned against a biotinylated peptide (BP) equal to the second extracellular loop of CCK-BR, subsequently phage-antibody binders were captured by streptavidin-coated magnetic beads. After four rounds of selection with 100 nM (rounds 1&2) and 50 nM (rounds 3&4) concentrations of BP, polyclonal phage ELISA was used for monitoring the progress of biopanning. Individual soluble antibody producing clones were obtained from the fourth round of selection and screened for their peptide-binding activity by ELISA. The ELISA-positive clones were sequenced and then analyzed for binding to native CCK-BR protein by Western blotting. Results: Polyclonal phage ELISA confirmed enrichment of peptide-specific phages with significant increasing signal after four rounds of selection (14 folds greater than round 1). Screening of 196 soluble antibody clones by ELISA revealed nine antibody fragments (4.68%) with binding activity to the peptide. Interestingly, sequencing of these soluble antibody fragments showed eight different scFv and one V_L single domain. Five out of eight scFvs are able to detect a 80 kDa band protein corresponding to recombinant CCK-BR in Western blot analysis. Conclusion: The results of this study showed that phage display technology together with solution-phase biopanning can be a useful method for production of scFv antibody fragments specific to CCK-BR protein.
Keywords: ScFv, CCK-BR/gastrin Receptor, Phage Antibody Library

299. Production of Nanobody (VHH) Derived Camelid Heavy Chain Antibody against PSMA Antigen in Prostate Cancer by Phage Dislay Technique

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Background: Prostate cancer is the most cancer in men. Antibody therapy offers promise for cancer treatment. Antibody therapy depends on the identification of molecular targets (antigen). Prostate-specific membrane antigen (PSMA) is a potential molecular target in prostate cancer that abundantly expressed on prostate cancer epithelial cells. The interesting type antibody fragments find in *Camelidae* are VHHs. Antibody fragments could be displayed on the surface of filamentous phages, called phage-display. After displaying an antibody fragment on the protein surface of the phage, antigen specific phages can be selected and enriched by multiple rounds of affinity panning. Finally, monoclonal nanobody against PSMA was produced. Design and production of an epitope of PSMA antigen, production of immune camel nanobody phage library, screening of library against PSMA and finally production of monoclonal nanobody (VHH) against PSMA was performed in this study.
Materials and Methods: A DNA fragment encoding dominant epitope of PSMA was synthesized and expressed on *E.coli.* Camel was immunized with purified rPSMA. Following mRNA isolation and CH2 gene specific reverse transcription, two successive PCRs are performed. VHH fragments are cloned and displayed on phage for selection. Bound phages can be reinfected into bacteria and re-grown for further enrichment and eventually for analysis of binding. After phage Elisa and selection some clony, production of soluble nanobody was performed. Result and Conclusion: Purified rPSMA protein was confirmed by Western blot and SDS-PAGE. Amplification of VHH gene was confirmed with electrophoresis. Confirmation of VHH library was done with colony PCR. Increased affinity of library was confirmed with Elisa. Production of recombinant monoclonal nanobody (VHH) was confirmed by Western blot and SDS-PAGE. Affinity of nanobody against rPSMA was 5.7×10^{-7}. Respect to this point that this nanobody has high level of specifity and affinity, so it can be an effective tool for curing and diagnosing prostate cancer.
Keyword: Nanobody, VHH, Phage display, PSMA, Prostate cancer

300. Alginate Nanoparticles: Noval Vaccine Delivery Systems for Infection Diseases

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Background: Today, nanotechnology as a multidisciplinary field covers sciences especially medicine sciences. Nanoparticles (NPs) as engineered structures with diameters of <100 nm produced by physicochemical processes. Among polymers, sodium alginate due to its unique properties (adjuvant, biodegradable and mucoadhesive polymer) has been used as carrier for different biological agents such as genes, drugs and antigens that cause to sustain release in the human body. The aims of the present study were to synthesis of DT-loaded nanoparticles as novel vaccine delivery system. Materials and Mathods: Alginate nanoparticles were prepared by ionic gelation technique. In order to manufacture of NPs, CaCl2 solution was added to sodium alginate solution dropwisly under homogenization. In different steps, influence of physicochemical factors such as different concentrations of polymer and CaCl2 solutions, homogenization time and stirring speed were studied. The NPs were characterized for their morphology and size distribution by SEM and DLS, respectively. Diphtheria toxoid (DT) was loaded in optimum NPs and loading efficiency and loading capacity were assessed. Invitro release profile of DT were investigated in (PBS, pH7.4, 37°C). The antigen activity was evaluated by double immunodiffusion test Results and Conclusion: Results showed that, concentration of 0.3%w/v polymer and 0.1%w/v CaCl2, stirring speed 1300 rpm and homogenization time 45min were obtained as optimum conditions that redounded to desirable size and spherical shape (Table 1). DT_ loaded NPs have good monodispersity (PDI: 0.32) and Zeta potential (-36.1). SEM photograph of loaded NPs showed that NPs were spherical without any aggregation. The invitro release profile of DT_ loaded NPs shown the prolong release (over 120 hours). Result confirmed that the integrity and antigen activity of the encapsulated DT remains intact. It is concluded that, these alginate NPs due to favorable size and monodispersity, great LC (over 81%), sustain release profile and antigen stability, could be used as remarkable carrier for novel vaccine delivery system.
Keywords: Alginate Nanoparticles, Noval Vaccine Delivery Systems, Infection Diseases

301. Preparation Conjugate Vaccine of *Haemophilus Influenzae* Type B Polyribosylribitol Phosphate with KLH Protein and PLGA Nanoparticle and Its Immunological Evaluation in Animal Model

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Background: Today,nano-materials are themostwidely usedmethods ofmakingmodernmedicine. Thesematerialsarevery usefulinincreasing theaccessibilityof drugstotargetanddestroycancerlineages. Materials and Methods: Inthisstudy,thePRPantigen of*Haemophilus influenza* conjugatedto keyhole limpet hemocyanin (KLH) that isapowerfulimmunogenmolecule andananoparticle withhighadsorption called poly lactic co-glycolic acid(PLGA).Thetwo-partandthree-partconjugatewasinjectedintoSW1 mices. Therefore,themiceswere divided into6 groups.Group 1 consideredasacontrol. Groups of 2-6 were injected PRP,PRP+KLH,PRP-KLH,PRP-KLH-PLGA andRP-TT with complete Freund's adjuvant respectively. 28daysafterinjection, blood wasobtainedandtheincrease inserumantibody titerwas determinedwithELISAtechnique. Results: Resultsshowedincreasedtitersof IgGandIgMantibodies. Conclusion: The resultsindicatethattheconjugatedtriplex-containing PLGA hadmore absorptionandhigherpathogenicity. Theconjugated antigencanswitchtheimmune responsestowardsT cell-dependent responses;induce highaffinityantibodyandformation ofmemoryB cell.Therefore conjugated antigen can bemore powerfulvaccineagainst*Haemophilus*meningitis.

Keywords: Conjugate Vaccine,*Haemophilus Influenzae* Type B, KLH Protein, PLGA Nanoparticle

302. Immunological Evaluation Antibody against rh EPO (human recombinant Erythropoietin), Produced in Pasteur Institute of Iran, as a Solid Phase in ELISA Assay as an in-house Antibody Detection

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Background: Human recombinant erythropoietin is a hormone which is administered when a patient is not producing enough erythropoietin on his or her own. This hormone is typically produced and activated in the kidneys, and may be used in patients who are in kidney failure. It can also be used to treat people with anemia, and in cases where patients need to build up their hematocrit but cannot receive a blood transfusion. During production of EPO, it has to do some exam to detect and monitoring the amount of product like concentration of EPO and contamination to host cell. Most of the pharmaceutical manufactory use its (in house) kit and antibody in quality control and in process control. this method is more reliable to controlling whole production process. Materials and Methods: in this experience EPO in deferent doses with complete freund's adjuvant and incomplete freund's adjuvant was injected to rabbit with special protocol. 1 month later the blood of the rabbit was taken and centrifuge. the serum was separated and with ionic chromatography and affinity IgG column antibody was purified. This antibody was compared with commercial kit to approve its activity. Results: in each step SDS PAGE %12 was used to calculated the purity and after purification WHO protocols ICB Q2(R1) and ICH Q2B used to evaluated the antibody and compare with commercial kit. Conclusion: using n house antibody and kit is a normal method in most biological manufactory and we try to check this method to produce a an antibody special for our biological product and we will exam its stability at the future.
Keywords: rh EPO, ELISA

IMMUNODEFICIENCY

Oral Presentation

303. Immunophenotyping in Subgroup of B lymphocytes in CGD patients

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Background: Chronic granulomatous disease (CGD) is a rare primary immunodeficiency syndrome characterized by a greatly increased susceptibility to severe fungal and bacterial infections. These patients with not knowing causes are also at risk of chronic inflammatory manifestations, including inflammatory bowel disease and autoimmune diseases. In this study, we investigated immunophenotype altrations of naïve B cells, memory B cells and B1 cells in peripheral whole blood of CGD patients in comparison with healthy controls. Materials and Methods: Flow cytometry analysis was performed on 31 Patients and 23 controls for investigating onmemory B cell, B1 cell and naïve B cell and were measured human sCD27 by ELISA and Immunoglobulins by nephelometric method. Results: We found the increase of naïve B cells (IgD+/CD27-), diminish of memory B cells (CD27+) and increase of B1 cells (CD5+) in CGD patients. However, it was not found significant alterations in sCD27 between CGD patients and healthy controls. The mean of IgA and IgG serum levels were more than normal range in some patients, whereas a mean of IgM serum levels were in the normal range. Conclusion: It is concluded that NADPH oxidase deficiency in CGD could be affected subsets of B cells in phenotype level and impaired conversion of naïve B cell to memory B cell during infection and result in decrease of memory B cell. On the other hand, increase of CD5+ B cells could be consistent with autoimmune frequency in these patients.
Keywords: Immunophenotyping, B lymphocytes, CGD patients

304. Recent Update on Genetic Defects Associated with Congenital Defects of Phagocyte

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Congenital defects of phagocyte is a group of primary immunodeficiency diseases (PIDs), which could be divided into defects of neutrophil differentiation, defects of motility, defects of respiratory burst, and mendelian susceptibility to mycobacterial diseases according to recent classification from the International Union of Immunological Societies Expert Committee for PIDs. Several gene mutations have been identified in severe congenital neutropenia, including *ELANE, HAX1, GFI1, G6PC3*, while cyclic neutropenia (*ELANE*), poikiloderma with neutropenia (*C16orf57*), p14 deficiency (*ROBLD3*), glycogen storage disease type 1b (*G6PT1*), Barth syndrome (*TAZ*), and Cohen syndrome (*COH1*) are other inherited disorders of this group of neutrophil differentiation defects. Leukocyte adhesion deficiency is the prototype of defects of motility, with three subtypes (*INTGB2, FUCT1, KINDLIN3*), while Rac2 deficiency (*RAC2*), β-actin deficiency (*ACTB*), specific granule deficiency (*C/EBPE*), localized juvenile periodontitis (*FPR1*), Papillon–Lefèvre syndrome (*CTSC*), and Shwachman–Diamond syndrome (*SBDS*) can also be categorized in this group of phagocyte defects. Chronic granulomatous disease (CGD) is due to defects of respiratory burst. Mutations in *CYBB* lead to x-linked form of disease, while mutations in *CYBA, NCF1, NCF2,* and *NCF4* lead to autosomal recessive CGD. Mendelian susceptibility to mycobacterial diseases (MSMD) is a group of PIDs that highly vulnerable to weakly virulent species of mycobacterium. *IL-12Rβ1, IFN-γR1, IFN-γR2, IL12B, STAT1, CYBB,* and *IRF8* are the genes that their mutations have been introduced as cause of MSMD.GATA2 deficiency (*GATA2*) and pulmonary alveolar proteinosis (*CSF2RA*) are two new genetic defects that have been classified as congenital defects of phagocyte.
Keywords: Genetic Defects, Congenital Defects, Phagocyte

305. The Study of Class Switch Recombination (CSR) in B Lymphocytes from Patients with Common Variable Immunodeficiency (CVID)

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Background: Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by hypogammaglobulinemia and recurrent bacterial infections. Most CVID patients have normal numbers of circulating T cells and surface immunoglobulin-positive B cells. However, CVID B cells fail to differentiate into immunoglobulin-secreting plasma cells in vivo. A specific genetic defect that accounts for all CVID phenotypes has not been identified yet. Defect in class switch recombination is reported in some cases of CVID. This study was done to investigate the frequency of class switching defect in Iranian patients. Materials and Methods: Fourteen CVID patients, (twelve males and two females) and 14 age-matched healthy controls were enrolled into the study. Briefly, PBMC were isolated from blood by Ficoll-Hypaque density gradient centrifugation. For the molecular analysis of class switching this cells were cultured in the presence of IL-4 and CD40L and cultured cells mRNA was analyzed for the presence of IgE mRNA by PCR method after 5 days of culture. Results: RT-PCR for IgE transcripts mRNAs showed that all controls express IgE normally. However 4 patients among the 14 were unable to switch to IgE in response to IL-4 and CD40L. Conclusion: Our results showed that CVID patients are heterogeneous in class switching ability like other aspects of B cell activities.

Keywords: CSR, B Lymphocytes, CVID

306. $CD4^+CD25^+FOXP3^+$ Regulatory T cells (Treg) Abnormality in Patients with Common Variable Immunodeficiency (CVID)

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Background: Common variable immunodeficiency (CVID) is a heterogeneous syndrome characterized by defective immunoglobulin production and high frequency of bacterial infections, autoimmunity and chronic inflammation. $CD4^+CD25^+FOXP3^+$ regulatory T cells (Treg) play an essential role in immune responses including down-modulation of immune response to pathogens, allergens, cancer cells as well as self-antigens. Abnormalities of $CD4^+CD25^+FOXP3^+$ regulatory T cells (Treg) have been associated with autoimmune and inflammatory disorders. In this study we investigate the frequency of $CD4^+CD25^+FOXP3^+$ Treg cells in CVID patients and compared the results with healthy controls. Materials and Methods: Sixteen CVID patients and 6 healthy individuals were enrolled in our study. Peripheral blood mononuclear cells were isolated and the frequency of Treg cells was determined using flow cytometric assay. Results: A significantly lower proportion of $CD4^+CD25^+FOXP3^+$ T cells was observed in CVID patients compared with healthy controls (P=0.008*). Conclusion: Our results indicate that the reduced frequency of Treg cells might be responsible for impaired immune function specifically autoimmune manifestations in CVID patients.
Keywords: Autoimmunity, Common variable immunodeficiency, FoxP3, regulatory T cells

307. Proapoptotic Genes "BAX and BIK" Alterations in Ataxia Telangiectasia

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Background: Ataxia telangiectasia is an autosomal recessive multisystem disorder that characterized by variable immunodeficiency, progressive neurodegeneration, occulocutaneous telangiectasia, and an increased susceptibility to malignancies. Chromosomal breakage and radiation hypersensitivity are main features of AT, which could lead to an increased susceptibility to cancers. T cell leukemia and B cell lymphoma are the most common cancers in this group of patients. We studied the role of proapoptotic BAX, and NBK/BIK genes in the AT patients to elucidate the possible role of these genes in progression of malignancies in this disease. Materials and Methods: Fifty Iranian patients with AT were investigated in this study. The entire coding regions of the NBK/BIK gene (exons 2–5), and BAX gene (exons 1–7) were amplified using polymerase chain reaction (PCR). The PCR products were separated by 2% agarose gel electrophoresis, and all positive samples were verified by direct sequencing of PCR products using the same primers used for PCR amplification, BigDye chemistry, and Avent 3100 Genetic Analyzer. Result: Higher frequency of nucleotide substitution in the noncoding region of BAX exon 7 (g.6855G>A) was also identified in 68% of the patient group (60%homozygotes and 8% heterozygotes) versus 24% in the controls, P value<0.0001, OR 6.73; 95%CI 2.99–15.36). There was not any exonic alteration in the NBK/BIK gene. Sequence alteration in intronic region of NBK/BIK gene IVS4-12delTC was observed in 23 of 44 cases (52.3%) of AT patients (13 heterozygotes and ten homozygotes). Conclusion: we observed different alterations of proapoptotic genes, NBK/BIK, and BAX. The results from our study would be in line with the proposed involvement of the mitochondrial pathway mediated apoptosis in accelerating and developing of cancers and in immunopathogenesis of AT, which would offer basics to establish mitochondrion-targeted therapeutic interventions in AT.
Keywords: BAX, BIK, Ataxia Telangiectasia

308. Classification of CVID Patients Based on Naïve CD4+ T cell Levels and Clinical Features

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Background*:* Common variable immunodeficiency (CVID) comprises a heterogeneous group of disorders with unknown etiology. In this study, we aimed to evaluate naïve CD4+ T in CVID patients and compare them with healthy controls and classify patients according to their naïve CD4+ T cells. Materials and Methods*:* Twenty patients with CVID and 20 age- and sex-matched healthy controls were included in this study. CD4+ T cells were negatively isolated from peripheral blood mononuclear cells by magnetic beads. Cell surface markers (CD45RA, CD62L) were assessed by flow cytometry.Results*:* Naïve CD4+ T cells (CD45RA+ CD62L+) were significantly lower in CVID patients compared to healthy controls. CVID patients were classified into 2 subgroups based on naïve CD4+ T cell levels (lower and higher than 30%naive CD4+ T cell levels). Complications including splenomegaly, bronchiectasis, lymphadenopathy and autoimmunity were more significantly seen in the group with lower-than-30% naïve CD4+ T cell levels. Conclusions*:* The classification of patients based on naïve CD4+ T cell levels seems to be consistent with clinical features.
Keywords: CVID, Naïve CD4+ T Cell, Clinical Features

309. Mutations in ELA2, HAX1, G-*CSFR, GFI1 and G6PC3* in Iranian Patients with severe Congenital Neutropenia

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Background: Severe congenital neutropenia (SCN) is a rare primary immunodeficiency disease. Different genes are found to be associated to SCN, including *ELA2, HAX1, WAS, GFI1, G-CSFR and G6PC3.*Patients with severe congenital neutropenia who referred to Immunology, Asthma and Allergy Institute between May 2007 and Dec 2011 enrolled the study.Materials and Methods: Genomic DNA of the patients were extracted from peripheral blood samples using standard procedures. Neutropenia related exons and flanking regions of *ELA2, HAX1, WAS, GFI1, G-CSFR and G6PC3* were amplified by PCR and the sequences were analyzed. Twenty one patients with SCN during the study were evaluated (14 men, 7 female), Results:The result showed 2 ELANE mutation, 9 HAX1 mutation and 2 G6PC3 mutation. None of the patients had GFI1 mutation and also one mutation found in G-CSFR in one patient with ELANE mutation. Conclusion: According to these results most of the patients had HAX1 mutations and about 35 percent of patients had unknown genetic diagnosis.
Keywords: ELA2, HAX1, G-*CSFR, GFI1, G6PC3* Neutropenia

Poster Discussion Presentation

310. Report of Two Siblings with c1q Deficiency and Systemic Lupus Erythematosus

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C_1q deficiency is the strongest known genetic factor for lupus. We describe two siblings with lupus and different presentation, associated with c_1q deficiency. The older sibling presented with prolonged fever, malar rash, and oral lesions at the age of 6 years. He had auto antibodies to the extractable nuclear antigens, double stranded DNA, and Anticardiolipin. The younger sibling presented with developmental regression, generalized spasticity, and some erythematosus rash on her face at the age of 9 months. She was suspected to lupus according to result of skin biopsy and had auto antibodies to extractable nuclear antigens, double stranded DNA, Anticardiolipin, Sm, and RO. According to presentation of younger sibling with central nervous system and skin manifestation we evaluated them for hereditary complement deficiencies. Both of them had undetectable titers of c_1q level. Patients with c_1q deficiency present nearly with early onset lupus. Lupus in these patients is more severe and has an earlier age of onset and also they have increased rate of infection relates to compromised opsonisation and decrease in B-cell co-stimulation.C1q deficiency should be suspected in every young child with lupus.
Keywords: Siblings, c1q Deficiency, Systemic Lupus Erythematosus

311. Consanguinity in Families Who Have Two or More Children with Autosomal Recessive Chronic Granulomatous Disease

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Backgraound: Chronic granulomatous disease (CGD) is a hereditary primary immunodeficiency disorder characterized by recurrent bacterial and fungal infections. CGD results in a defect in Nicotinamide Adenine Dinucleotide Phosphate oxidase enzyme in leukocytes. This study assessed consanguineous marriages in families who had two or more children with AR CGD. Materials and Methods: Families who had at least two children affected with AR CGD entered this study. We evaluated families according to the parental marriages into 3 groups: First cousin marriages, second cousin marriages and non consanguineous marriages. The diagnosis of CGD was based on nitro blue tetrazolium (NBT) test and DHR oxidation reduction (less than 10%) in the neutrophils after stimulation with phorbol myrisates acetate (PMA). Results: Twenty four families who had 61 patients including 29 (41.3%) male and 31 (51.7%) female with mean age of 17.82 ± 9.15 range from 1 to 39 years old entered this study.
The rate of first cousin, second cousin and non-consanguineous marriages in these families were 13 (54.2) families with 31 children, 7 (29.2) families with 22 children and 4 (16.7) families with 8 children respectively. Of these 24 families, 18 families had at least two consecutive siblings with CGD. Conclusion: In Iran and most of middle-east countries, rate of AR CGD is increased due to high rate of consanguinity. Our results showed the high prevalence of consanguineous marriages especially in families with more than two children with CGD. It is recommended to warn families with consanguineous marriages and particularly first cousin marriages about the risks of genetically inherited disease. Consanguineous families with one CGD child should also be aware of the high risk of CGD occurrence in their following children and prenatal diagnosis can be diagnostic and preventive for these families.
Keywords: Autosomal Recessive, CGD, Consanguinity

312. Comparison of Anti-pneumoccocal Antibody and Its Relation to IgG2 Level in Patients with Recurrent Sinopulmonary Infections and Controls Pre and Post Pneumococcal Vaccine

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Background: Selective antibody deficiency with normal immunoglobulines (SANDI) may be identified as part of district primary or secondary immunodeficiency disorders. It is a condition in which the production of specific antibodies to polysaccharide antigens is impaired in a patient with normal immunoglobulin and IgG subclass concentrations .The clinical manifestations include recurrent, often severe or prolonged , upper or lower respiratory tract infections. In our study to evaluate SANDI in patient with recurrent sinopulmonary infections the serum level of anti-pneumococcal antibody, IgG_2 subclass and correlation between them pre and post pneumoccocal vaccine has been measured. Materials and Methods: In a case control study, 46 cases and 54 controls, IgG_2 and anti-pneumococcal antibody titers evaluated in their serum before pneumococcal vaccine (23 valent polysaccharide vaccine, Merk, Germany) and also anti-pneumococcal antibody titers at least 4 weeks post injection. The levels were measured by enzyme-linked-immunosorbent assay (ELISA). Multiple regression analysis has been used to for relations. Results: There was a significant correlation between age and anti pneumococcal antibody before and after vaccination in cases (P=0.005) and controls (P=0.09) also between age and IgG_2 levels (P=0.001) in controls. A positive relation between pre and post vaccination titer of anti-pneumococcal titer was founded in cases (P=0.002) but no co- relation between anti-pneumococcal antibody titer and IgG2 serum level pre and post pneumococcal vaccine observed. Anti pneumococcal antibody titers mean before and after vaccination significantly were different in cases and controls (P=0.01, P=0.001 respectively) and were higher in control group. Conclusion: Evaluation of anti-pneumococcal antibody titer in Patients with recurrent, chronic and severe respiratory infections with normal immunoglobulins levle seems to be necessary and early diagnosis could prevent the later sequels such as chronic sinopulmonary infection, mastoiditis and bronchiecstasia.
Keywords: Selective antibody deficiency with normal immunoglobulins (SANDI), recurrent infections, pneumococcal vaccine.

Poster Presentation

313. Alterations in Immune Responses of Classic Phenylketonuric Patients: A Systematic Review

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Background: Classic phenylketonuria (PKU) is a disorder of amino acid metabolism which leads to the accumulation of phenylalanine and its metabolites in blood and tissues due to severe deficiency of phenylalanine hydroxylase activity. In some patients with PKU there is an increased susceptibility to infections. Changes in humoral and/or cellular immunity, due to different mechanisms, have been suggested. To review the available literature on alterations of immune responses in PKU patients a systematic review of all published articles was done. Materials and Methods: A computerized search strategy using Medline with MeSH headings ("Phenylketonurias/immunology" as one of the main headings) and appropriate keywords was performed. For additional potentially relevant publications, the references of all identified relevant studies were searched. The articles were analyzed by two reviewers. Results: The computerized searches in Medline between years 1960-2012 yielded 12 citations of which 10 (83%) were potentially suitable (2 articles were about non-classic PKU) for inclusion, and reference checking of these publications added another 8 articles. Of a total of 18 potentially appropriate articles, four were about the autoimmune disorders such as asthma, eczema or scleroderma in which there were no specific immunological evaluation. Finally, 14 of these were included in the review, of which no abstract or full-text were available for two of them. In the last 12 articles, 75% (9/12) reported at least a change in acquired cellular and or

humoral immunity. In 25% there were no changes in acquired and/or innate immunity. One third (4/12) of articles reported both acquired cellular and humoral immune changes in affected phenylketonuric patients. Conclusion: This is the first review about alterations of immune responses in phenylketonuria. Although there were few valid articles in databases about this subject, it seems that phenylketonuria may affect immunity in different ways. More controlled studies are needed to demonstrate this hypothesis.
Keywords: Phenylketonuria, Immune Responses

314. Suggestion of Some Hypothesis for AIDS Treatment

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In the course of multiplicating and replicating of AIDS virus few vital factors are presented , which one part belongs to virus and another belongs to white blood cell (WBC). As it is known bone marrow cells are sources of these WBCs. It may be possible that by Genetic engineering enterfere, e.g making some changes in the genom of (these) WBCs create some differents behavior in WBCs function and change the vital factors of AIDS virus, or destroy its vital factors. According to molecular and cellular studies, a few hypothesis for this purpose is suggested theoretically: 1-Compensating missed WBCs by introducing a gene such as semitreated gene of Leukemia in to the genome of bone marrow cells, to produce increase number of healthy WBCs, which is more than need of a healthy person. 2- Preventing expression of introduced gene in to the WBCs genome by inhibiting proteins, to prevent for RNA polymerase and mRNA content viral genes production. 3- Producting of protease refractory of reverse transcriptase enzyme in two ways: a) drugs b) introduction of its manufacturing gene into bone marrow cells. 4- Distructing some parts of WBCs which are not vitally necessary for WBCs but is vital for multiplication process of virus.
Cunclusion: By this hypothesis we have tried to suggest new ways for AIDS treatment to improve immune system of the patients and create more resistance against over diseases.
Keywords: Hypothesis, AIDS Treatment, WBC

315. To Investigate Humoral Immune Response among Stainless Steel Welders

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Background: The humoral immune response is the aspect of immunity that is mediated by secreted antibodies. In industries,welders are exposed to a range of fumes and gases. Stainless steel (SS) welding fumes contain some toxic metallic compounds like chromium, nickle, manganese and iron. The aim of the present investigation was to study the effect of stainless steel welding fumes on the humoral immunity. Materials and Methods: 30 stainless steel male weldersand 30 male controls enrolled in our study. The serum level of IgA,IgG andIgM were determined by turbidimetry Stainless steel welding fumes were sampled during 8 hours of welding and were analyzed by ICP-AES method. Results: There were no significant differences in the mean levels of serum IgG, IgM and IgA between stainless steel welders and controls. However, IgG level in smoker welders was significantly lower than non-smoker welders (*P- value* 0.002). Conclusions: Several investigators have shown that long-term smoking significantly reduces serum levels of immunoglobulins in humans. Our results showing that smoking seems to be aetiologically more important than fume inhalation as one of the occupation risk in welders. Further studies are needed for determining the appropriateness of periodic check-ups of immune functions.
Keywords: Humoral immune response, Stainless steel welders

316. *Mycobacterium tuberculosis* Meningitis as the First Presentation of Chronic Granulomatous Disease

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Background: Chronic granulomatous disease (CGD) as an uncommon congenital phagocyte disorder is characterized by life-threatening bacterial and fungal infections. Several neurological complications have been reported in patients with CGD; among them, meningitis is mostly due to the Candida. However, there wasn't any report of meningitis due to *Mycobacterium tuberculosis* (TB) as the first presentation of CGD. Herein we describe a patient who was referred to our center with TBM that further investigations during the course of disease confirmed the underlying diagnosis of CGD.
Case Presentation: A 3 year-old girl with FTT was admitted to our hospital due to persistent fever, headache, vomiting and chills from 5 weeks ago. On the examination, patient did not have Kernig's sign, Brudzinski's sign and stiff neck. Work-up for Fever of Unknown Origin (FUO) were done. Serology results were all negative. Bone Marrow Aspiration (BMA) did not reveal any pathologic changes. Phenobarbital and Phenytoin was given to the patient following presentation of atonic seizures. Lumbar puncture was performed owing to suspicion to meningitis. Cerebrospinal fluid (CSF) revealed lymphocytic pleocytosis while presence of two acid fast bacilli was reported in CSF. Protein and glucose profile of CSF were in consistency with TBM. PCR was negative for TB DNA. Anti–TB agents were given to the patient with the addition of Streptomycin. CT-scan was performed which showed mild ventriculomegaly. Due to the suspicion to immunodeficiency, immunologic work-ups were done. The patient diagnosed with CGD regarding compatible results of Nitroblue-Tetrazolium and flow cytometric Dihydrorhodamine 123 assay. Therefore the patient was treated by interferon-γ and Co-trimoxazole and Itraconazole. Due to consistent headache, a ventriculoperitoneal shunting was done which resulted in dramatically improvement of headache and vomiting. After 1 week, fever and all of the symptoms improved. Conclusion: In this presented case, establishment of CGD diagnosis had an important role in successful treatment, although delayed. Unfortunately, unawareness of physicians is still an obstacle in diagnosis of patients at proper time. Prompt diagnosis of underlying immunodeficiency and appropriate treatments are the key elements that could survive the immunodeficient patients and avoid irreversible organ damage and death in this group of patients.
Keywords: *Mycobacterium tuberculosis*, Meningitis, Chronic Granulomatous Disease

317. Oral Manifestations of Immunodeficiency Disorders in Children

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Background: Immunodeficiency disorders involve malfunction of the immune system, resulting in infections that develop and recur more frequently, more severe, and last longer than usual. Facial, dental, and oral findings are frequently demonstrated in children with immunodeficiency disorders. The aim of this review article was evaluation of oral manifestations of immunodeficiency disorders in children. Materials and Methods: Electronic database search was done, including published information availabe until 2011 about oral manifestations in children with immunodeficiency disorders. Results: Mouth ulcers, gingivitis, stomatitis, periodontal disease (particularly prepubertal periodontitis), recurrent aphthous ulcers, mucosal candidiasis, petechiae in oral mucosa, bleeding of oral cavity, angular cheilitis are the most common oral findings in patients with immunodeficiency disorders. Conclusion: It is important that the dentist be able to identify and

differentiate various oral lesions and abnormalities that may signal a previously undiagnosed immunodeficiency disorders from those that are not associated with any concurrent, underlying systemic disease or associated health problem.
Keywords: Immunodeficiency disorders, oral manifestations, children

318. Leukocyte Adhesion Deficiency Syndrome: Clinical Presentation and a Brief Review
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LAD is a rare autosomal ressecive immunodeficiency disorder characterized by reccurent and chronic bacterial and occasionally fungal infections without pus formation despite persistant leucocytosis as well as gingival and periodontal disease. There are three types of LAD syndromes, LAD 1, 2 and 3. They are characterized by an inability of Leukocytes to adhere and migrate during inflammatory and host defense reactions.LAD 1 is caused by mutations in a beta 2 Leukocyte integrin which results in an increased susceptibility for early onset prepubertal periodontitis. LAD 2 is caused by mutations in a focus transporter protein leading to poor formation of Sialyl lewis x (CD15) the ligand for L-selectin.The only persistant clinical symptom is chronic severe periodontitis. In LAD 3 all the receptors fail to become activated to mediate Leukocyte and platelet adhesion and cell spreading. So bleeding is a major problem.
Keywords: Leukocyte Adhesion Deficiency, Clinical Presentation

319. Zinc Supplementation: Is It effective on CD4 Level in HIV Positive patients?
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Background: Sufficient zinc is essential in maintaining immune system function, however HIV infected individuals are particularly susceptible to zinc deficiency. In HIV infected patients, low serum levels of zinc have been associated with a more advanced stage of the disease and also with an increase in mortality. However, the HIV virus also requires zinc itself, and excessive zinc intake may stimulate the progression of HIV infection. This study was performed to further assess optimal zinc intakes for HIV infected individuals. Materials and Methods: This clinical trial study was conducted in Navab–Safavi urban health center, in 2009. The patients that referred to this center were randomly assigned to receive zinc supplementation (zinc sulfate 45 mg per day) for about two months. CD4 level before and after the oral administration was counted and data was analyzed using SPSS 16. The chi-square and T-paired tests were used for data analysis. Results: According to the results, mean CD4 levels before and after the intervention was 486.6±226 and 460.6±203.9, respectively. No statistically significant difference was seen in CD4 levels (P=0.19). Conclusion: Our findings are not in line with the results of the previous studies that suggested a connection between zinc supplement and treatment of HIV. Thus more studies with larger number of patients, different doses of zinc supplementation and longer administration of zinc supplement are suggested.
We would also like to acknowledge the national elite foundation that supported us attending the congress.
Keywords: Zinc, HIV, CD4

IMMUNODERMATOLOGY

Poster Discussion Presentation

320. Asociation of IL-8 (-251 A/T) Gene Polymorphism with Leprosy in Iranian Population
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Background 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 susceptibility to Leprosy. Chemokines are potent chemoattractors of specific leukocyte subsets and therefore, are 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. The expression of IL-8 has been found in skin biopsies from lesions across the leprosy spectrum. Interestingly, production of IL-8 can be controlled by the −251 A/T polymorphism in the promoter region of this chemokine. The A-allele in this single nucleotide polymorphism was found to be related to higher in vitro levels of IL-8 production after stimulation with lipopolysaccharide or cytokines such as IL-1β and TNF-α. In the present study, for the first time in the world, we examined polymorphism in the IL-8 −251 A/T gene promoter region with respect to Leprosy in a population-based case-control study in Iran. Materials and Methods: One hundred and seventy-five Leprosy patients and 467 healthy and ethnic-sex-age matched controls were included in this study. IL-8 promoter (−251 A/T) gene polymorphism was genotyped via allele specific PCR (ARMS-PCR) method. Results A significant difference was found in IL-8−251 A/T polymorphism between Leprosy patients and controls ($p = 0.002$). This deference was a result of a lower incidence of the low producer allele of IL-8 (T allele) in Leprosy patients compared to controls. Conclusion In summary, carriers of IL-8 −251 T/T genotype may have decreased susceptibility to Leprosy because of their lower ability to IL-8 production compared to other two genotypes carriers.
Keywords: IL-8, Gene Polymorphism, Leprosy

Poster Presentation

321. A Report of Sever Shoe Contact Dermatitis
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Delayed hypersensitivity reactions are inflammatory reactions initiated by mononuclear leukocytes 48-72 hours after antigens exposure. This case report is about a 21years old boy Allergic contact dermatitis in feet. Symptoms were inflammation and necrosis in the center of ulcers. Allergy to preservative chemicals in shoe leather was diagnosed as the reason. For reduction and deletion of this problem should be used shoes that have no this compounds.
Keywords: acute Allergic contact dermatitis, shoe leather, necrosis and infection

322. The Prevalence ofRespiratory AirborneAntigensin Patients withChronicUrticariaReferred toAllergy and Asthma ClinicofKerman
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Background: Chronicurticaria, is an annoying case ofallergic diseases and almostwith the unknown causes. However,environmentalantigenscan be a cause of hives reactionsinpatients withurticaria. The purposeofthis study was to investigate the Prickskin testreactiontoairborneantigensin

patients withchronicurticaria, referred to asthma andallergyclinicat theKerman. Materials and Methods: This is a descriptive–cross sectional study. It was done by study of 19 patientswithchronicurticaria, asthmaandallergyclinicreferred toKerman. Aftergetting confirmed the chronicurticaria, skin prick testwas done by 20airborne antigens. For positive control, the histamineskin test was done for all patients. Finally the reslts were analyzed using the SPSS17. Results: Intotal of19subjects, 5 malesand14 femaleswithaverage age of 24± 12.2 were studied. Of the studied airborne antigens,the highest prevalence of were belong to Spinachantigens (33%),andantigenssunflower, alfalfa, cuminandmites (22%),amongpatients withchronicurticaria in Kerman. Conclusion: Theresultsof thisstudy indicated that, encounterwith the plantsrespiratoryantigensand mites, can causeallergicsymptoms of chronicurticariainthepopulation ofKerman.
Keywords: airborneantigens, chronicurticaria, Kerman

323. The Effects of Immune-Stimulation of Macrophage-Derived-NO on Dermal Healing of Diabetic Wounded Rats Using Low Level Laser
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Background: Diabetic wounds have been the area of challenge since many years. Nitric Oxide (NO) has been shown to play a crucial role in wound healing. In addition, application of laser on wound healing have already been examined. Thus, this study was designed to investigate the efficacy of a combination of 670nm and 810 nm lasers irradiation for possible immune-stimulation of macrophage-derived NO for healing of diabetic rats. Materials and Methods: 36 Sprague-Dawley male rats were used in this study. Diabetes was induced by IP injection of 55mg/kg of STZ. A full-thickness circular wound was made on the back of all rats. Rats were irradiated directly upon their wound with a combination of 670 nm and 810 nm every other day and upon wounding day. Wound imaging were performed on days 0, 7, 12, 16, 20 and 22. Some rats were sacrificed after taking a pathology sample on day 12 of the study. The wounds margin and context were scored pathologically. NO was measured by NO analyzer. Results: Percent open wound area (POWA) was significantly lower in the Diabetic Laser group in comparison to the Diabetic Non-Laser group. Also the POWA decrease in DML group was quicker than DMNL group (P=0.021; mean difference=19.7%). Total pathologic scores of wound margin were higher in DML group compared to DMNL group both on days 12 and 22 (P=0.049 and P=0.013, respectively); while these scores were not different in CL and CNL groups (P=0.882 and P=0.065, respectively). NO production was increased in DML group as compared to DMNL group. In addition, NO production increased dramatically in CL group as compared to CNL (P<0.005). Conclusion: Our study showed that the irradiation of diabetic wounds with a combination of low dose 670 nm and 810 nm lasers accelerates wound healing possibly by stimulation of macrophage-derived NO.
Keywords: low level laser, diabetic wound, (NO), Streptozotocin (STZ)

324. The Unusual Presentation of Food Allergy in a Child with Atopic Dermatitis
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Atopic dermatitis (AD) is a chronic eczematous skin disease with a prevalence of about 10% among children. Food allergies have a significant impact on the course of AD. The prevalence of food allergy in children with moderate to severe AD is about 35%, mostly to eggs, milk and peanuts. In this study, we describe a case of AD with unusual exacerbation. A 3 year-old girl admitted because of an exacerbation of AD with poor response to medication. She was the 4th child of consanguineous parents and had healthy siblings. Her condition begun at early infancy but became more severe after supplementation at 6 months. At 2.5 years old, it was found that she had allergies to some foods and laboratory data were remarkable for blood eosinophilia of 17850 cells/µl, serum IgE of 2250 kU/L and elevated specific IgE to egg white, milk, and wheat. Skin biopsy revealed pathologic changes consistent with AD. After dietary restriction, the disease became under good control, however she experienced an AD exacerbation with unknown cause 6 months later. The detailed examination revealed the presence of a wheat grain in her left external ear canal as the allergic stimulus. Her symptoms relieved after removal of the wheat grain. This signifies that food allergy could elicit AD through parenteral root as well.
Keywords: Food Allergy, Atopic Dermatitis

325. Identification of Autoimmune Urticaria in Patients with Urticaria by skin autoreactivity to serum and plasma Tests Versus Basophile Activation Tests
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Background: In about 45-65% of patients with chronic idiopathic urticaria (CIU), the presence of circulating histamine-releasing factors in serum and plasma, is detected by performance of autologous serum and plasma skin test, and production of wheal and flare. This will led to identification of anti-FceRI and/or anti IgE autoantibodies which can activate basophiles and mast cell degranulation. The aim of current study is comparing the diagnostic value of autologous skin tests with expression of CD 63 surface marker, to identify an agreement between the two methods in order to introduce a more reliable method for the diagnosis of patients with CIU. Materials and Methods: serum and plasma from patients with CIU and sera from healthy control subjects were incubated with donor basophiles. Activation of basophiles was determined on the basis of CD 63 surface expression, as analyzed on a FACScan flow cytometer. Results: Positive basophiles activation test result was found in 22(47%) of patients with CIU. The autologous serum skin test was positive in 29(63%) and autologous plasma skin tests was positive in 33(71.7%) of the patients. Conclusion: The data provide a reliable test for detection of these autoantibodies and association of them with the results of autologous skin serum and plasma tests.
Keywords: Chronic Idiopathic Urticaria(CIU), autoantibodies;autologous serum skin test(ASST), autologous plasma skin test(APST), basophil activation tets, CD63

IMMUNOENDOCRINOLOGY

Oral Presentation

326. Effect of Estrogen on Pro- and Anti-Inflammatory Cytokines Secretion by Proteolipid Protein and PHA Activated Peripheral Blood Mononuclear Cells Isolated from Multiple Sclerosis Patients in Comparison to Healthy Control Group
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Background: A large body of studies have been shown that E2 (17-β estradiol) has a protective effect on susceptibility to experimental autoimmune encephalomyelitis (EAE). Clinical improvement in multiple sclerosis patients and, it's animal model, EAE, during Pregnancy, when strogen levels are high suggests an immunomodulatory role for strogens. The immune basis for this protection is poorly understood. Materials and Methods: In this study we evaluated the effect of E2 on the synthesis of IL-4, IL-10, IL-17, TNFα and IFNγ cytokines produced by proteolipid protein (PLP) or mitogen phytohemaglutinin (PHA) activated peripheral blood mononuclear cells isolated from 20 Multiple Sclerosis patients (14/6 F/M with mean age 33.9±6.5) in Comparison to 19 healthy individuals (15/4F/M with mean age35.2±6.8).We used Real Time-PCR and ELISA to detect the level of cytokines. Results: In this study, we found that E2 significantly increased IL-10 secretion in peripheral blood mononuclear cells (PBMC) isolated from relapsing remitting Multiple Sclerosis (RRMS) patients in active phase and healthy individuals

stimulated with PLP or PHA ($p < 0.05$). E2 also significantly decreased expression of TNFα and IL-4 in these cells ($P<0.05$). Conclusion: These data indicate that E2 can affect on expression and secretion of inflammatory and anti-inflammatory Cytokines and can regulate Immune responses especially in the differentiation towards Th2 responses, and it can be useful at least in Treatment of relapsing remitting Multiple Sclerosis.

Keywords: Multiple Sclerosis, Estrogen, Cytokines

327. Evaluation of Humoral and Cellular Immunity in Diabetic Patients, Are Diabetic Patients Susceptible to Infections?

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Background: It has been widely thought that diabetic patients are prone to infections due to hyperglycemia induced immunodeficiency. Materials and Methods: For all the diabetic and normal subjects enrolled in this study, by single radial immunodiffiusion (SRID), total serum immunoglobulins (IgG, IgA, IgM), C3, C4 and CH50 were determined to evaluate humoral immunity and by lymphocyte proliferation test (PLT), T-Cell reactivity to hsp-60 molecule, tetanus toxoid recall antigen (TT), as well as phytohemagglutinin-A (PHA) were used for evaluating of cell mediated immunity. Results: The mean serum concentration of IgA in diabetic patients was 245.86±115.05 mg/dl versus192.96 ±105.33 mg/dl in healthy control group ($p<0.018$). The mean +/-SD serum concentrations of IgG(2540.82+/-1528.62)and IgM(305.54+/-121.66) in diabetic patients were lower than the control subjects(IgG;3035.76+/-1588.01,IgM;323.58+/-110.78). These differences were not statistically significant. Diabetic patients had higher levels of serum C3 and C4 when compared with healthy control subjects; in diabetic group, the mean+/-SD concentration of C3 and C4 were 101.68 +/- 4.31 mg/dl, 43.12 +/-3.51 mg/dl and in normal controls group, the values were 93.12+/-3.67,38.46+/-2.51 respectively. However, these differences were not statistically significant. Also, between the two groups there was no significant difference in CH50 activity. Most of both diabetic and normal subjects showed a circulating natural T-cell reactivity to human recombinant hsp60 (stimulation index; SI>2). Meanwhile, diabetic patients demonstrated a significant higher response than the normal control subjects ($p<.001$). In response to TT, the diabetic patients showed a trend of having a higher reactivity in comparison to normal subjects. However, this difference was not statistically significant. There was no significant difference between two groups in response to PHA. Conclusion: The data generated by the present study demonstrates that in diabetic patients there is no substantial mitigation in both humoral and cellular arms of immune system, thus, the common idea of believing that patients with diabetes are prone to infections should be revised.

Keywords: Diabetes, humoral, cellular, immunity, complement, infection

328. The Effect of Vitamin D on sCD26 Level in Mothers with Gestational Diabetes Mellitus

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Background: The role of vitamin D as an immune modulator has been emphasized in recent years. Vitamin D receptor with hight concentration has been shown in T cells and Macrophges. CD26 is a transmembrane glycoprotein which is expressed in higher amounts by Th1 cells and used as a Th1 cell marker. The purpose of this study was to determine the effect of a single mega dose of 300,000 IU of vitamin D after delivery in women with gestational diabetes after 3 months follow-up on the status of sCD26, Materials and Methods: This randomized clinical trial study with the follow-up period of 3 months included 45 women who had been diagnosed for the first time in their recent pregnancy as diabetics and were randomly divided into 24 women as intervention group (IG) and 21 women as control group (CG). The IG group received an intra muscular (IM) dose of 300,000 units of vitamin D. Fasting and two-hour glucose, Glycosylated hemoglobin A1C was assayed. The serum concentrations of adiponectin, 25-OH vitamin D and sCD26 were measured by ELISA method. Results: The mean age in case group was 30.7±6.2 years and for the ones in control group, it was 29.5±4 years. There was no significant difference in the age of women in two groups. Median concentration of 25-OH vitamin D in the study group before and after intervention was 24.25 and 62.1 nmol/L, respectively ($P <0.001$), while the figures in the CG were 25.3 and 24.1 nmol/L, respectively. Mean adiponectin levels before and after intervention showed no significant difference. The level of sCD26 was 153.2±65.5 ng/ml in case group and it was 164.9±62.7 ng/ml in control group before intervention ($P=0.5$).sCD26 concentration was 234.5±70.2 ng/ml in case group and it was 237.3±66.9 ng/ml in control group after 3 months follow up ($P=0.8$). There was significant difference in the sCD26 concentrations before and after intervention in two groups ($P=0.001$). Conclusion: Our findings show that IM administration of vitamin D to women with gestational diabetes after delivery improves vitamin D status. sCD26 concentrations were increased in two groups after 3 month follow up. It means that vitamin D had no effect on sCD26 production. Increase in sCD26 productionmay bedue tothe removalof immunosuppressiveeffects on Th1 cellsduringpregnancy.

Keywords: GDM, sCD26, Vitamin D

329. Effects of L-Glutamine as a Heat Shock Proteins Inducer in Autoimmune Diabetes on Male C57BL/6 Mice

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Background: Using of heat shock proteins (HSPs) and their inducers are an effective approach in treatment of autoimmune diaease such as diabetes type 1.L-glutamine, a hsp inducer drug such as hsp70,showed that could protects tissues,cells and all organs from damaged and stressful condition. Materials and Methods: Diabetes was induced in male C57BL/6 mice by streptozotocin (STZ), mice randomly allocated in 4 groups (N=5): control positive (A), pre induction treatment (B), post induction treatment (C), normal group (D). (groups B and C received L-glutamine every 12 hours,pretoneally). 14 days after final stz induced dosage,mice were euthanized and blood samples collected for evaluating of fasting blood suger (F.B.S.) by automatic glucometer and serum hsp70 levels by standard ELISA kit. in addition, the spleens of mice were sterilitely collected and were used to evaluating of frequency of T regulatory cells (CD4+CD25+FOXP3+) by flowcytometric standard kit and flowcytometer device, of course,after separating of spleen cells and 72 hours cell culture, their supernatant were picked up for measuring of interleukin 10,17(IL-10,IL-17) and interferon gamma(IFN-γ) by used of standard ELISA kits. Moreover, mice pancreases were used to evaluating of Caspase-3 enzyme activity by used of standard immunohistochemisrty kit. Results: For first time our data showed ,the mice that received L-glutamine their levels of splenic IL-10 and serum hsp70 were significantly higher than other mice ($p<0.05$) and their splenic IL-17 and F.B.S levels were markedly lower than others ($p<0.05$).in addition, their IFN-gamma levels were lower than mice in groups A but this was not significant. Of course, the activity of Caspase-3 enzyme in groups B and C was lower than group A. Conclusion: As a hypothesis we suggest for first time L-glutamine be able of inducing serum hsp70 and due to its can induce T regulatory cells and splenic IL-10 that can suppress IL-17 and it follows suppress of T helper 17 cells (TH 17) and relieving of Caspase-3 enzyme activity and maybe used in new future for treatment of human diabetes type1.

Keywords: L-Glutamine, Heat Shock Proteins, Autoimmune Diabetes, C57BL/6 Mice

Poster Discussion Presentation

330. Do Resistin and Tumor Necrosis Factor-α Relate to Changes in Insulin Resistance in Normal Pregnancy?

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Background: The purpose of this study was to evaluate the role of resistin and Tumor Necrosis Factor-α (TNF-α) in insulin resistance during pregnancy. Materials and Methods: Serum resistin and TNF-α concentrations were measured by ELISA in 86 healthy pregnant women (26, 23 and 37 of them in the 1st, 2nd and 3rd trimesters, respectively) and in 21 healthy non pregnant women in a cross sectional study. Results: Resistin concentration was significantly higher in the third trimester (9.5±3.3 ng mL^{-1}) as compared with non pregnant women (7±3.3 ng mL^{-1}). Serum TNF-α level were also significantly increase in pregnant women (2.6±1.9 pg mL^{-1}) as compared with maternal healthy controls (0.8 ±0.7 pg mL^{-1}). There were significant correlation between gestational age and BMI (r = 0.28, p = 0.01), resistin (r = 0.36, p = 0.002) and TNF-α (r = -0.44, p<0.0001). There was not significant correlation between gestational age and insulin resistance (IR). We also did not found correlation between IR and resistin as well as between IR and TNF-α in pregnant women. Conclusion: TNF-α and resistin do not appear to contribute greatly to pregnancy induced insulin resistance in healthy pregnancy.
Keywords: Resistin, TNF-α, Insulin Resistance, Pregnancy

331. Immune-Endocrine Interactions in Hashimotos thyroiditis

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Background: Autoimmune thyroid diseases (AITD) are the most common organ-specific autoimmune disorders affecting approximately 5% of the overall population. Aims of this study were determine of thyroid antibody levels in correlation to ultrasonography feature and laboratory parameters. Materials and Methods: Forty seven newly diagnosed women with Hashimoto thyroiditis (age 12−45 old years) were recruited. As a control group, 57 women with no history of thyroid malfunction were recruited. TSH, fT4 and Anti-TPO were assayed by ELISA. Thyroid volume (TV) was determined by ultrasonography.Results: TSH, fT4 and Anti-TPO were significantly different Hashomoto individuals than in controls (19.53±13.04 mIU/L, 0.83±0.38 ng/dL and 362.87±350.56 IU/mL vs. 1.42±1.15 mIU/L, 1.15±0.31 ng/dL and 37.07±113.72 IU/mL). TV was higher in hypothyroid individuals than in controls (14.7±9.2 ml vs. 10.3±4.1 ml). In Hashimoto subjects 70% were recognized reduced echogenicity. Positive correlation were seen between TSH with TV and Anti-TPO in hypothyroid subjects (r=0.620; P<0.05 and r=0.267; P<0.05). Hypoechogenicity was associated with large goiters, increased TSH levels (subclinical hypothyroidism) and extremely elevated TPO-antibodies. Conclusion: This study suggested that hypoechogenicity in Hashimoto's thyroiditis signifies high autoimmune activity with goiter, subclinical hypothyroidism and preferential elevation of TPO antibodies. Hypoechogenicity may reflect the grade of lymphocytic tissue infiltration, which is known to change the morphological appearance of the thyroid gland in autoimmune processes.
Keywords: Immune-Endocrine Interactions, AITD, Hashimoto thyroiditis

332. Relations between Thyroid Antibody and Thyroid Volume in Puberty Girl School Children in Urmia–West Azerbaijan

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Backgrounds: It has been suggested that in areas of iodine deficiency the transient to sufficient or excess iodine in take may precipitate the emergence of thyroid autoantibody. Despite sufficient iodine intake, the prevalence of goiter is still high in some regions of Iran, so causes other than iodine deficiency, such as autoimmune thyroid disease (AIT) have to be considered in children with goiter. Materials and Methods: According to previous estimation of TGP, 500 schoolchildren (Gender: female; 11-15 years) were recruited from the two education districts in Urmia. Of these, hundred forty children returned signed consent form for thyroid Ultrasound examination. Casual urine Samples were collected for UIE estimation. Thyrotropin (TSH), thyroperoxidase (TPO-ab) and thyroglubolin (Tg-ab) antibody levels were determined by ELISA. Results: School children were subdivided according to ultrasound; nonegoiteric (n=117) and gioterogenic (n=23) that exhibited a thyroid volume (TV) of 5.42±1.66mL vs 9.75±3.18mL respectively. The corresponding values for TPO-ab and Tg-ab were 14.2±35.43, 8.1±7.51 and 135.82±150.41 vs. 21.21±116.79, 65.35±114.15 and 282.17±455.07. Median urinary iodine concentration (UIC) in the children was 144µg/l, indicating sufficient iodine intake. 10.7% and 6.5% of subjects were thyroid antibody positive. Significant correlation were seen between TV with TPO-ab and Tg-ab (P<0.001) and TSH levels with TPO-ab and Tg-ab (P<0.001). We were not seen significant correlation between UIC with TPO-ab and Tg-ab. Conclusion: These findings reveals iodine supplementation has results in the elimination of iodine deficiency, and this has been accompanied by an increase in the prevalence of autoimmune thyroiditis and thyroid dysfunction.
Keywords: Thyroid Antibody, Thyroid Volume, AIT

333. Increased Circulating Levels of CXCL12 (SDF-1) Is Associated with Its SDF-1 3′A Genetic Variant but Elevated CXCL10 (IP-10) Is Unrelated to -1443 Promotor Polymorphism of IP-10 Gene in Type-1 Diabetes: A Study in Iranian Type-1 Diabetic Patients

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Background: Type 1 diabetes (T1D) is determined as an autoimmune disease. Chemokines are involved in the pathogenesis of several autoimmune diseases, including T1D. Therefore, the purpose of this study was examining the association between the circulating CXCL10 and its promoter polymorphism at position -1443 alongwith CXCL12 level and the known SDF-1 3′A genetic variant with T1D the objectives of this project were also to explore weather if elevated levels of these chemokines is accompanied with T1D complications and duration of diabetes. Materials and Methods: Blood samples were collected from 209 T1D patients and 189 healthy controls on either EDTA pre-coated or ordinary tubes for serum collection. Serum samples were isolated. DNA was extracted from EDTA containing samples. The extracted DNA samples were analyzed for CXCL10 and CXCL12 polymorphisms using PCR-RLFP. The serum levels of CXCL10 and CXCL12 were also measured by ELISA. Results*:* A significant difference was found between the *A/A, A/G* and *G/G* genotype and *A* and *G* alleles of CXCL12 at position +801 in T1D patients and control. Both CXCL10 and CXCL12 were markedly elevated in T1D patients with or without complications compared to controls. Conclusion: According to these results circulating CXCL10 and CXCL12 may play important roles in T1D pathogenesis and these factors can be considered as useful therapeutic, prognostic and/or diagnostic biological markers in T1D.
Keywords: Type 1 diabetes, CXCL10 (IP-10), CXCL12 (SDF-1), Polymorphism.

334. Evaluation of Angio-Geneic/ Anti-Angiogeneic CXC Chemokines CXCL10(IP-10), CXCL12 (SDF-1) and CXCL9 (Mig) in Gestational Diabetes Mellitus (GDM) Patients

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Background: Gestational diabetes mellitus (GDM) is the most frequent metabolic disorder in pregnancy, affecting 1–10% of all pregnancies. GDM is considered a prediabetic state, therefore it may display many abnormalities that possibly appear in the very early stages of type 2 diabetes mellitus (T2DM). Inflammation is an important component of the metabolic syndrome. Gestational diabetes mellitus (GDM) has been recognized as a significant risk factor for MetS and an inflammation component has been described in this disease. 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 whether changes in pro-inflammatory chemokines CXC (interferon-gamma-inducible protein (IP-10) and CXCL9 (MIG) are involved in pathogenesis of GDM. Material and Methods: This cross-sectional study was conducted at the Rafsanjan UniversityofMedical Sciences , Molecular Medicine Research Center during 2010 to 2012. The study group consisted of 54 diabetics in the third trimester of pregnancy.54 healthy woman matched for gestational age served as a control group. The serum,milk and blood-cord levels ofCXCL10(IP-10), CXCL12 (SDF-1) and CXCL9(Mig) were measured by ELISA (R&D systems, UK) in patients and healthy controls. The demographic information was also collected in parallel with experimental part of the study by a researcher designed questionnaire. Results: Our results showed decreased level of pro-angiogenic chemokine (CXCL12) in serum,milk, blood-cord and elevated levels of anti-angiogeneic cxc chemokines(CXCL10 and CXCL9) in serum,milk and blood-cord of GDM patiants. Conclusion: According to the results of this work it could probably be concluding that GDM followses a pattern of inflammatory in pregnant woman.
Keywords: CXCL10(IP-10), CXCL9(Mig), Gestational diabetes mellitus (GDM)

335. Counterpoising Leptin Hormone as a New Therapeutic Procedure in Multiple Sclerosis

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Regulatory T lymphocytes play the most important role in controlling the autoimmune processes and disease progression in neuroimmune disorders such as multiple sclerosis (MS). Many recent invitro studies have shown that leptin hormone interferes with the inhibitory function of these cells. Furthermore, strong relations between the elevated levels of this hormone and more susceptibility to MS disease and also more activity and progression rate of disease have been shown. In this regard, we hypothesize that there can be a new possibility for more successful treatment of the disease through invivo counterbalance of leptin hormone utilizing particular neutralizing antibodies or antagonist reagents, or specific sieving of patients serum for reducing the leptin concentrations in the patients body. For evaluating this hypothesis, we wish to suggest some in-vitro and in-vivo studies to analyse the effects of varied methods of leptin neutralization on the induction of regulatory T cells or on the function of isolated and purified regulatory T cells. Clarifying the effects of leptin compensation on regulatory T cells as the major controllers of autoimmune responses may provide us with strong evidence to use this procedure as a trustworthy treatment in severe relapses of MS which don't response to current therapies. In addition, this may be useful as a disease-modifying treatment in remission phase for reducing the rate and severity of future attacks.
Keyword: Leptin Hormone,Therapeutic, Multiple Sclerosis

336. Relation of High Sensitive CRP and Insulin Resistance to Retinopathy in Type 2 Diabetes

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Background: Retinopathy is the commonest long-term complication of diabetes mellitus. Diabetic retinopathy is a complex disease. The aim of this study was to evaluate the association between insulin resistance, high sensitive CRP level as inflammation markers and diabetic retinopathy. Methods and materials: patients with type 2 diabetes were enrolled.The following data were recorded: age, sex, duration of diabetes, HbA1c, FBS, HSCRP, lipid profiles and insulin level, ophthalmologic examination and systemic treatment.Insulin resistance was calculated by HOMA-IR formula. Relation between HSCRP levels, HOMA-IR was evaluated with diabetic retinopathy. Results: A total of 342 patients (108 male , 234 female) were enrolled. The mean age of patients was 55.05± 9.8 years. 35% of all patients had diabetic retinopathy that non-proliferative diabetic retinopathy was the commonest of them. There was a differences between the serum hsCRP levels of those with and without retinopathy however this difference was not significant (3.4±3.8 mg/dl in patients with retinopathy Vs 2.7 ± 3.7 mg/dl in patients without retinopathy , p=0.06) . Homa –IR was significantly higher in patients with diabetic retinopathy (1.8±1.39 Vs 1.6±0.76, p=0.004). A significant association was found between diabetic proliferative retinopathy and insulin resistance. Conclusion: This data suggests that the inflammatory process may play a role in diabetic retinopathy in type 2 diabetes.
Keywords: CRP, Insulin, Retinopathy, Type 2 Diabetes

337. Expression of the Growth Hormone (GH)/Insulin-Like Growth Factor (IGF) Axis during Balb/C Thymus Ontogeny and Effects of GH Upon *Ex-Vivo* T-Cell Differentiation

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Using quantitative RT-PCR, the expression profile of the somatotrope GH/IGF axis components was measured in different thymic cell types during thymus embryogenesis in Balb/c mice. Transcription of *Gh*, *Igf1*, *Igf2* and their related receptors predominantly occurred in thymic epithelial cells (TEC), but a low level of *Gh* transcription was also evidenced in thymic T cells (thymocytes). *Gh*, *Ghr*, *Ins2*, *Igf1*, *Igf2*, and *Igfr1*, displayed distinct expression profiles depending on the developmental stage. The protein concentration of IGF-1 and IGF-2 were in accordance with the profile of gene expression. In fetal thymus organ cultures (FTOC) derived from Balb/c mice, treatment with exogenous GH resulted in a significant increase of double negative $CD4^-CD8^-$ T cells and $CD4^+$ T cells, with a concomitant decrease in double positive $CD4^+CD8^+$ T cells. These changes were inhibited by concomitant treatment with GH and GHR antagonist pegvisomant. However, T-cell differentiation was not significantly affected in FTOC treated with GHR antagonist alone, suggesting a different role for endogenous thymic GH.
Keyword: Growth Hormone, Insulin-Like Growth Factor (IGF), Thymus Ontogeny, T-Cell Differentiation

338. Impact of Growth Hormone (GH) Deficiency and GH Replacement upon Thymus Function in Adult Patients

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Background: Despite age-related adipose involution, T cell generation in the thymus (thymopoiesis) is maintained beyond puberty in adults. In rodents, growth hormone (GH), insulin-like growth factor-1 (IGF-1), and GH secretagogues reverse age-related changes in thymus cytoarchitecture and increase thymopoiesis. GH administration also enhances thymic mass and function in HIV-infected patients. Until now, thymic function has not been investigated in adult GH deficiency (AGHD). The objective of this clinical study was to evaluate thymic function in AGHD, as well as the repercussion upon thymopoiesis of GH treatment for restoration of GH/IGF-1 physiological levels. Materials and Methods:

Twenty-two patients with documented AGHD were enrolled in this study. The following parameters were measured: plasma IGF-1 concentrations, signal-joint T-cell receptor excision circle (sjTREC) frequency, and sj/□ TREC ratio. Analyses were performed at three time points: firstly on GH treatment at maintenance dose, secondly one month after GH withdrawal, and thirdly one month after GH resumption. Results: After 1-month interruption of GH treatment, both plasma IGF-1 concentrations and sjTREC frequency were decreased ($p<0.001$). Decreases in IGF-1 and sjTREC levels were correlated ($r=0.61$, $p<0.01$). There was also a decrease in intrathymic T cell proliferation as indicated by the reduced sj/□ TREC ratio ($p<0.01$). One month after reintroduction of GH treatment, IGF-1 concentration and sjTREC frequency regained a level equivalent to the one before GH withdrawal. The sj/□□TREC ratio also increased with GH resumption, but did not return to the level measured before GH withdrawal.Conclusions*:* In patients with AGHD under GH treatment, GH withdrawal decreases thymic T cell output, as well as intrathymic T cell proliferation. These parameters of thymus function are completely or partially restored one month after GH resumption. These data indicate that the functional integrity of the somatotrope GH/IGF-1 axis is important for the maintenance of a normal thymus function in human adults.

Keywords: Thymus, T cells, GH, IGF-1, Thymopoiesis, TREC

IMMUNOGENETICS

Oral Presentation

339. Increased NF-κB Activity in Hct116 Colorectal Cancer Cell Line Harboring *TLR4* Asp299Gly Polymorphism

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Background: Toll-like receptors (*TLRs*) are the most important receptors in innate immunity. TLR4, considered one of the most important TLR, recognizes lipopolysaccharide of Gram-negative bacteria. Recognition of ligands by TLRs induces signaling pathways lead to activate transcriptional factors such as NF-κB which involve in the expression of inflammatory cytokines and chemokines. To prevent an inappropriate or an overactive immune response, a complex network of molecules negatively regulates TLRs and their associated signaling pathways. Two cosegregating single nucleotide polymorphisms of the human TLR4 gene, namely Asp299Gly and Thr399Ile, have been associated with hyporesponsiveness to inhaled LPS. The purpose of this study was to determine the impact of variations of TLR4 on NFkB activity in colorectal cancer cell line. Materials and Methods; HCT116 cells were Transfeced with wild-type and mutants Flag-CMV1-TLR4 expression vectors. Western blot analysis was performed to evaluate some molecules involved in TLR4 signaling. NFkB activity was assessed by Dual-luciferase reporter assay and Cytokine profiles were evaluvated by ELISA kit and Cytometric bead array. Results; Higher level of pNF-kB activity was observed in cells with TLR4 D299G polymorphism compared with other cells. However, higher level of pAKT, pERK1 and pIRAK activity was observed in wild-type cells. The results of cytokine measurements showed about four fold higher level of IL-8 in cells with wild-type *TLR4*. Conclusion; This study suggesting that Asp299Gly polymorphism impact on TLR4 signaling and intestinal homeostasis due to impaired control signals at the epithelial cell level. This may lead to chronic intestinal inflammation and interrupted intestinal homeostasis which may increase susceptibility to colorectal cancer disease.

Keywords; Colorectal cancer, Toll like receptors, polymorphism

340. Activating *KIR* Genes *2DS1*and *3DS1,* and their Combination with Specific *HLA class Iligands* Have Protective Effect on HBV Infection Outcome

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Background: Natural killer (NK) cells are one of the key players in the eradication and control of viral infections. NK effector functions are dependent on activation of NK cells which is determined by surface receptors interaction with ligands on target cells. Of these receptors, the killer immunoglobulin-like receptors (KIR), which interact with HLA class I molecules, have both inhibitory and activating roles. Owing to high polymorphism and independent segregation of *KIR* and *HLA* genes, individuals can present various KIR-HLA combination profiles. As each KIR-HLA interaction may have differential effects on NK cell activation and inhibition, this diversity has important potential influences on the host response to infections. The aim of this study was to explore the possibility of the inheritance of compound *KIR-HLA* genotypes as a candidate for HBV infection outcome. Materials and Methods: The sequence specific primers- polymerase chain reaction (SSP-PCR) was employed to identify 17 *KIR* genes and their three major *HLA class I ligand* groups (*-C1*, *-C2* and *-Bw4*: *-B Bw4^{Ile80}*, *-B Bw4^{Thr80}* and *-A Bw4*) in 151 infected subjects (chronic carrier *n=50*, chronic hepatitis *n=50*, cirrhosis *n=25*, and spontaneous recovery *n=26*) and 200 healthy controls. Results: The frequencies of telomeric cluster genes *KIR2DL5A*, *KIR2DS1* and *KIR3DS1*, and Bx genotypes were significantly increased in recovered individuals compared to controls and other infected subjects (*Pc*<0.05). Moreover, *KIR2DS1+HLA-C2* and *KIR3DS1+HLA- B Bw4^{Ile80}* combinations were significantly more frequent in recovered persons than in controls (*Pc*<0.05). Other comparisons between each subgroups of infected subjects or sum of persons with persistent infection (n=125) and control individuals, have showed no major dissimilarity. Conclusion: We conclude that possession of activating *KIR* genes *2DS1*and *3DS1*, beside of their specific *HLA ligands* may facilitate virus clearance after HBV infection.

Keywords: KIR, *HLA class Iligands*, HBV Infection

341. Investigation of CCR4 Genetic Variation at Position 1014 in Patients with Breast Carcinoma

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Background: CCR4 (CD194) is a chemokine-receptor selectively expressed by Th2 and Regulatory T (Treg) cells; both with inhibitory function in tumor immunity. Furthermore, expression of CCR4 by tumor cells has been indicated to potentiate their metastatic activity. Genetic study of CCR gene on chromosome three revealed a genetic variation in CCR4 gene (CCR4 1014) with potential effects on CCR4 function/expression. Materials and Methods: In the present study the association of the CCR4 polymorphism at position 1014 C/T has been investigated in 154 patients with breast carcinoma (mean age 49.3±11.6) and 160 age-sex matched healthy individuals as control group 49.8±12.9). Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method was used for genotyping; and the accuracy of genotyping were

verified by direct automated sequencing of PCR products using Big-dye sequencing technology. Results: Arlequin analysis showed no deviation from Hardy-Weinberg equilibrium neither in the patients nor in control group. The frequencies of CC, CT and TT genotype in patients and control group were respectively 89 (57.8%) versus 87 (54.4%), 54 (35.1%) versus 63 (39.4%) and 11 (7.1%) versus 10 (6.2%). The frequencies of C and T alleles at this position in patient and control groups were respectively 232 (75.3%) versus 237 (74.1%) and 76 (24.7 %) versus 83 (25.9%) respectively. Statistical analysis revealed no significant differences in the frequencies of genotypes and alleles between patients than controls. Statistical analysis also indicated no correlation between genotypes at position 1014 C/T with the clinicpathological characteristics of the patients including tumor type, tumor size, clinical stage, histological grade, LN involvement, lymphovascular invasion (LVI), distant metastasis and Nottingham prognostic index (NPI) (P>0.05). Conclusion: These data collectively indicate that the polymorphism at position 1014 in CCR4 gene does not affect the susceptibility and the progression of breast cancer in Iranian population.

Keywords: CCR4, polymorphism, Breast Carcinoma

342. PDCD1 (PD1) Gene Polymorphism in Iranian Patients with Thyroid Carcinoma

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Background: PD1 receptor is a co-signaling molecule with an important role in regulation of T-Lymphocyte activity. Correlation between PD-1 gene (PDCD1) polymorphisms and autoimmune, as well as, malignant diseases, has been found before. In current study, we aimed to investigate the association of PD-1 polymorphisms and haplotypes with susceptibility to thyroid carcinoma. Materials and Methods: 105 patients with confirmed thyroid cancer were investigated for the two important single nucleotide polymorphisms at positions +7146 G/A (PD-1.3) and +7785 C/T (PD-1.5) and the results were compared to 160 healthy people as control group. Genotypes were identified using Nested PCR-RFLP and PCR-RFLP methods. Results were analyzed by Arlequin and SPSS software packages. Results: There were no significant differences in the frequencies of genotypes and alleles at locus PD-1.3 between patients and control group. Results, however, revealed significant differences in the frequencies of T mutant allele and also in frequencies of CT and TT genotypes with odds ratio of 2 to 3 for thyroid carcinoma. GT haplotype (PD-1.3 G and PD-1.5 T) has also been observed with significant different frequency between patients and controls. Conclusion: As the first study to investigate two mentioned polymorphisms in thyroid cancer, current study confirmed the association of PD-1.5 C/T polymorphism and a haplotype resulted from both loci, PD-1.3 and PD-1.5 with susceptibility of Iranians to thyroid cancer.

Keywords: Thyroid cancer, PD-1, Polymorphism

343. Determination of ERAP1 Polymorphisms in Iranian Patients with Ankylosing Spondylitis

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Background: Ankylosing spondylitis (AS) is an inflammatory rheumatic disease that predominantly affects the spine and may be associated with peripheral arthritis. AS has the highest association with genetics (MHC system) among all autoimmune diseases. ERAP1 has the second highest association with AS, HLA-B27 is the most associated gene. The purpose of this study was to determine the association of 12 important SNPs of ERAP1 with the disease. Materials and Methods: 399 patients suffering from AS and 528 healthy controls were sampled. All patients fulfilled the modified New York Criteria and were clinically examined. All 12 SNPs of ERAP1 were genotyped using Real-Time PCR TaqMan Genotyping Allelic Discrimination and PCRSSP was utilized to genotype HLA-B27 SNPs. Results: 7 SNPs named: rs27434, rs27044, rs10050860, rs2287987, rs30187, rs28096 and rs13167972 were significantly associated with AS. rs10050860 and rs2287987 were highly linked together (r^2= 0.96, D'= 0.99, LOD=132). Conclusion: In contrast to other performed studies, the SNPs studied in ERAP1 in Iranian pathients were not associated with HLA-B27, which could be due to the lower frequency of HLA-B27 in Iran compared to European populations. ERAP1 might affect on the onset of AS in HLA-B27 negative patients.

Keywords: ERAP1 Polymorphisms, Iranian Patients, Ankylosing Spondylitis

Poster Discussion Presentation

344. Study of Programmed Cell Death 1 (PDCD1) Gene Polymorphims in Iranian Patients with Ankylosing Spondylitis

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Background: Ankylosing spondylitis (AS) is a chronic inflammatory disease, characterized by axial arthritis in which the genetic-environmental factors seem to be involved in the pathogenesis of the disease. This study was performed to investigate the role of polymorphisms of the programmed cell death 1 (PDCD1) gene on susceptibility to AS. Materials and Methods: In this study, 161 Iranian patients with AS and 208 normal controls were enrolled. two single-nucleotide polymorphisms (SNPs) of the PDCD1 gene; PD-1.3 (G, A) in nucleotide position +7146 of intron 4 and PD-1.9 (C, T) in nucleotide +7625 of exon 5 were studied in RFLP PCR method. Results: Analysis of PD-1.3 revealed that 82% of patients and 79% of controls had GG genotype, while GA and AA genotypes were detected in 17% and 0.6% of patients, respectively, and 20% and 1.4% of controls, respectively. Moreover, the genotype CC (PD-1.9) was present in 92% of patients and 97% of controls. Although these differences were not statistically significant between patients and controls, comparisons of genotypes frequencies in the AS patients and (HLA)-B27 revealed that all patients who had CT genotype (PD-1.9) were HLA-B27 positive, whereas 30% of patients with CC genotype were HLA-B27 negative. Conclusion:

There was no evidence of association for PDCD1 SNPs with AS in our study, but CT genotype (PD-1.9) seems to be associated with HLA-B27 positivity in the patients with AS.

Keywords: PDCD1, Polymorphims, Ankylosing Spondylitis

345. Phenotypic Frequencies of HLA-A, -B, -DRB1 Antigens in SLE Patients from Pakistan

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Background: The first genetic factors to be identified as important in the pathogenesis of Systemic lupus erythematosus (SLE) were those of the major histocompatibility complex (MHC) on chromosome 6. It is now widely accepted that MHC genes constitute a part of the genetic susceptibility to SLE. Materials and Methods: The study population comprised 61 SLE patients and control subjects were 61 healthy blood donors. The study was carried out after ethical approval was obtained and patients were included after informed consent. Ethylene Diamine Tetra Acetic acid (EDTA) blood samples were drawn by venepuncture from each patient and control for DNA-based HLA Typing. HLA-A, HLA-B, and HLA-DRB1 typing was carried out by Polymerase chain reaction with sequence specific primers (One Lambda Lot#006) using genomic

DNA from 61 SLE patients fulfilling at least four of the American college of Rheumatology criteria for SLE. Total 22 alleles have been studied at locus A, 37 alleles at locus B and 17 DRB1 alleles. Results: The allelic frequencies in patients and control subjects were compared using Odd-Ratio. The phenotypic frequencies of HLA-A, HLA-B, and HLA-DRB1 antigens in SLE patients from Pakistan were compared with the controls. A significant increase was observed in the frequency of HLA-A*01, A*03, A*11, A*23, A*26 A*69, HLA-B*27, B*40, B*49, B*51, B*52, B*53, B*54, B*95, HLA-DRBI*01, DRBI*03, DRBI*11, DRBI*14 among total SLE patients. HLA-A*24, A*29, A*31, A*34, A*68, A*92, HLA-B*18, HLA-DRB1*12, were found to be decreased in the patient group as compared to controls. Conclusion: This study showed that SLE is associated with certain MHC alleles in Pakistani population. As identification of genes involved in the development of SLE will provide important insight into the development of autoimmune disease, and opportunities to improve diagnosis and treatment.

Keywords: Systemic lupus erythematosus, Major histocompatibility complex, Human leukocyte antigen, autoimmunity, Polymerase chain reaction, primers

346. Strong Linkage Disequilibrium between Asp299Gly A>G and Thr399Ile C>T Polymorphisms in Toll Like Receptor-4 Gene

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Background: TLR-4 plays a major role in the innate immunity against Gram-negative bacteria. Asp299Gly A>G and Thr399Ile C>T are two well known polymorphisms in TLR-4 and their association as the genetic predisposition factors with autoimmune diseases have been determined in several studies. The aim of this study was investigation of the linkage disequilibrium of the above mentioned TLR-4 polymorphisms in the Iranian population. Materials and Methods: 341 subjects including 256 healthy controls and 85 patients with ulcerative colitis disease have been enrolled in our study. Molecular techniques based on PCR-RFLP were used to detect the above mentioned polymorphisms.Finally the frequency and genetic inheritance patterns of the polymorphisms were investigated. Results: Allelic frequencies for Thr399Ile C>T and Asp299Gly A>G in the 341 subjects were 14.7% and 16.1% respectively. The frequencies of the genotypes with LD inheritance were 74% (Thr399Ile C>T) and 82% (Asp299Gly A>G), and all followed the Hardy-Weinberg equilibrium. Therefore, the Toll-like receptor-4 Thr399Ile C>T and Asp299Gly A>G was in strong linkage disequilibrium (correlation coefficient: 0.742, *p value*< 0.001). Additionally, no significant differences between frequencies of these polymorphisms were seen in UC cases and healthy controls in our population (*p value*: 0.61). Conclusion: We found that Thr399Ile C>T and Asp299Gly A>G polymorphisms are in strong linkage disequilibrium in our study population, consistent with findings from an earlier studies in other populations such as Europeans and Caucasians. No significantly associations between polymorphisms and ulcerative colitis were observed. To study on the other polymorphisms in the Toll-like receptor gene is suggested for future investigation in our population.

Keywords: Linkage disequilibrium, toll like receptor-4, polymorphisms, ulcerative colitis

347. Effect of ACE Gene I/D Polymorphism on Lupus and Noise Associated Hypertension in a Pakistani Population

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Background: Angiotensin-converting enzyme (ACE) was first identified as a key component of the rennin-angiotensin system, as its main role is to process angiotensin I to angiotensin II and degrade bradykinin. Human ACE maps to chromosome 17q23 spans 21Kb, includes 26 exons and 25 introns. In humans, ID, DD, and II polymorphism is located in intron 16 of the angiotensin gene. The purpose of this study is to investigate the frequency of ACE gene insertion/deletion (I/D) polymorphism genotype in Systemic Lupus Erythematosus (SLE) patients and to study the association between ACE I/D polymorphism with the hypertensive patients exposed to different sound levels. Materials and Methods: Sixty one (61) SLE patients and 476 hypertensive patients were recruited from Punjab-Pakistan. These subjects were studied for ACE I/D polymorphism by using Triple primer method with nested polymerase chain reaction (PCR). Results: The frequency of DD, ID and II genotypes was 54.3 and 4% in SLE patients' and 23.32 and 6% in healthy controls, respectively. The frequency of DD allele in SLE patients with lupus nephritis is 100%, Sjogren's syndrome 100%, Raynaud's phenomenon 88.88%, and with rheumatoid arthritis it is 78.94%. The frequency of ID allele in SLE patients with Raynaud's phenomenon is 5.55%, and with rheumatoid arthritis it is 10.52%. The frequency of II allele in SLE patients with Raynaud's phenomenon 5.55%, rheumatoid arthritis is 10.52% but the important thing to note is that the frequency of II allele in SLE patients with vasculitis is 100%. Conclusion: It can be concluded that Lupus Nephritis, Sjogren's syndrome, Raynaud's phenomenon, Rheumatoid Arthritis and Vasculitis, which are common among Pakistani SLE patients are related diseases and ACE gene is involved in lupus susceptibility. Furthermore, allelic frequencies in hypertensive patients exposed to different sound levels were according to Hardy Weinberg equilibrium. Thus the frequency of DD allele was found to be higher in SLE patients as well as in hypertensive patients exposed to different sound levels as compared to ID and II alleles.

Keywords: Systemic Lupus Erythematosus, Angiotensin Converting Enzyme I/D Polymorphism, Sjogren's Syndrome, Raynaud's phenomenon, Rheumatoid Arthritis, Vasculitis.

348. Determination of HLA Class I and HLA ClassII Haplotypes in Systemic Lupus Erythematosus Patients

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Background: Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease with unknown etiology. Genetic susceptibility and some environmental triggers may play a role in its pathogenesis. HLA molecules are one of the key molecules in this disease and the expression of autoantibodies and immune responses to autoantigens is controlled by HLA haplotypes. Materials and Methods: We studied 93 patients with SLE, attending the rheumatology unit. Blood samples were collected in EDTA and DNA was extracted using salting out method. HLA class I and HLA classII were typed at genomic level by using a commercial SSP-PCR kit, then amplified DNA was run on 2% agaros gel and visualized by gel documentation. Abundance of HLA haplotypes between SLE patients and control group compared. Because of the variations in associations between SLE and HLA around the world, the analysis of allele or haplotypes as risk factors and its extent for each population is necessary. Results and Conclusion: In this study, significant different allele frequencies were observed for HLA-A*30, A*02, HLA-B*52, HLA-DRB1*03, DRB1*07, DRB1*15, that were increased in SLE patients. Results indicate a positive association between these alleles and SLE. HLA-A*31 was found to be decreased in the patients group as compared to controls.

Keywords: SLE, HLA class I and II, Haplotypes

349. Association of Three Polymorphisms of IL-18 Genes (137G/C, 607C/A, 133C/G) in Patients with Allergic Rhinitis in the Iranian Population

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Background: Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa induced by an IgE-mediated reaction, following exposure to an allergen. AR is a global health problem, with a prevalence of between 9-42% among the general population. Inflammatory reactions in allergic rhinitis are regulated by many cytokines.The pleiotropic role of IL-18 in Th1 and Th2 responses is known to be controlled by the cytokine

milieu. IL-18 is a member of the IL-1 family. It was originally described as IFN-γ –inducing factor (IGIF),and is known to influence the balance of Th1/Th2 immune response. The *IL-18* gene has two promoter regions, promoter 1 and promoter 2. Three SNPs are located in promoter1, two are in exon 1, and three are in the promoter 2. This study aimed to examine the association of three different (SNPs) located in IL-18 gene (–607 C/A, –137 G/C and–133 C/G) on chromosome 11q22 with allergic rhinitis.Materials andMethods: Genomic DNA was obtained from the blood samples of 300 AR patients and 300 healthy control volunteers. The IL-18 polymorphism was analyzed by polymerase chain reaction and restriction fragment length Polymorphism (PCR -RFLP) analysis.Results: Frequency of these three SNPs in IL-18 genotypes arising from combinations of the three common polymorphisms (–607 C/A, –137 G/C and–133 C/G) were significantly different between patients and control group.Conclusions: This study suggests that IL-18 gene variants may be participate as a risk factor in the pathogenesis of AR or in intermediary phenotypes. SoFurther studies are needed to reveal the associations between the IL-18 promoter polymorphism and allergic diseases in other populations.

Keywords: Allergic rhinitis, IL-18, SNP

350. The Synergistic Effect of Donor Bx genotype with KIR2DS3 and/or KIR3DS1 and cGVHD Occurrence on Survival after non-T-cell Depleted HLA-Identical Sibling Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia

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Background: Natural killer (NK) cell allogenic reaction, defined as lack of interaction between donor inhibitory killer cells immunoglobulin-like receptors (KIRs) and recipient human leucocyte antigen (HLA) class I molecules, may affect T-cell depleted hematopoietic stem cell transplantation (HSCT) outcome. However, influence of donor and recipient activating KIRs in HSCT has poorly been investigated. Materials and Methods: In this study, HLA and KIR genotypes were determined in 40 recipients with acute myeloid leukemia (AML) and 38 recipients with acute lymphoblastic leukemia (ALL) undergoing non-T-cell depleted HSCT from HLA-identical sibling donors. Then, overall survival (OS), disease-free survival (DFS) and relapse were evaluated in recipients. Results: No impact of 'missing KIR ligand' was found on OS, DFS and relapse for AML and ALL recipients. In AML patients, however, presence of KIR2DS3 and/or KIR3DS1 in donor genotype and cGVHD occurrence were associated with a higher two-year OS (P=0.006 and P=0.024, respectively) and DFS (P=0.021, P=0.033, respectively) in a univariate analysis. In these patients, bivariate analysisshowed thatthesefactorshave asynergistic effectonOS (P=0.01)andDFS (P=0.001). Conclusion: These findings may imply that 'missing KIR ligand' in recipients is of little importance in our matched non-T-cell depleted HSCT outcome. However, lack of KIR2DS3 and/or KIR3DS1 in donors and non-occurrence of cGVHD in recipients synergistically decrease survival in AML patients.

Keywords: KIRs, HLA, Acute Myeloid Leukemia

351. IL-22 Gene Variants Are Associated with Brucellosis in Iranian Population

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Background: Interleukin-22 is an IL-10 family cytokine member that was recently discovered to be produced by Th17 cells. Recent studies have shown the importance of Il-22 in the host defense against Gram-negative bacteria in the gut and lung. Since the route of the entry of Brucella is mucus surfaces and IL-22 has a critical role in the mucosal immunity, this study aimed to find any probable relationship between IL-22 gene variants and brucellosis.
Materials and Methods: One hundred and ninety two patients with brucellosis and 81 healthy farmers who consumed contaminated raw milk and dairy products from animals with brucellosis, were included in this study. All individuals were genotyped for 12 polymorphic sites of IL-2 gene (rs2227501 A/T, rs1179246 A/C, rs2046068 A/C, rs1012356 A/T, rs17224704 A/T, rs2227503 A/G, rs2227513 A/G, rs1026786 A/G, rs1182844 A/T, rs2227485 T/C, rs1179251 C/G and rs2227491) using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: IL-22rs1026786 AA genotype and A allele were significantly more frequent in the patients than the controls (P=0.000037 and 0.00026, respectively). Also, the frequency of IL-22rs1179251 CC genotype and C allele were significantly higher in the controls than the patients (P=0.0022 and 0.003, respectively). Conclusion: According to the data IL-22rs1026786 AA genotype and A allele could be considered as susceptibility factors for brucellosis while the inheritance of IL-22rs1179251 CC genotype and C allele might be a resistance factor against the disease.

Keywords: IL-22, Brucellosis, Iranian Population

352. Haplotype Analysis of IL-18 Gene in Iranian Patients with Thyroid Cancer

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Background: Thyroid cancer is the most common malignancy of the endocrine system, and genetic factors have been shown to be associated with its risk. Interleukin-18 (IL-18) is a pleiotropic pro-inflammatory cytokine that induces IFN-γ production, and is involved in Th1 development. Materials and Methods: To determine the role of IL-18 gene in thyroid cancer susceptibility, we conducted a case-control study and genotyped three single nucleotide polymorphisms (SNPs) in IL-18 promoter region (-656 G/T (rs1946519), -607 C/A (rs1946518), -137 G/C (rs187238)) and two SNPs in IL-18 5'-untranslated region (+113 T/G (rs360718), and +127 C/T (rs360717)), in 105 patients with thyroid cancer and 148 healthy controls from Iranian population. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and allele specific-PCR (AS-PCR) were used for genotyping. The association of different alleles, genotypes and haplotypes with thyroid cancer, tumor type, and the tumor stage were analyzed. Results: Comparing cases and controls, the allele frequencies and genotype distribution at position -656, and allele frequencies at position +127 were significantly different (p = 0.003, 0.002, and 0.003 respectively). Genotype TT at position -656 was associated with a significantly increased risk of thyroid cancer compared with G/G genotype (OR= 2.246, CI: 1.07-4.70, p= 0.03) and also compared with GG and GT genotypes combined (OR= 2.782, CI: 1.451-5.331, p= 0.002). No association with thyroid cancer was found at other positions (-607, -137, and +113). Also no association was found between the SNPs and stage of tumor, and thyroid tumor type, but at position -656 the genotype distribution was significantly different between PTC subtypes (p= 0.023). Haplotype analysis showed 21 different haplotypes and only TAGTT haplotype was significantly more frequent in patients (P= 0.001). Conclusion: Our results suggest increased susceptibility to thyroid cancer in subjects with genotype T/T at position -656 of IL-18 gene promoter, as well as TAGTT haplotype.

Keywords: Haplotype Analysis, IL-18, Thyroid Cancer

353. Genetic Polymorphism of *interleukin-17A* Genes in Gastric Carcinogenesis

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Background*:* Gastric cancer is one of the most common types of human cancers globally, which remains an important public health burden worldwide. There is a wide variation in the incidence of gastric cancer in different geographical regions. In Iran, while the northern and northwestern regions are high risk areas for gastric cancer, there are several intermediate and low risk populations in other geographical areas. Interleukin-17 (IL-17) is a pro-inflammatory cytokine secreted by activated T-cells. This study in implemented to indicate the polymorphism of

IL-17A in patients affected with Gastric cancer.Materials and Methods*:* in this case control study 300 samples collected from patients affected with gastric cancer and healthy controls. Genomic DNA extracted from peripheral blood samples was used. Genetic variation in IL-17A was evaluated using PCR RFLP method. An adjusted analysis was also performed by logistic regression analysis after adjustment for gender and age. Results*:* The prevalence of G-197A (rs2275913) polymorphism of IL-17A gene was significantly differed in GC patients and controls (p=0.001). The presence of an adenine in -197 position in promoter of IL-17A gene increased the risk of gastric cancer approximately up to 2.8 fold (1.48-5.17; p=0.001). Conclusion*:* the present findings demonstrated that the polymorphism in IL-17A gene was associated with development of gastric cancer. This allele carrier may be associated with an increased risk of subsequent development of gastric cancer

Keywords*:* Gastric cancer, IL-17A, carcinogenesis, polymorphism

354. Transcription Factor FoxP3 Gene Polymorphisms -2383 C/T and IVS9+459 T/C Are not Associated with Lung Cancer

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Background: *FoxP3* gene is an X-linked gene that encodes FoxP3 protein, an essential transcription factor in $CD4^+CD25^+FoxP3^+$ regulatory T (Treg) cells. Suppressing immune responses by the Treg cells may contribute in tumorogenesis. Moreover, role of FoxP3 as a tumor suppressor gene has been documented. Regarding the above mentioned dual roles of FoxP3, investigation of probable association of *FoxP3* gene polymorphisms in cancers may open new windows to potential gene and molecular therapeutics of cancer. We have chosen lung cancer for this study as it is one of the most fatal malignant disorders all over the world and most patients are diagnosed at a late clinical stage, accordingly, identification of lung cancer-prone individuals would reduce the cancer mortality rate. Materials and Methods: In a case-control study we analyzed the -2383 C/T (rs3761549) and IVS9+45 T/C (rs2280883), two SNPs of the *FoxP3* gene, in 156 patients with lung cancer and 156 age and sex matched controls in southern Iranian population, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) strategy. As *FoxP3* is an X-linked gene we analyzed males and females separately. Results: In male patients, male controls, female patients, and female controls rs3761549 genotype frequencies were 110C : 16T, 118C : 8T, 25CC : 4CT : 1TT, and 27CC : 3CT, respectively; and the frequencies of rs2280883 genotypes were 83T : 43C, 71T : 55C, 15TT : 14CT : 1CC, and 13TT : 13CT : 4CC, respectively. No significant differences in the frequencies of genotype and allele at the both loci were observed between patients and controls. Haplotype analysis has not shown any significant differences, too. Conclusion: This study indicates that these two SNPs of *FoxP3* gene are not associated with lung cancer in Iranian population.

Keywords: FoxP3, Polymorphism, Lung Cancer

355. The Influence of -330, -475 and -631 IL-2 Polymorphism on Multiple Sclerosis in Iranian Population

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We have assessed the role of -330 G/T, -475A/T, -631 G/A *interleukin-2* (IL-2) gene polymorphism in patients with multiple sclerosis (MS) and in healthy individuals. It was the first time that the-475 and -631 IL-2 promoter single nucleotide polymorphism were analyzed in Iranian MS patients. The study was carried out among 100 MS patients and 100 matched healthy controls. The RLFP-PCR method was employed to define the alleles and Bonfernio's corrections method was also adapted to analyses. A marked increase was noticed in the T allele and T/T genotype at -330 IL-2 location in MS patients. A very low frequency of T at−475 and A at−631was observed in each of the two groups. Our results indicate that -330T IL-2 SNP had the susceptibility effect on MS disease and -475 and -631 IL-2 gene polymorphisms do not cause any susceptibility to MS.

Keywords: IL-2, polymorphism, multiple sclerosis

Poster Presentation

356. Influence of Endothelial Nitric Oxide Synthase Gene Polymorphisms (-786T/C, 894G/T) in Iranian Liver Transplant Recipients

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Background: Nitric oxide (NO) is a major mediator in regulation of regional blood flow. Its production is catalysed by the enzyme endothelial nitric oxide synthase (eNOS). Protective actions of NO in ischemia and reperfusion are due to its potential as an antioxidant and anti-inflammatory agent. The eNOS gene polymorphisms affect eNOS activity. The aim of the present study was to find genotype frequencies of (SNP) in eNOS gene (rs 2070744, and rs1799983) in 90 healthy individual and compare these result with liver transplant patients who develop acute rejection. Materials and Methods: Ninety healthy persons as well as twenty- five liver transplant recipients, between June 2010 and March 2011, were included in this study. The polymorphism was determined by simple polymerase chain reaction and PCR-restriction fragment-length polymorphism analysis. Results: Recessive model of T-786C alleles (TT vs. TC+CC) revealed TT genotype 59 (65%) normal people; 14(56%) patient and TC+CC in 31 (35%) normal subjects and 11(44%) liver recipients. No significant difference was observed (p-value =0.32). For eNOS gene 894G/T, GG genotype was observed in 72(80%) normal people; 12(48%) patient and TG+TT in 18 (20%) normal subjects and 13(52%) liver recipients with significant difference (p-value =0.001, OR=4.27 and CI= 1.52-10.18). Conclusion: We conclude that recipient eNOS gene polymorphisms may alter the risk of acute rejection after liver transplantation. However, our study was done on small sample; therefore more study is needed for this evaluation.

Keywords: Nitric Oxide, Polymorphisms, Liver Transplant Recipients

357. Influence of Endothelial Nitric Oxide Synthase Gene Polymorphisms (-786T/C, 4a4b, 894G/T) in Iranian Kidney Transplant Recipients

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Background: Nitric oxide (NO) is a major mediator in vascular biology and regulation of regional blood flow. Its production is catalysed by the enzyme endothelial nitric oxide synthase (eNOS). Protective actions of NO in ischemia and reperfusion are due to its potential as an antioxidant and anti-inflammatory agent, along with its inhibitory effects on cell signaling pathway of nuclear proteins, such as NF-κ B. The eNOS gene polymorphisms affect eNOS activity and are associated with endothelial dysfunction. The aim of the present study was to examine the association between single nucleotide polymorphisms (SNP) in eNOS gene (rs 2070744, 27VNTR, and rs1799983) and the development of acute rejection in renal transplant patients. Materials and Methods: Sixty-six renal transplant recipients (33 patents with episode of acute rejection and 33 recipients without rejection), between June 2010 and March 2011, were included in this study. The polymorphism was determined by simple polymerase chain reaction and PCR-restriction fragment-length polymorphism analysis. Results: There is only a significant association of eNOS -786T allele and acute rejection (P = 0.03). Recessive model of T-786C alleles (TT vs. TC+CC) and acute rejection confirmed a significant association (P = 0.025; Odds ratio: 3.12; 95% CI: 0.01-9.83). Haplotype CbG was higher in recipients without rejection as compared to rejection group (OR: 0.42, 95%CI: 0.16–1.13, P < 0.05). In respect of eNOS gene 894G/T SNP and 27VNTR, no significant association between allele/ genotype and acute rejection was observed. Conclusion: We conclude that recipient eNOS gene polymorphisms do not alter the risk of acute

rejection after renal transplantation. Rejection is a complex immunologic event. Therefore, finding the associated genetic variants demand a multicentric larger sample size.
Keywords: Nitric Oxide, Polymorphisms, Kidney Transplant Recipients

358. Use of SNP Technologies in Medicine, Future Challenges and the Concept of Personalized Medicine in Autoimmune Disease such as Rheumatoid Arthritis and Multiple Sclerosis

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Studying genetic variation across autoimmune disease particularly allows us to systematically identify allele-specific relationships. Classification of diseases based on allelic differences may be used in the future to shed light on potential new therapies. Certain drugs like anti-TNF have positive effects in RA and psoriasis as compared to MS. Genome-wide association studies (GWAS) across autoimmune diseases provide a vast prospect to study the genetic architectures across autoimmune disease. We published our data in the field of autoimmune disease recently and we examine two Case Control studies together with 232 cases and 170 controls for 2 complex human autoimmune disease (RA and MS) in Iranian patients by using PCR-RFLP method, which comprises four programmed cell death related genes including: Fas, FasL, TRAIL and PD-1 SNPs.
In this work, we are going to define a novel concept of a disease variation profile and personalized medicine accomplish comparative analyses to find similarities and differences in the genetic architectures of autoimmune diseases. With regarding the importance of SNPs and GWAS in the field of autoimmune disease, using SNP technologies such as microarrays, and microfluidic chips, we were able to set up and classified personalized medicine in the field of autoimmune disease. In addition, it will be highly useful in diagnostics and drug development .In the future we should explore individual SNPs and genes that play an important role in defining similarities and differences between
disease and individuals and using this data to setting up a new SNP technologies for diagnostic and therapeutic purposes.
Keywords: SNP Polymorphisms, autoimmunity, personalized medicine, Iranian patient

359. The Role of *TIRAP* S180L but not *TLR4* and *TLR9* in Host Resistance to Severe Malaria

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Background: Toll-like receptors (TLRs) play an important role in the initiation of the innate immune response to a wide variety of pathogens. Different studies showed that TLR4, TLR9 and the adaptor protein TIRAP are central mediators of pro-inflammatory responses to Plasmodiuminfection. Therefore, the current immunogenetic study was designed to investigate the frequency of the common polymorphisms of *TLR4*, *TLR9* and *TIRAP* in Baluchi population with mild malaria. The results were also compared with similar studies carried out in African countries with greater proportion of severe cases to evaluate the effect of these polymorphisms on susceptibility/resistance to malaria. Materials and Methods: The *TLR4* (D299G and T399I), *TLR9* (T-1486C and T-1237C) and *TIRAP* (S180L) polymorphisms were analyzed in 640 Baluchi individuals who are living in malaria hypoendemic region of Iran (320 *Plasmodium falciparum*-infected and 320 healthy individuals) by using PCR-RFLP and sequencing analysis. Results: The results showed that *TLR4* (D299G and T399I) and *TLR9* (T-1486C and T-1237C) SNPs were distributed equally among *P. falciparum*-infected and non-infected groups (*P* value > 0.05). Furthermore, the *TIRAP* S180L heterozygote frequency for infected and control group was 33.8% and 25.6%, respectively (OR, 1.479; 95% CI, 1.051-2.081; *P* = 0.024). The results also revealed that *TIRAP* S180L heterozygote frequency in Baluchi population was considerably greater than African populations (0%-6% in Gambia, Ghana and Kenya). Conclusion: These data showed that *TLR4* (D299G and T399I) and *TLR9* (T-1486C and T-1237C) common SNPs were not associated with mild malaria (*P*> 0.05) in Baluchi population. In addition, significantly higher frequency of heterozygosity for *TIRAP* S180L in Baluchi infected individuals than control group might suggest increasing risk of mild malaria. Also the greater heterozygote frequency for *TIRAP* S180L variant in Iranian mild malaria patients than African populations might support the role of this SNP in protection against severe malaria.
Keywords:*TIRAP*, *TLR4*, *TLR9*, Host Resistance, Severe Malaria

360. The *FasL -844T* Allele Increases Recovery after HBV Infection in Iranian Individuals

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Background: The outcome of hepatitis B virus (HBV) infection can be affected by host immunogenetic factors. This study was undertaken to investigate the association between HBV infection outcome and single nucleotide polymorphisms (SNPs) of genes for *FAS/FASL* death signal pathway used by cytotoxic T lymphocytes to eradicate virus from the liver. Materials and Methods: The case group consisted of 151 infected subjects (chronic carrier *n=50*, chronic hepatitis *n=50*, cirrhosis *n=25*, and spontaneous recovery *n=26*) who were diagnosed and classified by serologic and molecular HBV markers, and if needed liver biopsies. Additionally, 100 ethnicity and age matched healthy controls negative for HBsAg, HBsAb and HBcAb were included in this study. *FAS -1377G/A* and *FAS -670A/G*, and *FASL -844C/T* SNPs genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays in case and control groups. Results: Our data showed that prognosis of HBV infection can be influenced by *FASL -844 C/T* polymorphism. The *T* allele and *CT+TT* genotypes were significantly higher in recovered individuals than healthy controls (*T* allele: 69.2% vs. 49.5%, *Pc*=0.017, OR=2.296, 95%CI=1.20-4.40; *CT+TT* genotype: 100% vs. 75%, *Pc*=0.01). Comparison between recovered subjects and cirrhotic patients regarding this polymorphism was also showed similar results (*T* allele: 69.2% vs. 38.0%, *Pc*=0.003, OR=3.671, 95%CI=1.62-8.34; *CT+TT* genotype: 100% vs. 60%, *P*=0.0003). Analysis of other polymorphisms among case subgroups and between each case subgroup with controls indicated no significant difference. Conclusion: As the presence of *T* allele in -844 position of *FASL* gene promoter reduces expression rate, we conclude that modulation of apoptosis through FAS/FASL interaction may be effective in reduction of immunopathology and facilitating recovery after HBV infection.
Keywords: HBV, *FasL -844T* Allele, SNP

361. Role of KIR-HLA Combination in Hematopoietic Stem Cells Transplantation

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Allogeneic hematopoietic stemcells transplantation (HSCT) is a valuable therapy for refractory acute leukemias, leukemias with a high risk for relapse, myelodysplastic syndromes, and chronic myeloid leukemia. HSCT outcome is dependent on several factors, including the stage of disease, degree of human leukocyte antigen (HLA) identity between donor and recipient, conditioning regimen, and development of graft-versus-host disease (GVHD). Recent studies have indicated that another potential factor influencing transplantation outcome is the presence of donor-derived alloreactive natural killer (NK) cells. NK cell alloreactivity has been defined as a mismatch between the donor and recipient killer immunoglobulin-like receptor (KIR) ligands (ligand-ligand model), or as recipient lacking KIR ligands for donor inhibitory KIR (receptor-ligand model), or as a mismatch between the donor and recipient KIR genes (gene-gene model). Conclusion: The anti-leukemic effects of NK cell alloreactivity include lower rates of relapse, graft failure, and GVHD, ultimately translating intohigher overall survival (OS). However, the effects of NK cell alloreactivity on the outcome of HSCT in malignant hematopoietic diseases is a topic of debatable.
Keywords: KIR-HLA, Hematopoietic Stem Cells, Transplantation

362. Association Study of Rs7270101 SNP of ITPA Gene with Multiple Sclerosis in an Iranian Population

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Background*:* Human multiple sclerosis (MS) is an autoimmune and a complex disease that making MS the most common CNS disorder to cause disability in young people. This disease affects approximately two million people worldwide. These disease variants likely result from the involvement of different effectors arms of the immune system such as CD4+ and CD8+ T cells as well as autoantibodies. The azathioprine (AZA) drugs and derivatives are amoung the common drugs used to maintain clinical remission in MS. Among polymorphic enzymes of thiopurines'metabolic pathway, recent studies point to inosine triphosphate pyrophosphohydrolase (ITPA) polymorphism as an important enzyme in metabolism pathway of AZA drug. The cytosolic enzyme ITPase (EC 3.6.1.19) hydrolyses the rough deaminated purine nucleotides d/ITP to corresponding mono phosphate forms. It has been reported that an allelic variant of, ITPA IVS2+21A>C in the gene is associated with decreased ITPase enzyme activity. To study the possible association of ITPA activity with MS, we decide to verify the association rs7270101 SNP with multiple sclerosis. Materials and Methods*:* Peripheral blood was collected from 100 subjects with MS and 100 healthy controls. All subjects were diagnosed with definite MS by a specialist. The ITPA IVS2 +A21C polymorphism was determined by a PCR-RLFP technique or Real Time PCR in the genomic DNA.Results*:* We found a difference in allele frequency for this SNP of ITPA between MS patients and control group.Conclusion*:* Our result shows that the SNP rs7270101 of ITPA may has association with multiple sclerosis in Iranian population.
Keywords: Multiple Sclerosis, SNP, ITPA

363. Association between T-991C Genetic Polymorphism of *Ku70* and Susceptibility to Breast Cancer

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Ku antigen which is also named ku70 is encoded by *XRCC6* gene, which is located on chromosome 22q13.2. Ku70 as a member of NHEJ (Non-Homologous End Joining) pathway has an important role in repair of DNA double-strand breaks. Moreover, Ku70 can function as a DNA sensor and induce IFNL1 (IL29) activation, which is a type III interferon and has significant immunoregulatory properties. Previous studies have reported association between polymorphisms of this gene and susceptibility to different types of cancer. *Ku70* promoter T-991C polymorphism (rs5751129) has been reported to be associated with susceptibility to gastric cancer, oral cancer and pterygium. However, the association between this polymorphism and susceptibility to breast cancer has never been reported. In this case-control study, we included 111 females with breast cancer and 140 frequency age-matched healthy females. RFLP-PCR method was used to perform the genotypes. Analysis revealed that there was no significant difference between cases and controls for distribution of T-991C genotypes (TT, TC and CC), before and after adjustment for breast cancer risk factors. In conclusion, our study did not support any association between susceptibility to breast cancer and T-991C polymorphism of *XRCC6* gene.
Keywords: Polymorphism, *Ku70*, Breast Cancer

364. FOXP3 and TGF-β Gene Polymorphisms in Allergic Rhinitis

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Background: Regulatory CD4+T (Treg) cells are effective in maintaining immune tolerance. Objective: To investigate single nucleotide polymorphisms (SNPs) of Transforming Growth Factor β-1 (TGF-β1) and Forkhead Box Protein 3 (FOXP3) genes in Iranian patients with allergic rhinitis (AR). Materials and Methods: Variations at codons 10 and 25 of TGF-β1 and FOXP3 at positions -3279 A>C and -924 A>G were evaluated in AR patients and compared with controls. In a case-control study, 155 AR patients and 163 allergy-free controls were genotyped using polymerase chain reaction sequence-specific primer (PCR-SSP) technique. Results: The analysis of the frequency of these SNPs showed that the haplotype formed by FOXP3 -3279 A allele occurred significantly more frequently in patients than controls (odds ratio=1.44, 95% CI=1.312-2.66; p=0.001). Conclusion: Our results suggest that polymorphism in FOXP3 gene is associated with susceptibility to AR.
Keywords: FOXP3, TGF-β, Polymorphisms, Allergic Rhinitis

365. Bioinformatic Analysis for Allergenicity Assessment of Cry1Ab Proteins in Iranian Genetically Modified Rice

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Background: Recently, Iranian biotech researchers succeeded to introduce transgenic rice resistant to rice stem borer larvae. Based on researches, this transgenic rice has all traits like other rice cultivars and one trait as resistance against stem borer larvae different it from other rice cultivars. New produced protein causes plant resistance against stem borer larvae pests. This transgenic rice, with Cry1Ab gene transfer from the bacterium *Bacillus thuringiensis* (Bt), has created using gene (Cry1Ab) bombardment into cultivated Tarom Mola'i and Caspian cultivars. Cry1Ab gene produces toxic protein for rice stem borer larvas. Materials and Methods: Bioinformatic analysis *(In silico*) was implemented in order to approval of non allergenic Cry1Ab protein. Hence Cry1Ab protein sequence was extracted from NCBI. Cry1Ab gene protein contains 1155 amino acids. This sequence was aligned using the FASTA program in protein databases FARRP, SDAP, Algpred. Sequence alignment was implemented with the allergen proteins in three matches including: the full sequence matching sequence, matching the 80 amino acids and eight amino acids. Results: The results showed no similarity between Cry1Ab protein and allergen proteins in the full sequence matching, matching the 80 amino acid (Domain) and matching an 8 amino acid to determine the epitope potential. Conclusion: We conclude that Cry1Ab protein has non-allergenic potential. Hence Iranian transgenic rice is non-allergen for consumers.
Keywords: *In silico* assessment, Allergenicity, *Cry1Ab*, Iraniantransgenic rice, Tarom Mola'i cultivar

366. No Association between CCR5 Δ 32 Mutation and Multiple Sclerosis in Patients of South-Eastern of Iran

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Background: Multiple sclerosis (MS) is considered as a complicated autoimmune disorder. Evidences are in favor of involvement of chemokines and their receptor in autoimmune diseases. Hence, this project aimed to analysis the known CCR5-Δ32 mutation in MS patients. Material and Methods: In this experimental study, blood samples were collected from 100 MS patients and 300 healthy controls on EDTA pre-coated tubes. DNA was extracted and DNA samples were analyzed for CCR5-Δ32 mutation by Gap-PCR in patients and controls. Demographic data were also collected by questionnaire. Rsults: Our results showed that, none of MS patients displayed CCR5 Δ32 mutation, while two healthy controls showed heterozigotic form of this mutation. Conclusion: Several studies analyzed relation of this mutation with autoimmune diseases. Some studies failed but some succeeded to find an association between this mutation and autoimmune diseases. Based on the results of our study it could be probably concluded that this mutation do not play a role in etiology and pathogenesis of MS.
Keywords: Multiple sclerosis, CCR5, CCR5-Δ32 mutation.

367. Association of Interleukin-17 Gene Polymorphisms (rs4711998 and rs381025) with Visceral Leishmaniasis

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Background: Visceral leishmaniasis (VL) is a lethal disease mostly caused by *Leishmania infantum* parasite in the Middle East. The parasite can stimulate the production of IL-17 by Th17. It was shown that IL-17 is strongly and independently associated with the protection against VL. Then, it seems that IL-17 has a complementary role in the human protection against VL. Since the production of cytokines is under the control of their genes, this study aimed to study the probable relationship between VL and the polymorphisms of IL-17 gene. Materials and Methods: One hundred and seventeen patients with VL and 146 healthy individuals living in the same area as patients joined this study. All individuals' DNA samples were genotyped for IL-17 gene (rs4711998A/G and rs3819025A/G) polymorphisms using PCR-RFLP method. Genotypic and allelic frequencies were estimated by counting method. Associations were analyzed using Chi-square test with the level of significance set at < 0.05. Results: We couldn't find any statistical significant differences in the allele and genotype distributions of IL-17 (rs4711998 and rs3819025) gene among the patients and controls. Conclusion: Our analysis did not show a significant difference between the frequencies of IL-17 genotypes and alleles among the patient and controls. It might be the result of the limited number of cases and controls, so a study on the larger population is suggested. Since other single nucleotide polymorphisms (SNPs) are shown IL-17 gene, the analyses of those SNPs are recommended.
Keywords : Interleukin-17, Polymorphism, Visceral Leishmaniasis

368. Deletion of 53 Nucleotides from IFN-β mRNA 3´UTR for Increasing It´s mRNA Stability

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Background: Inteferons are small polypeptides proteins which are secreted by most animal cells in response to exposure to a variety of inducers. Natural interferon beta can be produced by most cells in the body. Interferon beta is used for treatment of multiple sclerosis. Experiments have shown that there is one destabilizing element in 3´UTR IFN-βgene. Deletion of this part can improve the protein stability. So, we deleted 53 nucleotides from IFN-β mRNA 3´UTR for increasing it´s mRNA stability.this aim was accomplished by SOEing PCR technique. Materials and Methods: 4 primers (F1,R1.F2,R2) were designed for amplification of IFN-β gene from PMI7 vector, using Oligo®6 software. These primers used during two PCR reactions, where the first PCR is just a reaction between F1R1 and F2R2. We purified the PCR products and then performed the second reaction using the purified PCR products and adding only primers F1 and R2. Part of R1 primer was overlap with F2 primer.so, the PCR product F1R1 and F2R2 were overlap. So, 53 nucleotides from 3´UTR were deleted. Different procedure such as gel electrophoresis and PCR analysis were performed on the amplified fragment in order to confirmation of its structure. EcoR1 site was selected as a cloning site in the pSVM vector, after cutting PCR product and this vector with this enzyme, we cloned this gene in to the vector. Different constructs were selected following transformation of *E. coli*. Results: The modified IFN-β gene was successfully integrated in to pSVM vector as illustrated by gel electrophoresis. Conclusion: Studies have shown that the 3' UTR exerts its inhibitory effect through a physical association with polyA tail. A new construct has been made in this project containing modified IFN-β gene. This construct has to be introduced into CHO cells and effects of the deleted fragment on IFN-β mRNA stability can be analyzed.
Keywords: Deletion, IFN-β, mRNA Stability

369. Constraints of the Allogeneic GL26/BALB/c Mouse Intracranial and Subcutaneous Glioma Models: Concepts for Evaluating Immunotherapy

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Background: Glioblastoma Multiforme (GBM) illustrates many challenges amongst the cancer patients survival, despite current therapies, covering surgery, radiation therapy, and salvage chemotherapy. Improvement of glioma patients outcome depend upon correspondent animal models, to studying disease mechanisms and evaluating novel therapeutic strategies, in order to reducingside effects and toxic consequences of new drugs. In immunegenetherapy of glioma, synegeneic models are excellent, but the lack of access to syngeneic model in glioma, in some countries, inspired us to design intracranial and subcutaneous allogeneic models with usage of varied amounts of mouse glioma cell line GL26 cells within female BALB/c mice. Perhaps, we could detect this fundamental inquiry, whether GL26 has sufficient capacity to induce glioma in BALB/c allogeneic system as an alternative in-vivo model. Materials and Methods: In order to achieve this Purpose, we induced the subcutaneous and intracranial tumors, then, tumors were evaluated in terms of macroscopic and microscopic characteristics. Results: Microscopic analysis of the intracranial model represented only a mild inflammatory reaction. In subcutaneous inoculation, tumors were induced faster within the groups with the higher number of cells inoculation. In macroscopic examination, the tumor was relatively large, thick and entirely full of blood. Moreover, in microscopic examination, cell proliferation, mitosis, abundant vessels and tumor necrosis were observed. Conclusion: Our data demonstrated that the use of GL26 cell line in BALB/c mice does not induce intracranial tumors while subcutaneous tumors are induced via high numbers of cells after an extended time period. It probably, can be considered as a successful model of allogeneic tumor rejection.
Keywords: Glioma , GL26, Intracranial, Subcutaneous, Immunotherapy, BALB/c mouse

370. Detection of New Allele of HLA-DRB1 Gene by High Resolution DNA Sequencing

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The HLA (Human Leukocyte Antigens) loci are part of the genetic region known as the major histocompatibility complex (MHC). HLA genes are major component of immune system ،and located on 6p21.3. There are various methods of HLA-Typing which are different in resolution, turn- around and work load. Resolution of technique is the most important factor to detect new alleles. SSP, SSO, RFLP and other methods can not detect new alleles. Only high resolution HLA-Typing by DNA sequencing is capable to detect new alleles. We identified a new undefined allele of HLA-DRB1 by DNA sequencing in two individuals of Iranian origin. This test was carried out by sequencing of exon 2 and 3 of HLA-DRB1 gene. Exon 3 at position 570 showed a double peak, indicating ' A ' and ' G ' clearly in heterozygous form. All the submitted data which have been reported so far to the IMGT/HLA library are homozygote for G at position 570. So, this is a new allele which we have found in Iranian population. We are submitting to the IMGT/HLA data base.
Keyword: HLA, HLA-typing, high resolution, sequencing, new allele.

371. The Influence of the HLA-DRB, HLA-DQB and Polymorphic Positions of the HLA-DRβ1 and HLA-DQβ1 Molecules on Risk of Iranian Type 1 Diabetes Mellitus Patients

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Type 1 Diabetes mellitus (T1D) is an autoimmune and multifactorial disease. HLA-DRB1 and DQB1 loci have the strongest association with T1D. This study was aimed at investigating a) susceptibility or protection of alleles, genotypes and haplotypes of HLA-DRB1 and DQB1 loci and

b) highly polymorphic amino acid residues of HLA-DRβ1 and DQβ1 in 105 Iranian T1D patients and 100 controls. The results indicated DRB1*04:01, 03:01, DQB1*03:02, 02:01 alleles, DRB1*03:01/04:01, 03:01/13:03, DQB1*02:01/03:02 genotypes, DRB1*04:01-DQB1*03:02, DRB1*03:01-DQB1*02:01, DRB1*07:01-DQB1*03:03 haplotypes had positive association with T1D. In contrast, HLA-DRB1*15:01, 13:01, DQB1*03:01, 06:01 alleles, DRB1*11:01/15:01, DQB1*03:01/06:01, 03:01/05:01 genotypes and DRB1*15:01-DQB1*06:01, DRB1*11:01-DQB1*03:01 haplotypes had negative association with T1D. Analysis of amino acid sequence of HLA-DRβ1 and DQβ1 revealed that $DR\beta1^{Lys71+}$ and $DQ\beta1^{Asp57-}$ were significantly more frequent in patients than controls and had a positive effect in development of T1D. Haplotype analysis demonstrated that HLA-$DRB1^{Lys71+}$ allele provided major susceptibility for T1D and $DQ\beta1^{Asp57-}$ had an additive effect. We designed an allele-specific primer to develop an easy, quick and cost-benefit method (called "Simple method") to detect the $DR\beta1^{Lys71+}$. This method can identify all 114 DRB1 alleles encoding$DR\beta1^{Lys71+}$ by three PCR reactions. The PcPPV and PcNPV Were also calculated to determine the impact of HLA genotype testing at amino acid positions. It showed that the $DR\beta1^{Lys71+/+}$ genotype carrier had 1% absolute risk to develop T1D.

Keywords: Type 1 diabetes, DRB1, $DR\beta1^{Lys71}$, $DQ\beta1^{Asp57}$, HLA amino acid

372. OX40 Genetic Variations (rs2298211 A/C and rs17568 A/G) in Laryngeal Squamous Cell Carcinoma

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Background: OX40, also known as CD134, is a member of the TNF receptor superfamily that predominantly expresses on activated T cells and provides a co-stimulatory signal for T cell activation. In addition, ligation of OX40 appears to be contributed in the proliferation and survival of memory cells. This molecule is also critical for differentiation and activity of regulatory T cells as well as differentiation of activated B-cells into highly immunoglobulin-producing cells. Objective: Present study aimed to investigate the association of two OX40 gene polymorphisms, rs2298211 A/C in intron 5 and rs17568 A/G in exon 5, with susceptibility of Iranians to laryngeal squamous cell carcinoma. Materials and Methods: Two hundred women with laryngeal squamous cell carcinoma and 135 age and sex matched controls without any history of cancer and autoimmune diseases in themselves and their first degree relatives were recruited in this study. An informed consent was obtained before the recruitment. PCR–RFLP methods were used for genotyping of both positions. Results: Frequency of rs2298211 AA, AC and CC genotypes in patients and control group were respectively 86% vs. 81%, 10% vs. 17% and 4% vs. 2%. In the case of rs17568, frequency of GG, GA, and AA were 39%, 44% and 17% in patients and 50%, 31% and 19% in controls, respectively. No statistically significant differences were found in the frequencies of genotypes and alleles between patients and control group at both positions (P>0.05). In addition, there was no association between genotype frequencies and cancer progression factors including tumor type, size and distant metastasis. Conclusion: As the first study in head and neck cancers, results of this investigation conclusively suggest that OX40 gene polymorphisms are not associated with susceptibility of Iranians to laryngeal squamous cell carcinoma.

Keywords: OX40, Laryngeal Squamous Cell Carcinoma, polymorphism

373. MicroRNAs in Multiple Sclerosis

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Multiple Sclerosis (MS) is a chronic inflammatory disorder in the central nervous system (CNS). In MS, the myelin cover of nerve cells is lost or damaged, leading to nerve cell exposure or destruction. Nevertheless the etiology of MS is not clearly known, both genes and environment play the central role in pathogenesis of MS. One of the newest fields of genetic research in MS is the study of effect of MicroRNAs (miRNAs) on disease pathogenesis. miRNAs are small (20- 22 nucleotides) non-coding regulatory RNAs that regulate gene expression at the post-transcriptional level. Since the discovery of miRNAs in 1993 by Lee and his colleagues, it has been shown that deregulation of miRNAs expression and function is associated with a variety of human diseases including cancer, neurodegeneration and autoimmunity. Also many studies are performed about miRNAs in MS indicating that miRNAs have unique expression profiles in cells of the innate and adaptive immune systems and have pivotal roles in the regulation of both cell development and function. For example, miRNA-181a contributes in B cell development. Alternation in expression of these small RNAs has a putative role in pathogenesis of MS and serves as a biomarker for the fast diagnosis of MS. Finally, all studies proposed that miRNAs can serves as a new therapeutic approach for MS. The number of new MS cases has significantly increased during the past decade in Iran. The new approaches for disease detection and prevention are necessary. In this review, we aimed at updating the new findings about miRNAs in MS and its potential role in diagnosis and prognosis as well as the better understanding of the pathogenesis of the disease.

Keywords: Multiple Sclerosis, MicroRNAs

374. RANTES -403 G/A Polymorphism Allele and Genotype Frequencies in Association with Coronary Artery Disease

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Background:The chemokine RANTES is normally expressed in T leucocytes and located on chromosome 17q11.2-q12. Functional polymorphisms within RANTES promoter region have been shown associated with wide range of immune and inflammatory conditions. There are controversial findings regarding the association between different polymorphisms in RANTES promoter and coronary artery disease (CAD), the aim of this study was to examine RANTES -403 G/A polymorphism in patients with CAD compared to patients without CAD in an Iranian population. Materials and Methods: The study was performed on 319 patients who underwent coronary artery angiography and patients with >50% stenosis in vessels considered as case groups (CAD^+) N=191 and normal vessels group as control (CAD^-) N=128.Five ml of peripheral blood was collected in EDTA tubes from patients. The DNA was extracted from WBCs by using salting-out method and PCR-RFLP technique was performed to determine allele and genotype frequencies for RANTES -403 G/A polymorphism. Results: No significant difference for allele and genotype frequencies of RANTES -403 polymorphism was found between cases and controls. No significant differences were also found after adjustment for sex, age, smoking and diabetes (GG vs GA+AA, p=0.3, OR: 0.7, 95%CI = 0.4-1.3). Conclusion: In this study we have found thatthe frequency of RANTES gene polymorphisms at position –403 was equally distributed between patients and controls, therefore it seems not implicated in susceptibility of CAD. There are a few studies that have previously described the association between RANETS gene polymorphism and CAD with controversial results. Consequently the discrepancies observed in association between RANTES gene polymorphism and risk of CAD in various studies might be partly due to the variable phenotypes associated with the disease which was not similar in different studies.

Keywords: RANTES, CAD, -403G/A polymorphism

375. RANTES Gene mRNA Expression in PBMCs of Patients with Coronary Artery Disease

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Background: RANTES gene plays a major role in two preliminary processes in atherosclerosis:chemoattraction of leucocytes to the endothelial wall and subsequent trans-endothelial migration of leucocytes to the inflammation site. The increased expression of RANTES in T leucocytes in

human atherosclerotic plaques as well as in lymphocytes, macrophages, endothelial cells and vascular smooth muscle cells suggest its role in the development and progression of atherosclerosis. The aim of this study was to examine the RANTES gene expression in patients with coronary artery disease (CAD) compared to patients without CAD in an Iranian population. Materials and Methods: Subjects with normal vessels considered as CAD^- (N=20) and patients with>50% stenosis in vessels categorized as CAD^+ (N=20).Total RNA was extracted from 5 ml of each individual's fresh peripheral blood collected in heparin-containing tubes. RNA extraction was carried out by Tripure and Phenol/chloroform method. The cDNA synthesized with Expand Reverse Transcriptase according to manufacturer instructions. RANTES mRNA expression was examined using quantitative real-time PCR. Results: Normal distribution of RANTES gene expression was observed both in CAD^+ and CAD^- patients (p=0.4 and p=0.3 respectively). RANTES mRNA expression was increased to 1.37 fold in CAD patients compared to the controls which was not statistically significant (p=0.1, 95%CI=-0.2-1.1). Conclusion: It has been shown that Increasing of RANTES expression associated with a number of inflammatory disorders and pathological conditions, including atherosclerosis, inflammatory airway disorders such as asthma. In this study we analysed the role of RANTES in promoting leukocyte infiltration to the inflammation sites. Our study showed expression of RANTES gene is increased in patients with CAD. This result introduce RANTES gene for both therapeutic and diagnostic researches. More studies on larger number of samples are required to further evaluate role of RANTES in pathogenesis of CAD.
Keywords: RANTES, CAD, real-time PCR

376. The 16C/A Genetic Variation (rs4359426) in Macrophage-Derived Chemokine/CCL22 is not Associated with Susceptibility of Iranians to Breast Carcinoma

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Background: CCL-22/MDC is a CC chemokine with a critical role in regulating and maintaining immune balance. CCL-22/CCR-4 ligation has been documented to participate in the migration of Regulatory T (Treg) cells and Th2 lymphocytes to the site of primary breast tumors. Accumulation of these cell types has already been indicated to be associated with poor prognosis in breast cancer patients. In the present study we aimed to investigate the association of a single neuclutid polymorphism (SNP) in CCL-22 gene; 16C/A (rs4359426), with the susceptibility to breast carcinoma in a population from the south of Iran. Materials and Methods: On hundred sixty one patients with pathologically confirmed breast cancer (mean age 49.3 ± 11.5) and 178 age-matched healthy women (mean age: 49.3±12.9) with no personal and familial history of cancer or autoimmune disease were enrolled. CCL-22 genotypes were investigated by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. RFLP results were verified by direct automated sequencing. Results: Arlequin analysis showed no deviation from Hardy-Weinberg equilibrium neither in the patients nor in control group. The most frequent genotype in both patients and control group was wild type CC genotype with frequency of 146 out of 161 (90.7%) among patients and 153 out of 178 (86.0%) in control group (P=0.24). The frequency of CA genotype was 15 (9.3%) and 23 (12.9%) in patients and controls respectively (P= 0.38). No AA genotype was observed among patients but this genotype was observed with the frequency of 2 out of 178 (1.1%) in control subjects. No correlation was found between investigated genotypes with clinicopathological characteristics of the patients. Conclusion: Results of this investigation does not support the association of 16C/A SNP (rs4359426) in CCL22 gene with susceptibility to, and progression of, breast cancer in Iranian population.
Keywords: Genetic Variation, CCL-22/MDC, Breast Carcinoma

377. Frequency of HLA-A Alleles in an Iranian Population

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Background: Major histocompatibility complex (MHC) is the most polymorphic system in the genome of different species. In the human beings, these genes are located on the chromosome 6 and named HLA. Recognition of the polymorphism of HLA alleles is useful in transplantation, disease, and anthropological studies. Among different antigens of HLA, HLA-A alleles are of specific importance. In this research, the polymorphism of these alleles was surveyed to analyze the frequency of these alleles in Iran. Materials and Methods: DNA was extracted from the whole blood of 1000 individuals registered in ISCDR (Iranian Stem Cell Donor Registry). Then the polymorphic region of HLA-A gene was studied using PCR-SSP and PCR-SSOP methods. Ultimately PCR products were visualized by electrophoresis in 2% agarose gel and reverse hybridization method, respectively. Results: The most frequent alleles were recognized as HLA-A*02 (15.43%), HLA-A*03 (12.29%), HLA-A*24 (11.79%), HLA-A*11 (9.09%), HLA-A*01 (8.49%). Some alleles had intermediate frequency: HLA-A*26 (5.54%), HLA-A*32 (6.19%). The least frequent alleles were determined as HLA-A*25 (0.35%), HLA-A*34 (0.3%), HLA-A*69 (0.15%), HLA-A*74 (0.1%). Additionally, HLA-A*43, HLA-A*36 and HLA-A*80 was not found in the studied population. Conclusions: The data of this study suggested that HLA-typing with the methods of PCR-SSP and PCR-SSOP could imply similarities between the results for the frequent alleles of HLA-A in Iranian population with caucasoid.
Keywords: HLA-Typing, HLA-A, PCR-SSP, PCR-SSOP

378. The rs28096 Affect ERAP1 Gene Expression in Ankylosing Spondylitis Patients

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Background: Ankylosing spondylitis (AS) is an inflammatory rheumatic disease that predominantly affects the spine and may be associated with peripheral arthritis. AS has the highest association with genetics (MHC system) among all autoimmune diseases. ERAP1 has the second highest association with AS, HLA-B27 is the most associated gene. The purpose of this study was to determine effect of rs30187 and rs28096 polymorphisms on the function and expression of ERAP1. Materials and Methods: 399 patients suffering from AS and 528 healthy controls were sampled. All patients fulfilled the modified New York Criteria and were clinically examined. Expression of the gene having homozygote genotype of rs30187 and rs28096 were performed using real-Time PCR TaqMan Gene Expression comparative CT ($\Delta\Delta$CT) and cell surface receptors shedding were measured by ELISA. Results: The rs28096 G/G had higher expression of ERAP1 than rs28096 A/A (1.5 fold), this polymorphism also had a highly significant association with BASMI index of the disease. Conclusion: The rs28096 had a great effect on ERAP1 expression, on the other hand, these genotypes had association with BASMI, an objective index to determine the severity of AS. The rs28096 is located in an intron between exons 19 and 20, which are noncoding, it seems that this SNP has no effect on regulation of ERAP1expression.
Keywords: ERAP1, Ankylosing Spondylitis

379. The rs28096 G/G is Related to Higher BASMI in Ankylosing Spondylitis Patients

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Background: Ankylosing spondylitis (AS) is an inflammatory rheumatic disease that predominantly affects the spine and may be associated with peripheral arthritis. AS has the highest association with genetics (MHC system) among all autoimmune diseases. ERAP1 has the second highest association with AS, HLA-B27 is the most associated gene. The purpose of this study was to determine the association of 12 SNPs in ERAP1 with susceptibility to disease and its severity in HLA-B27 negative and HLA-B27 positive patients and also different subtypes of HLA-B27. Materials and Methods: 399 patients suffering from AS and 528 healthy controls were sampled. All patients fulfilled the modified New York Criteria and were clinically examined. All 12 SNPs of ERAP1 were genotyped using Real-Time PCR TaqMan Genotyping Allelic Discrimination and PCRSSP was utilized to genotype HLA-B27 SNPs. Results: The rs28096 G/G and rs13167972 A/G polymorphisms had a highly significant association with BASMI index of the disease. The rs27044 was associated with BASDAI in ankylosing spondylitis patients. Conclusion: Association of the rs28096 with the disease might be due to its linkage with other SNPs effective in gene regulation. This result is highly important in studying the function of ERAP1, which is the main purpose of researches who work on the genetics of ankylosing spondylitis.
Keywords: BASMI, Ankylosing Spondylitis , ERAP1

380. Introducing a Useful Monoclonal Antibody for Thrombolytic Therapy

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Background: Human plasminogen is a single-chain glycoprotein of 92 kDa, consisting of 791 amino acid residues and contains five kringles. Several monoclonal antibodies (mAb) against specific parts of the human plasminogen molecule have been developed. Materials and Methods: In this study, we investigated the effects of an anti-human plasminogen monoclonal antibody, A1D12 on Glu-plasminogen activation in presence of u-PA, t-PA and streptokinase. The kinetic activation of Glu-plasminogen by it`s activators (u-PA, streptokinase and t-PA) in presence of A1D12 following the effects of the antibody and its comparative ligands on conformational changes of two plasminogen forms by fluorescence and circular dichroism spectroscopy were investigated. Enhancing of β-structure percentage of Glu-plasminogen in presence of A1D12 according to CD-spectra study was coinciding with higher fluorescence intensity in Glu-plasminogen-FITC than Lys-plasminogen-FITC by A1D12 inducing. Results: A high similarity between EACA and A1D12 in inducing of Glu-plasminogen conformational changes was concluded. The kinetic analysis of the time course of Glu-plasminogen activation at varying concentrations of S-2251 showed that the A1D12 increased catalytic efficiency of Glu-plasminogen activation by all plasminogen activators without changing in KM values significantly. Thermal inactivation studies also confirmed the conformational change results. We suggested that the binding of A1D12 from F(ab) region to N-terminal epitope of Glu-plasminogen following conformational changes to a more open structure enhanced catalytic efficiency of activation reactions. Conclusion: It may be useful to apply clinically monoclonal antibody A1D12 for the therapy of some thromboembolic events by humanizing the F(ab) region of the antibody.
Keywords*:* plasminogen, u-PA, t-PA, streptokinase, monoclonal antibody.

381. Evaluation the Role of Fibrinolytic systemin Angiogenesis by anti-plasminogen Monoclonal Antibodies

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Background: Angiogenesis, the formation of new blood vessels from existing vasculature, is involved in normal development but also in a variety of pathologies such as cancer, atherosclerosis and ocular diseases. The plasminogen activation system has been implicated in angiogenesis. Native Glu-plasminogen is the inactive precursor of plasmin, the central enzyme responsible for fibrinolysis. This molecule is a multidomain glycoprotein, consisting of 791 a.a., that is converted tolthe serinproteinase plasmin by cleavage of Arg^{561}-Val^{562} peptide bound by plasminogen activators (PAs). New information about the molecules responsible for Angiogenesis, has led to variety of novel approaches for development of angiogenic inhibitors. Among of these approaches, monoclonal antibodirs (mAb), as biological tools have a special importance. Materials and Methods: Hybridoma cells producing the antibody were injected to Mouse. After preparation of ascit liquid, A1D12 and MC2B8 antibodies were purified. Results and Conclusion: were reported at the time of congress.
Keyword: Fibrinolytic system, Angiogenesis , nti-plasminogen, Monoclonal Antibodies

382. Detremination of Immunity Status against Hepatitis B in Staff of Hazrate Maasuomeh in Kermanshah

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Background: Hepatitis B is one of the prevalent diseases in developing countries and the major of hygienic and financial cost of those countries. Because the frequent exposure to blood components and patients body fluids, the staffs of the health and treatment services are at risk of HBV infection. HBV vaccination recommended as the preventive method in at risk group. The criterion of appropriate immune is the protective level of HBS-Ab in serum. The determination of HBS-Ab in all Hazrate Maasuomeh hospital staffs in Kermanshah helps us to take appropriate approach. Material and Methods: In this descriptive study our population was all Hazarate Maasuome staff (dependent to social insurance organization). We collected 244 blood samples and checked for HBS-Ab level with Enzyme Immuno Assay. The times and frequency of vaccination, demographic and of analyzed by SPSS. Results: 32 % (78/244) was male and 68 % (166/244) female. The most our population was in 30-39 years group (43 %).The frequency of vaccination was 98.2 % and in 74.2 % completed their vaccination and in 22.9 % the HBs-Ab level was protective. Conclusion: According to our findings the HBV immunity of the hospital staffs was in moderate level and in some situation boost dose or re vaccination recommended.
Keywords: Hepatitis B, Vaccination, Health and treatment staffs

IMMUNOHEMATOLOGY

Oral Presentation

383. Evaluation of the Relationship Between Sialic Acid Content, Protein substances and the Biological Activity of the Recombinant Erythropoietin (*Pastopoietin*), Manufactured in Pasteur Institute of Iran

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Background: Erythropoietin is a glycoprotein hormone, which is naturally secreted by the kidney cells and stimulates erythrocytes production. In a biotechnological plant, it is manufactured by the recombinant DNA technology in eukaryotic cells. In human body, this hormone is cleared by hepatocytes, so the presence of sialic acid for protection against hepatic clearance is necessary. Not only sialic acid protects the hormone against serum proteases and increase its half-life in the serum, but also helps the molecule to form correctly. Materials and Methods: In this post-prospective study, 28 batches of active pharmaceutical ingredients (API) of Erythropoietin produced during 3 years were used. The assays of sialic acid, total protein and *in vivo* biological activity on normocythemic mice were all performed. Specific activity ratio was also obtained by dividing biological activity to total protein content. Results: After statistical calculations, pearson coefficient was obtained and a good relationship was shown between biological activity and total protein (80.9%). The relationship between sialic acid content and biological activity

was determined to be 78.7%. A significant correlation (92%) was observed between the total protein and sialic acid contents. Conclusion: By improving the cell culture and purification procedures, the amount of sialic acid in the protein will increase, so the biological and specific activity will increase, respectively. This will be an important quality characteristic for registeration and marketing of this biotechnological product and while releasing each batch of the product for public administration.
Keywords: Sialic Acid, Protein substances, Biological Activity, *Pastopoietin*

384. The Recombinant N-terminal Domain of Human Platelet GPIIIa Domain Revealed Potential Immunoreactivity
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Background: Human platelet HPA-1a and HPA-1b alloantigens are determined respectively by the polymorphism Leu or Pro at position 33 of the β3 chain (GPIIIa) of the platelet integrin αIIbβ3.The HPA-1 alloantigen system, most commonly responsible for eliciting an immune response in both post-transfusion purpura and neonatal alloimmune thrombocytopenic purpura. The aim in this study was to investigate the potential ability of the recombinant 62-mer N-terminal fragment of Human glycoprotein GPIIIa in antibody binding. Such experiments can show the accessibility of the HPA-1a epitope and the relative affinity of the anti-HPA-1 antibodies to the prepared recombinant N-terminal fragments of HPA-1a/-1b (62-mer). Materials and Methods: The ability of the recombinant HPA-1a and HPA-1b to bind the related antibodies was tested using different capture methods: ELISA, using peptide-coated wells versus polyclonal antibody, peptide coupled to sepharose beads to remove anti-HPA-1a antibodies from a plasma sample, Surface Plasmon Resonance technology (SPR) to monitor antigen–antibody interaction in a real-time assay, Luminex™ using peptide-coated beads to bind a monoclonal antibody. The recombinant GST-fused HPA-1a/-1b (62-mer) fragments bound the relevant antibody in ELISA. The ability of recombinant GST-HPA-1a to remove anti-HPA-1a antibody from plasma indicated the proper conformation of the HPA-1a epitope in the expressed fragments. Results: The results showed conclusively that immobilised recombinant GST-HPA-1a could be used for the recovery of anti-HPA-1a directly from plasma in a repeatable and reproducible fashion. The activity of such purified anti-HPA-1a was then also shown in the BIAcore affinity studies. The results showed incorporation of the HPA-1 epitope in the expressed recombinant 62-mer GST-HPA-1 peptide. The ability of the SZ-21 monoclonal antibody to react with our recombinant GST-HPA-1a was a reliable sign for the presence of the Cys16-Cys38 loop. The results also provided evidence that the SZ-21 antibody can differentiate Leu33 (HPA-1a) from Pro33 (HPA-1b) when using the Luminex methodology. As, the only difference between the two recombinant proteins is the amino acid at position 33, it is suggested that the SZ-21 epitope is close to or near the Leu/Pro polymorphism. The Luminex data confirms that the SZ-21 antibody binds strongly to the recombinant GST-HPA-1a but reacts only minimally with the recombinant HPA-1a in the absence of the GST moiety. As SZ-21 antibody binding can indicate the presence and accessibility of the basic structure required for anti-HPA-1 alloantibody binding, then the GST fragment may help correct formation or accessibility to the antigenic binding site. Conclusion: In our study the GST fused HPA-1/-1b recombinant peptides were able to mimic the native HPA-1a/-1b epitope. The results showed the feasibility of using recombinant GST fused HPA-1a, as a carrier of functional epitope, for HPA-1a antibody capture.
Keywords:HPA-1, HPA-1b GPIIIa

Poster Presentation

385. Comparison of phenotype of lymphocytes in ABO blood groups
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Background: Researcher have made considerable efforts to determine the significance of particular ABO antigens to diseases susceptibility such as cancer and infection. Individuals with blood group O have been found to be at a higher risk of contracting cholera than those with other blood groups. The relationship between ABO blood groups and disease susceptibility remain unknown. The aim of this study is to determine the lymphocyte phenotypic profile in ABO blood groups. Material and Methods: Peripheral blood samples from forty health individual with different ABO blood groups (each group=10). All individuals were male with similar age and genetic background. The samples were analyzed using a FACSort flow cytometr to determine phenotype of CD3+ T-lymphocytes and their subsets CD4+ , CD8+ and Treg cells (CD4+/CD25+/Foxp3+), CD19+ B-lymphocytes and their subsets CD5+ and CD5⁻ B cells. Results: There were no significant differences in the frequency of the lymphocyte subpopulations examined (CD4+ and Treg lymphocytes and B- lymphocyte subsets) between the ABO blood groups. But, the percentages of CD8+ T lymphocytes in B blood group was higher than that other groups (p=0.07). Conclusion: High frequency of CD8+ T lymphocytes in B blood group may reduced susceptibility to some disease such as cancer and viral infections.
Keywords: ABO blood group, Lymphocytes, flow cytometry

386. Prokaryotic Expression and Purification of the N-Terminal Domain of the Platelet Membrane Glycoprotein IIIa
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Background: Platelet membrane glycoprotein IIIa (CD61, β3 integrin) is a 90-kDa single chain glycoprotein with a large extracellular N-terminal region, a transmembrane domain and a short cytoplasmic C-terminal segment. The N-terminal Plexin-Semaphrin-Integrin (PSI) domain (residues 1-54) of the β3 structure is a distinctively, solvent exposed loop which carries clinically important alloantigens. Human platelet HPA-1a and HPA-1b alloantigens are determined respectively by the polymorphism Leu or Pro at position 33 of the β3 chain PSI domain. The HPA-1 alloantigenic system is involved in two clinical conditions of Post-Transfusion Purpura (PTP) and neonatal alloimmune thrombocytopenia (NAIT). Platelet antigens incompatibility and subsequent alloimmunisation responses cause platelet destruction during pregnancy or after blood transfusion in NAIT or PTP. Localisation of the functional HPA-1a epitope to the amino terminal PSI domain of platelet GPIIIa makes this small, extracellular loop suitable for use in both antibody binding and Tolerization studies. The aim was to express the N-terminus of human GPIIIa containing the Leu^{33}/Pro^{33} polymorphism as a recombinant fusion protein whilst maintaining the natural conformation of the HPA-1a epitope. Materials and Methods: Considering the first 62 amino acids of GPIIIa as a minimum requirement for forming the HPA-1a epitope, oligos were designed for the introduction of EcoR1 and Xho1 restriction enzyme sites by PCR to facilitate the subsequent cloning steps. The HPA-1a and HPA-1b PCR products cloned into the expression vector pGEX-6P-1 (Amersham Bioscience). *E.coli* BL21 (DE3) cells were transformed. The accuracy of the peptide sequences were determined as early as the generation of the PCR products, used to create the HPA-1a gene fragments, by DNA sequencing. The N-terminal fragment of GPIIIa HPA-1 was subsequently expressed as fusion protein with GST. Fusion proteins recovered from the bacterial lysate on Sepharose 4B (Amersham Biosience). Results: On average, 15 mg in 10 ml of eluate was purified from 1 litre of bacterial culture. The HPA-1 fragment was removed from the GST fusion protein by cleavage with PreScission protease. The efficiency of cleavage was shown by detection on an SDS polyacrylamide gel of an appropriate-sized band related to the cleaved protein. The MALDI-TOF Mass spectrometry analysis was carried out on a purified GST-HPA-1a sample to ensure its identity. The peptide sequence analysis was also obtained by LC-MS/MS from the tryptic digest of the whole purified protein. The peptide mass spectra data was searched against the NCBI database with MASCOT and both GST and the HPA-1 platelet protein were identified. Conclusion: In conclusion, high-throughput expression of GST fused HPA-1 in a prokaryotic system, generated soluble recombinant proteins, without the need for reducing agents such as Urea. Furthermore, over-expression of the antigen was achieved without affecting antigen solubility. The N-terminal fragment (PSI) domain of the GPIIIa, as a fusion protein will be used for the subsequent antibody binding and tolerization studies.
Keywords: Platelet membrane glycoprotein IIIa, pGEX-6P-1, HPA-1a, HPA-1b alloantigens

IMMUNOINFORMATICS & STATISTICS in IMMUNOLOGY

Oral Presentation

387. 3-Dimentional Molecular Modeling Studies of KSHV Vox2 Protein as an Example of Immuno-Regulatory Molecule

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Background: KSHV which is the etiological agent of Kaposi's Sarcoma encodes a protein by open reading frame (ORF) K14, called vOX2. This protein shares identity with the mammalian OX2 (CD200). Signal delivery of vOX2 occurs through binding to CD200R which contributes to maintaining the homeostasis of immune responses, in a manner similar to CTLA-4 and PDL-1/-2. Due to highly glycosylation and adhesive properties of vOX2, it failed to determine the 3D-structure of the protein by X-ray crystallography method. Therefore, probable crystal structure of vOX2 has been bioinformatically identified. Materials and Methods: In order to identify the templates, BLAST sequence homology searches (NCBI) were performed. The PD-L1 (PDB entry: 3FN3) was chosen for modeling. Multiple alignment process was carried out on the selected sequences by ClustalX2 (protein weight matrix: BLOSUM series). Model building was performed in the program MODELLER9v5 using model-ligand algorithm and models at various refinement levels were generated. Finally the refined structures were minimized under molecular mechanic AMBER method (RMS gradient = 0.5) in HyperChem7.5. All models were validated using the program ERRAT and PROCHECK at UCLA. Results: The best 3D model had an Errat score of 94%. In the modelled protein all of the glycosyl-linked residues (Asn 83, 91, 138, 157 and 166) are situated in the external region of the 3D structure with steric flexibility of their side chains. Conclusion: Our modeling supports the data which indicates that vOX2 has two Ig-like domains, located between residue 11 to 125, and 131–220, respectively and the integrin-binding motif, RGD, at residues 191–193 is exposed.
Keywords: KSHV, Kaposi's Sarcoma, vOX2

388. In Silico Analysis of a Chimeric Vaccine Candidate against Enterotoxigenic *Escherichia Coli*

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Background: Enteric infections resulting in diarrhoeal disease remain a leading global health problem. Among bacteria enterotoxigenic*Escherichia coli* (ETEC) cause the largest number of diarrhoeal cases. Based on the great health impact of infections with ETEC, there is a great interest in developing an effective ETEC vaccine.An ETEC vaccine should probably contain colonization factor antigens present on the most prevalent ETEC pathogens and LT toxoid or nontoxic LTB. Chimeric proteins carrying epitopes, linkers or adjuvant sequences increased immunogenicity of the recombinant antigen and the possibility to elicit a broad cellular or humoral immune response. *In-silico* tools are highly suited for both the discovery of new and development of existing vaccines. Materials and Methods:A synthetic chimeric 1800 bp gene encoding containing CfaB, CstH,CotA ,LTB and hydrophobic amino acid linkers, was designed; the gene, named L2C3, encoded a total of 599 amino acids. The gene was modified with regard to codon usage to optimize gene expression in *E.coli* and plant system.Modeling was done to predict the 3D structure. This model was validated with the program PROCHECK using Ramachandran plot statistics .The predicted B-cell epitopes were mapped on the surface of the model.Results:A chimeric gene containing colonization factors and LTB was designed. Tertiary structure was predicted and evaluated. Validation result showed that 97.2 % residues lies in favored and additional allowed region of Ramachandran plot.VaxiJen analysis of protein showed high antigenicity.Linear and conformational B-cell epitopeswas identified.T-cell identified epitopes were expected to bind MHC molecules.
Conclusions:The chimeric protein has epitopes that are likely to induce both the B-cell and T-cell mediated immune responses. The chimeric protein could be produce in microcapsulated form or in plant system and has potential as a valuable vaccine candidate for oral immunization against ETEC.
Keywords: Chimeric Vaccine, ETEC, Chimeric proteins

389. In Silico T-cell and B-Cell Epitope Prediction in MLV Pseudotype Virus with VSVG for Epitope Based Vaccine Design against Rabies Virus

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Background: The conventional approaches to develop vaccines are killed, live or inactivated organisms. The problems with this approach are that many of the proteins are not necessarily expressed in vitro, meaning good candidate antigens can be overlooked and it might not be possible to cultivate a particular pathogen in the laboratory, moreover, conventional approaches require longer times for identifying candidate antigens as targets. The development of epitope-based vaccines is one example of reverse vaccinology. These peptide epitopes represent the minimal immunogenic region of a protein and allow for precise direction of immune responses. A critical requirement of epitope based vaccine design is the identification and selection of T-cell and B-cell epitopes. Materials and Methods: We have produced MLV pseudotype virus with vesicular stomatitis virus glycoprotein (vsvg). We retrieved the sequence of vsvg from NCBI database. A BLAST search, subcellular localization, T and B-cell epitope prediction were performed for vsvg. Results: Homologus proteins obtained by BLAST studies were specific for vsvg. Prediction of subcellular localization of protein and presence and location of signal peptide cleavage sites that were performed using PSORT b,TMHMM, Subloc and Signal P respectively, confirmed the protein to be extracellular and transmembrane , with a signal peptide between positions 16 and 17. Characterization of epitopes on vsvg with BcePred and NetCTL-1.2 servers identified four peptide sequences NQKGNWKNVPSNYHY, PSVEQCKESIEQTKQ, TVHNST TWHSDYK and CPEGSSISAPSQTSV and three peptide sequences of ELWDDWAPY, VLRTSSGYKF and SWKSSIASFFF that are B-cell and T-cell epitopes respectively, are good candidate epitopes for vaccine design against rabies virus. Conclusion: The present study is a computational approach for identification of candidate T-cell and B-cell epitopes from vsvg using immunoinformatics tools. We report seven epitopes which have good binding affinity for MHC and antibody molecules. However, these should further be tested by lab studies for a targeted vaccine design against rabies virus.
Keywords: MLV Pseudotype Virus, VSVG, Rabies Virus

390. A Computational Study of Novel Fusion Protein (Cfab-LTB) for Use as ETEC Plant Based Vaccine

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Background: Computational studies were performed to ensure that the epitopes of the constructed novel CFA/I-LTB fusion protein were not changed during the linkage of the two molecules by 3 Dimensional structures of CfaB-LTB fusion protein and prediction of its epitopes. Materials and Methods: Protein homology based modeling was carried out using MODELLER 9 V7 software. For CfaB-LTB fusion protein homology modeling, the 3F83, 3F84 and 3F85 and the 1B44, 1LTR were selected as templates, respectively. Template models were generated for each segment of fusion protein construct based on the one template procedure of MODELLER software, and for fusion protein the multiple template procedure of MODELLER software was used. After constructing the three structures (i.e. CfaB, LTB, and CfaB-LTB), the epitopes

propensity was predicted using Prediction of Antigenic Epitopes on Protein Surfaces by Consensus Scoring (EPCES) service at Chi Zhang's systems biology lab University of Nebraska-Lincoln (BMC Bioinformatics. 2009 Sep 22;10:302). In addition, the possibility of the N-Glycosylation presence in CfaB-LTB was examined by NetNGlyc 1 server. Results: Based on computational studies, secondary structure models of CfaB, LTB and CfaB-LTB fusion protein were predicted. There was no difference between the <90 EPCES score of CfaB antigenic epitopes in the fusion and independent form, indicating no significant dissimilarities between epitopes of the fusion and independent structures of CfaB. Software based figures and data predicted an independent form of LTB. The antigenic epitopes with <95 EPCES score were 59%, and these epitopes in fusion form were decreased to 53%. This may indicate that a few LTB epitopes with <95 EPCES score (%6) in the fusion structure were altered. As shown, the fusion protein may have undergon N-glycosylation in asparagines residues (residue 27, 96, 157 and 286) but these glycosylations were occurred in antigenic epitopes with low (50 EPCES) score, suggesting that the probable N-glycosylation has happened in protein regions that were not predicted as main epitopes. Conclusion: Taken together, our results propose that our novel CfaB-LTB fusion protein as a plant based vaccine may be suitable for vaccination against ETEC infection.
Keywords: Cfab-LTB, ETEC Plant, Vaccine

391. Nonparametric Simulation of Signal Transduction Networks with Semi-synchronize Update: A Modular Oriented Approach

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Dynamic modeling provides a modeling approach, to test a hypothetical signaling network, to assess and interpreting signal transduction. In this study, we developed the computational framework to describe the profile of evolving process and the time course of the proportion of active form of molecules in signal transduction networks. This continuous model, do not require biochemical or experimental parameters in order to capture the system dynamics. The activity of nodes will change step by step acceding to the specific function. During the iteration nodes are updated to their new values in a semi-synchronous manner. We also incorporate the possibility of perturbation experiments on model. Validation on four signaling networks shows that this model can effectively uncover the quantitative (proportion of active molecules) and qualitative (trends of responses) properties of signal transduction process and results were in agreement with the results of experimental studies. This model is an efficient procedure for analysis of signaling networks where parametric modeling is impractical because of the scarcity of known details and kinetic parameters. We place particular focus on the evaluation and demonstrate the correlation between simulation results and experimental in four examples. The results serve as reliable predictions, provide insights into signal transduction mechanisms and can guide experimental follow-up studies.
Keyword: Nonparametric, Signal transduction, Modular oriented

Poster Presentation

392. Available Allergenic Databanks

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Background: Immunoinformaticsis one of the branches of bioinformatics which has main role in management and analysis of immunological information based on immunological databank information. Immunological databanks prepare to data access, derivation and analysis of these data. The efficiency of databanks information which is used for assessment of engineered protein biosafety is related to nature and capacity of data bank which has been used. In recent years, some databanks have been developed for assessment of protein's safety based on specific goals. These tries have caused databanks with different accession ability, organization and information content. Materials and Methods: For accessing to Immunoinformatics data the available databank such Evaller, WebAllergen, AllerTool, AlgPred, Allermatch, ALLERDB, SDAP, ADFS, FARRP, AllergoPharma, InformAll, Protall, CSL, Allergome, IUIS and AllAllergy are used.
Results and Conclusion: These differences have caused some limitations on users for accession, transfer, derivation and mix of data. Hence, user's awareness of the goals of designers or available tools within each databank can help researchers for optimum utilization of available data. In current study, we reviewed available allergenic databanks on the web.
Keywords: Available, Allergenic, Databanks

393. Evaluation of Allergencity of Cry Proteins Using Allergenic Databanks

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Background: The family of Cry proteins has been considered because of their resistance induction to some important crop pests. Hence, evaluation of food immunity of these proteins based on in vivo, in vitro and in silico methods is necessary. Allergenic databases can estimate protein's allergenicity by different algorithms. Materials and Methods:In current study allergenicity-research of 199 Cry proteins was studied by available searching methods in five allergenic databases including Allermatch, AlgPred, IEDB, Evaller and SDAP. Results: The results of six continuous amino acids method in Allermatch database showed that these proteins are allergenic. Because of the occurrence of six identical contiguous amino acids accidentally, obtained results was tested by a further method including eight contiguous amino acids. Allergenecity of these proteins was disapproved based on obtained results by the further methods. Moreover, for additional study, allergenicity of 199 Cry proteins was investigated by advanced allergenecity search methods in four databases (AlgPred, IEDB, Evaller and SDAP). The results of advanced search methods showed that investigated proteins were non-allergenic proteins. Conclusion: Based on the current results and our knowledge about allergenic sequences and structures, we can conclude that these Cry proteins are non-allergens due to absence of sequential and structural identities to known allergenic sequences and epitopes.
Keywords: Allergencity, Cry Proteins, Allergenic Databanks

394. *In Silico* Prediction for Identifying, HLA-DR Binding Hotspot and Epitopes of *Helicobacter pylori* Urease B Subunit Protein

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Background: Currently *Helicobacter pylori* is recognized as the most widespread human pathogen and approximately half of the world's population is infected. Thisinfection is the most important cause of gastritis, peptic ulcer disease and gastric MALT lymphoma, and also a risk factor for the development of gastric adenocarcinoma. There is great interest in developing a vaccination method to prevent *H. pylori* infection. Protective immunity against the gastric carcinogen *H. pylori* is considered to be $CD4^+$ T-cell dependent. Therefore, the ideal *H. pylori* vaccine

should comprise a well conserved and characterized *H. pylori* antigen(s), which will trigger mainly Th1-like immune responses in the stomach of vaccinated hosts without any harmful effects. So we suppose that triggering the immune response different from that triggered by natural infection and constructing the vaccine antigen different from native antigen may be effective to combat against *H. pylori*. This goal probably can be achieved by promoting different immunity with *H. pylori* epitopes. The aim of this study was identification of the $CD4^+$ T cell specific epitopes derived from *H. pylori* urease B subunit protein by Immunoinformatic software. Materials and Methods: *In silico* experiments was performed to predict potential Class II HLA-DR binder of *H. pylori* UreB which can stimulate $CD4^+$ T cells as a hotspot by using the MULTIPRED and Hotspot Hunter. Results: Two potential hotspot epitopes of about 20 amino acids (P404-423 and P444-463) in length were selected according to the results of prediction software. These were consensus among UreB sequences of at least 4 strains. Future studies will need for *in vitro* and *in vivo* evaluating of these peptides. Conclusion: In conclusion, we have defined two potential hotspots in UreB protein of *H. pylori* (P404-423 and P444-463). These data will provide much information for further study of the epitope-based vaccine for human use.
Keywords: *In Silico*, HLA-DR Binding, *Helicobacter pylori*, Urease B

395. Peptide Antigen Design on Basis of B-Cell Epitope Prediction for Production of Monoclonal Antibody against Cow's Milk Beta-Lactoglobulin

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Background: Beta-lactoglobulin (BLG) is one of the major allergens in cow's milk. Monoclonal antibodies against this protein are important for its characterization, localization and purification. Synthetic peptides are often used to gain monoclonal antibodies. For this purpose, bioinformatics tools were employed for B-cell epitope prediction and the sites of molecules that are recognized by antibodies of the immune system were detected to design potential peptide antigens. Materials and Methods: Several server applications such as Discotope, Ellipro, Epitopia were used for B-cell discontinuous epitope prediction and their results were compared. Prediction was conducted according to protein structure with default setting of each server. Result: One discontinuous epitope was selected as candidate for production of monoclonal antibody based on the results obtained from different software tools. This epitope were in the first domain of BLG. Conclusion: Monoclonal antibodies against BLG are useful tools. However, construction of monoclonal antibodies is expensive and time-consuming. Hence, designing synthetic peptides with potential antigenic properties to induce the immune response in mice, by means of bioinformatics tools is critical for obtaining high-quality monoclonal antibodies.
Keywords: Monoclonal Antibody, Beta-Lactoglobulin, Bioinformatics

396. Computational Prediction of a Mutation in the a Chain of the Hgm-CSF Protein Affects Its Binding Affinity to Its Cellular Receptor

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Background: Human Granulocyte-macrophage colony-stimulating factor (GM-CSF), a glycoprotein with molecular weight of 14.477 kD comprises 144 amino acid residues. The encoding gene of this glycoprotein is located on chromosome 5 in human. N-terminally seventeen amino acids are serving as a signal peptide and the rest of 127 amino acids are known as molgramostim. GM-CSF acts on mature macrophages, eosinophils, and neutrophils to stimulate various functional activities. Bioinformatics data have revealed a high affinity of this protein for binding to a heterodimer receptor on surface of the cell. The respective receptor includes α and β chains which the β chain is similar to interleukins 3 and 5 receptors. Due to this similarity, interleukins 3 and 5 are able to compete with GM-CSF in binding to the receptor. Materials and Methods: In the present study we have predicted a mutation in glutamic acid 21 substituting with arginine using Molegro software. In the present study, to compare binding affinity of native GM-CSF and mutant GM-CSF to the related receptor, a computational prediction study carried out using Modeller, Hex and Molegro softwares. Results: According to this study, results showed that native GM-CSF with 301.20 kJ/mole and mutant GM-CSF with -535.04kJ/mole energy binds to the α chain of receptor. Conclusion: As a conclusion, this mutation enhanced binding affinity (kcal/mol) of GM-CSF protein to α chain of GM-CSF receptor.
Key words: GM-CSF, bioinformatics, docking, mutation, receptor, immunology

397. B-Cell Epitopes Prediction of Kiwi Fruit Actinidin by Bioinformatics Tools

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Background: Allergy to kiwi fruit was first described in 1981, and there have since been reports of the allergy presenting with a wide range of symptoms from localized oral allergy syndrome (OAS) to life-threatening anaphylaxis. According to earlier studies, at least 12 IgE binding proteins (allergens) could be detected in kiwi extract. One of the most important allergens of kiwi fruit is Act c 1, a protein of molecular mass about 30 kDa, commonly known as actinidin is a cysteine protease related to papain. There is clear evidence that green kiwi fruit can elicit allergic cross-reactions in some individuals with allergies to natural latex, other fruits and specific pollens due to similarities between kiwi fruit proteins and hevein-like proteins or chitinases from those sources. Materials and Methods: The amino acid sequence of Kiwi Act c 1 (accession number: P00785.4) was fetched from National Center for Biotechnology Information (NCBI). B-cell epitopes of protein were predicted by several web servers including BepiPred, BCPREDS and ABCpred. Results: Sequences of the consensus epitopes are TEENYPYTAQDGE (position 211-223) and AFSMSKDGPVGVDDGQR (position 361-377) with the best predicted binding affinities. Conclusion: Act c 1 has been identified as an important cross-reactive allergen for patients suffering from allergy. So the determined peptides are useful for further vaccine development because they can reduce the time and minimize the total number of required tests to find the possible proper epitopes, the target for vaccine development.
Keywords: B-Cell Epitopes, Kiwi, Actinidin, Bioinformatics

398. Bioinformatics Analysis for Allergenicity Assessment of CP4 epsps Proteins in Glyphosate Resistant Crops

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Background: Nowadays, genetic engineering researchers increase food and agricultural products through the production of transgenic plants seriously. Transgenic plants have many characteristics such as resistance to herbicide, pests, water stress, salinity and etc. Genetic engineering researches after commercialization introduce the consumption cycle. One of the most important issues in the consumption of GM products is to ensure safety, health and non-allergens. About 80 percent of commercial transgenic plants have herbicide resistance genes. CP4 epsps gene is one of the important genes for herbicide (Glyphosate) resistance in transgenic plants such as soybean, corn, canola, cotton, sugar beet. Glyphosate (*N*-(phosphonomethyl)glycine) is non-selective and is abroad-spectrum herbicide. Glyphosate Inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyzes the reaction of shikimate-3-phosphate (S3P) and phosphoenolpyruvate to form 5-enolpyruvyl-shikimate-3-phosphate (ESP) and prevents shikimate pathway which produces phenylalanine, tyrosine and tryptophan. CP4 epsps gene causes shikimate pathway in another manner as the process mediate by phosphoenol pyruvate (PEP). In this research, allergenicity of CP4 epsps protein was studied. Materials and Methods: Protein encoded by gene CP4 epsps, contains 455 amino acids. This sequence was aligned using the FASTA program in protein databases FARRP, SDAP, Algpred. Sequence alignment was performed using the allergen proteins in three matches including: the full sequence matching sequence, matching the 80 amino acids and eight amino acids. Results: The results showed no similarity

between CP4 epsps protein and allergen proteins in the full sequence matching. Matching the 80 amino acid (Domain) in the SDAP database showed three little similarity (35, 36 and 37/25) which were not confirmed in the Algpred for Mapping of IgE epitopes search. Matching an 8 amino acids showed no similarity to determine the epitope potential. Conclusion: We conclude that CP4 epsps protein probably no allergenic potential.
Keywords: *In silico* assessment, Allergenicity, Glyphosate, CP4 epsps

399. Using Human Coxsackie Virus B4 Epitope Prediction for Designing a Novel Type I Diabetes DNA Vaccine
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Background: Coxsackie virus a nonenveloped, linear, positive-sense ssRNA belongs to entroviruses which can cause autoimmune diseases such as type I diabetes. The structure of this virus resembles to the human glutamic acid decarboxylase (GAD). GAD is a pyridoxal 5'-phosphate-dependent enzyme that catalyzes the decarboxylation of glutamate to GABA (gamma-Aminobutyric acid) and CO_2. There are two isoforms of GAD that were encoded by two different genes (GAD1 and GAD2) in mammalian tissues: a 65 kDa form (GAD65) and a 67 KDa form (GAD67). Sequence similarity betweenGAD65 and coxsackievirus B4 includes 9 identical amino acid residues. It is supposed that coxsackie virus B4 has role in inducing type I diabetes. Therefore, it is possible to predict human Coxsackie Virus B4 Epitope for designing a novel type I diabetes DNA vaccine. Materials and Methods: To predict virus continuous epitopes ABCpred and BepiPred softwares were used and the results were compared with each other. Results: Data obtained from the study of viral epitopes and GAD protein showed the slightest resemblance between them. The peptide sequence 1137-1145 (EVKEKHEFL) in CVB4 P2C was similar to the peptide sequence 261-269 (EVKEKGMAA) in GAD65. These sequences in CVB4 bind with high affinity to HLA-B8 and with intermediate affinity to HLA-A2.1. The parts of these sequences were detected as epitopes with using above servers. Therefore these regions can't used for DNA vaccine design. Conclusion: Because of amount of homology between Coxsackie virus and GAD 65 these sequences isn't suitable for designing DNA vaccine against this virus.
Keywords: Coxsackie virus B4, type I diabetes, glutamic acid decarboxylase

400. B-cell Epitope Prediction for HIV-1 Matrix Protein p17
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Background: HIV-1 matrix protein p17 is a structural protein that is involved in the virus life cycle main stages. For example participate in the early stages of virus replication thus RNA targeting to the plasma membrane, incorporation of the envelope into virions and particle assembly. Besides its well established role in the virus life cycle, p17 may also be active extracellularly in deregulating biological activities of many different immune cells that are directly or indirectly involved in AIDS pathogenesis. Thus, p17 might represent a capable target for developing a therapeutic vaccine as a involvement to combating AIDS. Materials and Methods: Several server applications such as BCpred, Ellipro, Bepipred, ABCpred were used for B-cell linear epitope prediction and their results were compared. Prediction was conducted according to sequence of protein with default setting of each server. Results: One linear epitope was chosen based on the results obtained from different servers. This epitope is located in the sequence 68-74. Conclusion: In recent years many efforts have been done to design a vaccine against HIV-1 virus. Using Bioinformatics tools is useful because of its low cost and accessibility. Knowledge of B-cell epitopes may be used in the design of vaccines and diagnostics test. These servers assign a tendency value to every amino acid, based on studies of their physico-chemical properties.
Keywords: B-cell Epitope, HIV-1 Matrix Protein p17, AIDS

401. Antigenic Epitopes Prediction and MHC Class I Binder of Beta Toxin Clostridium Perfringenes (CBP) by Bioinformatic Tools
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Background: Beta-toxin (CBP) is one of the lethal toxins of Clostridium perfringens types B and C. Beta toxin, cause fatal diseases originating in the intestines of humans or livestock. Vaccine production and vaccination are very costly. Due to this problem the Scientists try to find simple and effective method of bioinformatics for predicting immunization peptids. Predicted MHC binding regions act like red flags for specific antigens and generate an immune response against the parent antigen. So a small fragment of antigen can induce an immune response against whole antigen. This them is implemented in designing subunit and synthetic peptide vaccines. Materials and Methods: Considering that the three-dimensional structure of protein CBP is not available. Using the Immune Epitope Database (IEDB) and other tools that includes algorithms based on primary sequence protein are predict epitopes. In this study, we analyzed secondary structure and antigenic determinants, which form antibodies against infection. The method integrates prediction of peptide MHC class binding, solvent-accessiblity and flexibility. Also usingthehomologymodelingwe showedepitopes of CBP in theThree-dimensional structureof protein. Results: There are 10 antigenic determinants in sequence CBP. These peptides are consensus output results of all softwares. The results show highest pick at position 5-29 (ISLVIVSSLLNGCLLSPTLVYAND), 80-91 (IDDKYSSEMTTL), 104-119 (DVIKKYNLHDVTNSTA), 253-279 (YQMSKLITGGLNPNMSVVLTAPN), 293-308 (YYLNWNGANWVGQVYS) amino acid residue. Conclution: Identification of antigenic sites on CBP are of vital importance for developing synthetic peptide vaccines, immunodiagnostic tests and antibody production.
Keywords: MHC Class I Binder, CBP, Bioinformatic Tools

IMMUNOLOGY & CLINICAL LABORATORY

Oral Presentation

402. Most Frequent Genotype of HBV in South Iranian Blood Donors
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Background: Hepatitis B virus (HBV) is one of the leading causes of acute and chronic liver disease worldwide which is responsible for a million of deaths annually. Based on an inter group divergence of 8% or more in the complete nucleotide sequence the genomes of virus has been classified into eight groups, designated as A to H. These genotypes show different geographical distribution. Some genotypes are associated with different clinical outcomes and particular viral mutations. The aim of this study was to investigate the genotype of pattern and prevalence of HBV in blood donors of south of Iran. Materials and Methods: In this experimental study we investigated the prevalence of HBV positivity in 198289 volunteer blood donors from south of Iran in Isfahan, Yard and Kerman provinces by both ELISA and PCR based methods. Among the donors 120 $HBsAg^+$ donors were selected. The HBV DNA was extracted using commercial kit and the p gene sequences were amplified by nested-PCR. HBV genotypes were determined by direct sequencing of the polymerase gene of HBV. Phylogenetic trees were constructed by the neighbor-joining (NJ) method. Results: findings of this study indicated that the rate of HBV positivity was 0.184%, 0.329% and 0.215% blood donors of

Esfahan, Kerman and yard provinces, respective. Sixty nine (57.5%) out of the 120 $HBsAg^+$ donors, were positive for HBV DNA. Sequence analysis was done on positive samples and the D was genotype detected among HBV infected donors.
Conclusions: The prevalence of genotype D was 100% in this study. These findings may have an impact on the immunological and genetic diagnosis of HBV, selection of diagnostic kits and viral quality control (VQC) panels to evaluate diagnostic methods.
Keywords: HBV, Blood donor, Genotype, Iran

403. A Sensitive Enzyme-Linked Immunosorbent Assay for Detection of Hepatitis B Surface Antigen

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Background: Hepatitis B virus (HBV) infection isthe 10th leading cause of death worldwide. The most important diagnostic and screening marker for HBV infection is hepatitis B surface antigen (HBsAg), and the most widely used HBsAg screening test is enzyme-linked immunosorbent assay (ELISA). Materials and Methods: In this study, an ELISA assay has been developed for detection of HBsAg using two novel monoclonal antibodies (mAb) as capture layer and a polyclonal biotinylated Ab as detector phase. We evaluated the sensitivity, specificity, detection limit, seroconversion time, positive and negative predictive values and reproducibility of our assay with standard panels and different serum samples. Results: The results were compared with a well established commercial kit. Both assays showed similar detection limit values of 0.5 to 0.7 ng/ml and the same seroconversion periods of 42 and 65 days for “ad” and “ay” serotypes of HBsAg, respectively. Sensitivity and specificity of the assay were 98.98 % and 99.6%, respectively. The positive and negative predictive values of our assay were also calculated as 99.49% and 99.2%, respectively. Analysis of reproducibility of the present assay demonstrated 3.96% and 5.85% intra-and inter-assay coefficient of variations, respectively, which were less than those obtained by the commercial kit. There was a highly significant correlation between our designed assay and the commercial ELISA kit ($p<0.0001$, $r = 0.957$). Conclusion: Altogether, our results indicate that the designed assay is comparable to the commercial kit in terms of sensitivity, specificity, positive and negative predictive values and reproducibility and could be employed for diagnosis of HBsAg in blood samples.
Keywords: ELISA, HBV, Surface Antigen

404. Defective Expressions of TRIF in Iranian Chronic HBV Infected Patients

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Background: TRIF is of the main intracellular adaptor protein for TLR3 and 4 signaling. Mal-expression of the molecule may lead to an defective immune responses against viral infections including hepatitis B infection. Thus, the aim of this study was to identify the mRNA levels of TRIF in the PBMCs isolated from chronic HBV infected (CHB) infected patients. Materials and methods: This study was undertaken on 60 CHB patients and 60 healthy controls and the mRNA levels of TRIF were examined in parallel with beta-actin (as housekeeping gene) using Real-Time PCR technique. Results: Our results demonstrated that expression of TRIF was significantly decreased in PBMCs isolated from CHB patients in compare to healthy controls. Conclusions: Based on the current results, it seems that CHB patients are unable to express TRIF gene firmly and in turn properly TLR3 and 4 signaling subsequent to HBV infection. Therefore, our results suggest a probable mechanism which almost partially may define a reasonable fact that why the infection is stable in the CHB patients.
Keywords: Chronic HBV infection, TRIF, Real-Time PCR.

405. CD93 is Highly Expressed on Myeloma Cells: a New Marker for Diagnosis of Myeloma by Flow Cytomeyery

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Background: CD93 is a highly glycosylated transmembrane protein which plays as one of the C1q receptors, is expressed on monocytes, neutrophils, endothelial cells, and stem cells. We have recently demonstrated that CD93 is strongly expressed on human hematopoietic stem cells from bone marrow, umbilical cord blood as well as mobilized peripheral blood stem cells (Transfusion 2010:50;2002). In addition, recent studies have shown that CD93 is expressed on B lymphocytes during their early development and is downregulated along B cell maturation. However, it was demonstrated that CD93 is expressed on mice plasma cells (PC) particularly long-lived PC. Moreover, animal studies have shown that CD93- deficient mice are impaired in antibody secretion and the number of plasma cells in the bone marrow, indicating that CD93 is crucial for maintenance of plasma cell in the bone marrow. However, the expression of CD93 on myeloma cells as long-lived malignant plasma cells have not been yet studied. Materials and Methods: First, by employing flow cytometry and RT-PCR we examined the level of CD93 on a myeloma cell line, U266, and found that it highly expressed on this cell line. Next, to determine the expression of CD93 on primary myeloma cells from patients with multiple myeloma, a three-colored flow cytometry analysis was performed and bone marrow samples were stained with anti-CD93-PE, Anti-CD38-PerCP and CD45-FITC. Results: We observed that CD93 is highly expressed on myeloma cells, $CD38^{hi}/CD45^{low}$ cells. Conclusion: We are demonstrating for the first time that CD93 is expressed on myeloma cells and that CD93 could be new marker for diagnosis of multiple myeloma. However, further experiments such as determining of CD93 in CD138 positive cells (sorted by magnetic beads) by RT-PCR and flow cytometry are currently on going in the Lab.
Keywords: CD93, Myeloma Cells, Flow Cytomeyery

406. Comparison of Diagnostic Value of Procalcitonin (PCT), Interleukin-6(IL-6) and CRP in Rapid Diagnosis of Neonatal Sepsis

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Background: Early diagnosis of neonatal sepsis is essential for the successful treatment and prevention of sepsis outcomes. A reliable marker existence is useful for early detection of neonatal sepsis. Blood culture is a gold standard but the final results are not available until up to 48-72 hours after collection.Curently C-reactive protein is in use for this purpose, but CRP is not a reliable early marker of infection because the level of this marker is low in the first 12 hours of sepsis. Thus, in the present study, we attempted to find new laboratory diagnostic markers for early diagnosis of neonatal sepsis. In recent years measurement of procalcitonin has been reported as sensitive parameters for the early diagnosis of neonatal sepsis. Interleukin-6 (IL-6) as a marker plays a critical role in the induction of C-reactive protein (CRP) synthesis in the liver,it was hypothesized that IL-6 and PCt could be detected earlier in blood than the CRP during the course of neonatal sepsis. Materials and Methods: Blood samples were collected at admission from 69 neonates with suspected infection. Patients were included in 2 different groups according to bacteriological and laboratory results: Group I consisted of 20 newborns' with positive blood cultures and other biological tests which suggests infection. Group II, 49 neonates with negative blood cultures but had two or three of clinical singes of sepsis. The control group(group 3) included 10 healthy neonates with no clinical and biological data of infection. CRP was determined by Nephlometry method, IL-6 was determined by Elisa method and PCT measured by Immunoluminometric assay. Results: mean levels of CRP before therapy were significantly higher in septic neonates (group I)than the levels in infants in the other two groups(23.16 mg/l versus 9.31 and 4.22). Sensitivity and specificity

and negative predictive value of CRP were 45% and 95% and 62%respectively (P Value<0.005). Mean levels of IL-6 were 119.26 pg/mL in group I neonates which was that higher than the other groups (P value< 0.005). With 85% sensitivity and 100% specifity and 87% negative predictive value. Mean levels of PCt were 5.70ng/ml in proved sepsis group which was high than the other groups (P value <0.05). Sensitivity, specificity and negative predictive value of PCT were 70% and 80% and 75% respectively. Conclusion: According to our results, measurement of IL-6 for early diagnosis of neonatal sepsis especially before in the first 24 hours of sepsis onset is more useful than CRP. The sensitivity of procalcitonin(75%) is higher than CRP(45%) for diagnosis of neonatal sepsis and PCT appears to be a useful marker for the severity of infection, while IL-6 appear to be as superior diagnostic sepsis marker compared to PCT and CRP(with high sensitivity and specificity and negative predictive value). It is concluded that evaluation of PCT and IL-6 in combine with CRP can be ideal tests for early diagnosis of neonatal sepsis.
Keyword: Procalcitonin, Interleukin-6, C-reactive protein, Neonatal sepsis

Poster Discussion Presentation

407. The Evaluated Of Relative Potency OfHuman Tuberculin Skin Test (Produced by Razi Vaccine and Serum Research Institute) in the Guinea Pigs Sensitized with *M.tuberculosis, M.bovis* BCG and *M.avium*

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Background: The tuberculin skin test is the most commonly used test for diagnosing TB. The basis of tuberculin testing is the induction of a delayed hypersensitivity reaction to the intradermal injection of tuberculin. Unfortunately, this test is incapable of distinguishing *Mycobacterium tuberculosis* (MTB) infection from bacille Calmette-Guérin (BCG) vaccination or infection with non-tuberculous mycobacteria. The aim of this study is to evaluate the relative potency ofhuman tuberculin skin test (produced by Razi Vaccine and Serum Research Institute) in the guinea pigs sensitized with *M.tuberculosis*, *M.bovis* BCG and *M. avium.* Materials and Methods: For skin test, different groups of guinea pigs were sensitized with *M. tuberclusis*, *M. avium* and *M.bovis* BCG. guinea pigs were injected intradermally with 0.1 ml of 10, 2 and 0.4µg/ml of PPD. Skin reactions (diameters of erythema, in millimeters) were independently measured 24 h after injection and Results were performed and calculated. Results: The resultsshowed that the relative potency of PPD for guinea pigs sensitized with *M.bovis* BCG in compare of guinea pigs sensitized with *M. tuberclusis* was reported as 107 (confine 80-125) and for guinea pigs sensitized with *M. avium* was reported as 767 in potency test. Conclusion: This study demonstrated that the human tuberculin skin test produced by Razi Vaccine and Serum Research Institute is more specific to diagnosis of *M. tuberclusis* andBCG vaccine than *M. avium* infection.
Keyword: human tuberculin skin test, tuberculosis, *M.tuberculosis* , *M.bovis* BCG and *M. avium*

408. Is Quantitative CMV IgM Assessing an Informative and Confirmatory Serologic Marker for Diagnosis of Primary Infection in Pregnant Women Population

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Background: Diagnosis of an active CMV infection is hampered by the fact that clinical symptoms are non-specific and may not distinguish it from other infection .The Diagnosis of CMV infection therefore is dependent on laboratory parameters, i.e.isolation of virus from clinical samples such as blood, urine and saliva, and the detection of a specific antibody response. Materials and Methods: In the period between April 2003 and December 2006 125 pregnant women longitudinally studied (CMV-ELISA) with respect to their humoral immune response against CMV and followed. Sera were obtained on the day of attending, if were positive for IgM, then taken at weekly intervals thereafter for at least 3 months. Qualitative and quantitative IgM assays were done simultaneously and compared. Results: of 125 cases studied 49 (%31) were seronegative and 54 (%44) were seropositive. of 49 seronegative, 15 (31%) contracted a primary CMV- infection which was diagnosed by ELISA test , in all of 15 and a subsequent humoral immune response, a quick rise of IgM,reached maximum values of 40-655 U within 2-3 weeks and gradually declined thereafter. Of 54 seropositive 35 (65%) had a secondary CMV infection. Of these, 31 were diagnosed by both qualitative and quantitative assays, and significant increase in IgG and IgM, while remaining four, repeatedly had IgM quantitative negative ,but qualitative positive , which could be resulted of false positive reactions . Conclusion: We conclude that quantitative determination of CMV antibodies together with periodically following up of qualitative IgM positive cases can give good indications about a patient's capability to surmount CMV infection.
Keywords: Primary infection, Serologic tests, Comparison

409. Tumor Markers (CEA, CA 125, CYFRA 21-1, SCC and NSE) in Patients with Non-Small Cell Lung Cancer as an Aid in Histological Diagnosis and PrognosisComparison with the Main Clinical and Pathological Prognostic Factors

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CEA, CA 125, SCC, CYFRA 21-1 and NSE were prospectively studied in 211 patients with non-small cell lung cancer and compared with clinical parameters (age, sex, Karnofsky Index, symptoms and smoking status), histopathological parameters (stage, histology, tumor size and nodal involvement), biological parameters (LDH and albumin) and the therapy used (surgery, chemotherapy or radiotherapy). Tumor marker sensitivity was CYFRA 21-1: 76%, CA 125: 55%, CEA: 52%, SCC: 33% and NSE: 22%. One of the tumor markers was abnormally high in 87% of the patients with locoregional disease and in 100% of the patients with metastases. Except for NSE, all tumor markers showed a clear relationship with tumor stage and histology and therefore enabled a better histological diagnosis. Abnormal CEA serum levels were mainly found in adenocarcinomas, CA 125 in large-cell lung cancers (LCLC) and adenocarcinomas and SCC in squamous tumors. Eighty-five percent of the patients with SCC levels >2 ng/ml had squamous tumors. Likewise, CA 125 levels <60 U/ml or CEA <10 ng/ml excluded adenocarcinoma or LCLC with a probability of 82 and 91%, respectively.
Keywords: CA 125, CEA, CYFRA 21-1, NSE, SCC, Non-small-cell lung cancer

410. Correlation of Antinuclear Antibody Immunofluorescence Patterns with Patients Referred for ANA Testing in West Azerbaijan, Northwest of Iran

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Background*:* Antinuclear antibody tests (ANA) have played a vital hallmark in the diagnosis and prognostic in several autoimmune disorders. Immunity status, individual response to disease and types of antibodies produced are well known to vary from person to person, population to population and probably from place to place. The aim of this study understands a definite association between ANA patterns and specific antibodies in the serum of patients referred for ANA testing by Indirect Immunofluorescence method in Medical of Aryan laboratory. Materials and Methods: This study has been performed on 512 patients. Serum samples of patients for rheumatic disease were subjected for ANA testing by indirect immunofluorescence method. Serum samples were processed in dilution of 1:100 using HEp-20-10 cells liver biochip (Monkey) (EUROIMMUN AG). Results: The antinuclear antibody indirect immunofluorescence patterns encompassed a certain spectrum of specific antibodies such as Homogenous (622%), Anti-mitochondria pattern (16.2%), Nucleoli homogenous pattern (5.4%) and Nuclear dots (16.2%). Conclusion: The presence of ANA is a hallmark of autoimmune disease. In this study is focused on the Correlation of antinuclear antibody immunofluorescence patterns, which play a crucial role in pathogenesis and follow up of rheumatic diseases.

Keywords: Antinuclear antibody tests, Correlation, Antinuclear Antibody Immunofluorescence Patterns

411. Diagnosis of Acute Appendicitis: Usefulness of Serum Level of C-reactive Protein

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Background: Accurate diagnosis of acute appendicitis is a common problem in emergency medicine. The accuracy of the clinical diagnosis of acute appendicitis is not always possible and many cases of over- or under-diagnosis succumb to resulting complications. The aim of this study was to evaluate the accuracy and cutoff points of the serum level of C-reactive protein (CRP) in the diagnosis of acute appendicitis based on the duration of symptoms. Materials and Methods: Preoperative CRP was assessed in patients undergoing surgery for suspected appendicitis. Sensitivity, specificity, cutoff levels, and area under the curve in different categories based on the duration of pain, were calculated by the receiver operating characteristic (ROC) curve analysis. Results: In a total of 307 patients who underwent appendectomies, 222 patients had histopathologically confirmed acute appendicitis. The mean ± SD CRP levels in patients with appendicitis were significantly higher than in those patients with normal appendix (62.99±49.33 VS 29.63±39.11) (p value<0.05). The cutoff points for CRP based on the duration of symptoms were: 4.1, 23.9, and 30.4 µg/mlfor <24 hr, 24-48 hr, and >48 hr, respectively. The accuracy of CRP increased over time. Conclusion: CRP concentration might be used as an adjuvant predictive factor in the diagnosis of acute appendicitis, especially in suspicious cases with symptoms lasting more than 48 hours. We therefore propose the use of CRP levels as a routine laboratory test in patients with clinically suspected appendicitis.
Keywords: Appendicitis, C- reactive protein, ROC curve, Sensitivity and specificity

412. Urine Trypsinogen-2; as a Valuable Factor for Diagnosis of Acute Pancreatitis

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Background: Acute pancreatitis is a common cause of abdominal pain, without any characteristic sign, symptom or a gold standard diagnostic modality. The purpose of this study was to evaluate diagnostic value of urine trypsinogen-2 strip test in acute pancreatitis. Materials and Methods: This cross sectional prospective study was planned on 76 patients with abdominal pain and suspected to acute pancreatitis who admitted in emergency department of Valiasr hospital in Arak. In 46 patients, acute pancreatitis confirmed and considered as "pancreatitis group". In 28 patients acute pancreatitis ruled out and considered as "control group". Two patients excluded of this study. In both groups serum level of Amylase, lipase.CRP and urine trypsinogen-2 by quantitative and qualitative methods were measured. Sensitivity and specificity of the tests were determined. Results: Urine trypsinogen-2 Dipstick was positive in 36 of 45 patients in pancreatitis group (sensitivity80%), and was positive in 2 0f 28 patients in control group (specificity 92.8%). Urine trypsinogen-2 ELISA test was positive in 41 of 45 patients in pancreatitis group (sensitivity91.1%), and in 4 of 28 patients in control group (specificity 89%). Amylase had a sensitivity and specificity equal to 82.6% and 75% respectively. Lipase sensitivity and specificity were 76% and 85.7% respectively. Conclusion: Urine trypsinogen-2 Dipstick can be used to differentiate acute pancreatitis from other causes of abdominal pain. This rapid, easy to use, and accurate test can be used in emergency departments and primary health care units with limited diagnostic facilities.
Keywords: acute pancreatitis, amylase, lipase, urine trypsinogen-2

413. The Role of Anti-cyclic Citrullinated Peptide (anti-CCP) Antibodies in Serologic Diagnosis and Evaluation of Disease Activity in Rheumatoid Arthritis

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Background: Anti-cyclic citrullinated peptide (anti-CCP) antibodies are used as highly specific and sensitive markers in the diagnosis of rheumatoid arthritis (RA), in recent years. The aim of this prospective and cross-sectional study was to measure the levels of anti-CCP and rheumatoid factor (RF) in patients with RA and healthy controls to evaluate the specificity and possible diagnostic value of anti-CCP and RF, as well as their correlations with parameters of disease activity. Materials and Methods: Fourthy-three patients with RA, and 43 healthy controls were evaluated RA diagnosis was done on the basis of criteria recommended by American College of Rheumatology (ACR). Clinical parameters, including disease activity score (DAS28) indices for physical capacity were detected for RA patients. Results: We found 72.5% of the patients with RA positive (>18 RU/ml) for anti-CCP (mean value: 214±27 RU/ml), while 81% were positive for serum levels of RF showed statistically significant increase in patients with RA in comparison with healthy controls that were 100% serongative for RF and 92% for anti-CCP Furthermore, there was no correlation between DAS28 and anti-CCP levels. Conclusion: There is no relation between disease activity and anti-CCP level in serum. antibodies against CCP were thought to be more specific than RF for RA, and the determination of anti-CCP in addition to RF could be helpful in serological diagnosis and monitorization of patients with RA. So for diagnostic RA it is essential to determine both RF and anti-CCP.
Keywords: anti-CCP, Rheumatoid Arthritis, Serologic Diagnosis

414. SerumInterleukin-23 (IL-23) and Interleukin-33 (IL-33) Concentrations in Patients with Rheumatoid Arthritis

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Background: Rheumatoid arthritis (RA) is a long- term autoimmune systemic disease that identified by inflammatory responses which mainly affecting joints and surrounding tissues. Although the etiology of RA is unknown, but cytokines that produced by different cells such as lymphocyte, monocyte, endothelial and epithelial cells are believed to play major roles in the induction and propagation of the inflammatory conditions. During recent years researches have been revealed that IL-23 stimulates particular T-cells to produce IL-17 which has a major role in autoimmune inflammation. Furthermore, IL-33 is the 11th and most recently discovered IL-1 cytokine family which is expressed in normal and diseased synovium but diseased tissue displays higher level of expression. A limited number of studies have focused on IL-23 and IL-33 in RA. The aim of this study was to measure the levels of IL-23 and IL-33 in the serum of patients with RA, before treatment, three month after treatment and compare to patients with osteoporosis as well as healthy volunteer controls. Materials and Methods: We measured the serum levels IL-23 and IL-33 of 30 RA patients, 15 patients with osteoporosis and 16 age and gender matched healthy controls by using ELISA assay. Results: The serum IL-23levels of the RA patients before treatment 1419.7±252.7 (pg/ml) were significantly higher than the Il-23 level three months after treatment748.1±209.7 (pg/ml) P=0.009 and the control group 634.07±204.3 (pg/ml) P= 0.007. Serum levels of IL-33 were significantly higher in patients with RA before treatment 5.47± 0.142 (pg/ml) versus three months after treatment 4.34±0.072 P=0.001, and control subjects 4.53±.076 (pg/ml) P=0.001. There were significant differences between patients with RA and osteoporosis before treatment in IL-33 levels 5.47± 0.142 versus 3.65 ± 0.08 P=0.002.Conclusion: Our results showed that IL-23 and IL-33 are highly active in RA and these cytokines might be closely connected to pathogenic mechanisms of the disease.
Keywords: IL-23, IL-33, Rheumatoid Arthritis

Poster Presentation

415. Comparison of Quantitative & Qualitative Serological Assessment for Diagnosis of Early Syphilis

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Background: The diagnosis of early or primary syphilis is difficult. Although dark field microscopy is a high sensitive test when performed by skilled personnel, thus method requires an experienced operator ,and is labor- intensive , for this reason some of serological tests such as VDRL, RPR, FTA ,or ELISA procedures are available, easier , and cost- benefit , and all of laboratories use of them for testing of doubtful patients. Materials and Methods: 26 suspected cases (19male), (7 female) have attended to laboratory for syphilis examination (2003-2006). 21 cases of them were young (28-32) and5 cases were older (≥50 years old). All of them have been examined by VDRL, RPR, FTA, then compared the results. All of patients in accordance of clinical symptoms included in group (1); they were symptomatic and have contacted with infected cases. Group (2): Asymptomatic without any contact or having an infected sexual partner. Results: out of 26 cases of group 1only in 8 case the results of three technique were positive , but in 13 case VDRL, RPR were negative and FTA positive .Patients of group 2 with FTA were negative and by VDRL, RPR techniques were positive , which could be false positive cases. Conclusion: we can explain that VDRL, RPR techniques for surveying primary or recent syphilis infection weren't sensitive procedures, but they may be replace by FTA or some of EIA IgM tests.
Keywords: diagnosis, syphilis, comparison

416. Importance of IgG Avidity for differentiation of Acute Toxoplasma gondi infection in early pregnancy

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Background: Toxoplasmosis has been well known as an important human infection to be considered in pregnant women. Although many serologic methods are available, diagnosis of early Toxoplasmosis may be extremely difficult. The aim of this study is detecting Toxoplasma IgG antibodies developed at early stage of infection in pregnant women. Materials and Methods: 225 pregnant women who were in 2-4 month of their pregnancy, enrolled in this study. They were categorized into three groups .Anti_ toxoplasma IgG, IgM and IgG Avidity were evaluated by ELISA method. Results: Group A: 124 cases (IgG+, IgM+), (55.1 percent), group B: 99 cases (IgG+,IgM-),(44percent) and group C: 2 cases (IgG -, IgM +), (0.9 percent). Fifty five percent of the pregnant women had positive IgG and IgM among whom 7.1 percent had low avidity that reveals the active infection in the pregnant women. In the current study 44 percent of pregnant women had positive IgG and negative IgM that all had high avidity, indicates that in our society the level of Toxoplasmosis infection is high and most women have contact with this parasite before pregnancy. Conclusion: In this study the low avidity Test was 7.1 percent which shows that the occurrence of Toxoplasmosis infection is still a serious issue. Observation of 45.8 percent high avidity among group A suggests that either IgM has a high half-life or the false existence of IgM in Rheumatologic disorders makes it falsely positive. Therefore, avidity test is valuable in predicting maternal toxoplasmosis infection so,can be a high value parameter in disease treatment.
Keywords: Acute Toxoplasmosis, Pregnancy, IgG, IgM, Avidity

417. The Implementation Study of Practical Immunoloy Schedules of Medical Students (Undergraduate) in Tehran University of Medical Sciences with Universities of Selected Countries

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Background: Teaching the immunological techniques is one of the way for familiarization the medical (undergraduate) students with knowledge of basic, clinical, applied immunology. So topics and contents comparative study with so many universities of selected counties may improve the teaching and learning activity of laboratory immunology in Tehran University Of Medical Sciences (School Of Medicine). Materials and Methods:
Methodology is a conceptual, in terms of modeling and implementation. In this purpose, the topics and contents of immunology laboratory schedules which were included in curriculume and syllabusses of universities of selected countries (5 Asia, 15 USA, 5 Canada respectively) were searched through internet. Results: At overall, The content of our course was nearly the same as Mutah Medical University (Jordan) and similar to (optional course of Maryland University, and both of the Austin And Clark state Community Colleges) in USA. These colleges train Medical laboratory technicians. The course teaching method in Tehran University Of Medical Sciences as well as the above mentioned universities and colleges were practical, versus most of the other universities that were experimental. Nevertheless, the taught contents in the two styles were based upon each of the objectives of universities and colleges in USA and Canada, and some universities in Asia. Conclusion: In spite of similarity of content there was difference between quality of teaching and learning activity, it means, other than course content, the applying standards for so many items such as lab reports, note books, seminar, poster, homeworks , *assessments and behoviour cods etc*,….also utilizing many kind of elements such as Quizzes, case study and paper research and tutorial sections will improve the quality of teaching and learning activities of the immunology laboratory courses.
Keywords: Laboratory immunoloy, course contents, undergraduate, medical students, practical, experimental, teaching and learning activity

418. Analysis of Anti ds-DNA Test Results in Patients from Imam Reza Hospital, Tabriz, Iran

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Background: Anti double stranded deoxyribonucleic acid test (Anti-dsDNA Test) helps to predict and control autoimmune diseases such as Systemic Lupus Erythematosis (SLE). SLE most often harms the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. Materials and Methods: Data was collected from the results of tests in Imam Reza hospital Laboratory. Patients referred from different units. Serum of patients were analysed by ELISA kits.Then, SPSS16 was used to analysis extracted data. Rate of one and more than it represent as positive and less than one as negative for Anti ds-DNA. Results: Totally, Anti ds-DNA was measured in 289 patients, which 69.6% was female and the remaining 30.4% male between 3-84 years old. Data analysis showed 9.3% of patients were positive who was referred from different units of hospital. Sex ratio among positive cases was 81.5% female and 18.5% male. 26% and 17% of positive cases were referred from Romatology and Kidney units. Conclusion: According to the result of current study, it seems that the side effect of SLE on joints and kidney was more than other organs. The data showed that incidence of autoimmune disease are more in our society and its incidence in females is about 81.5%.
Keywords: Anti ds-DNA, Systemic Lupus Erythematosis, Tabriz, Iran

419. Correlation of Autoantibodies Presence Detected by IFA-anti-dsDNA, IFA-AMAwith Final Clinical Diagnosis of Autoimmuone Diseases

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Diagnosis of patients with SLE (Systemic Lupus Eritematosus), autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), involves specific diagnostic tests, such as, IFA anti-dsDNA, and immunoblotting for the detection of autoantibodies for specific autoantigens (mitochondria, dsDNA, LKM-1, LC-1, SLA/LP). We established specific correlation between the detected autoan- tibodies and corresponding clinical findings. The total of 800 serum specimens were probed with IFA-anti-dsDNA, rather than 10 percent of which tested positive. We also performed dilution analysis to the end point for all the positive specimens. Numerous specimens were tested by IFA, AMA and immunoblotting. Our IFA-anti

dsDNA, AMA, imunobloting analy-ses of serum specimens, as a part of AIH PBC, SLE diagnostic approach show significant correlation with final clinical diagnosis of these diseases.
Keywords: SLE, AIH, PBC, IFA, AMA, anti-dsDNA, LKM-1, LC-1, SLA/LP

420. Prostate Cancer Screening and Prevention
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Prostate cancer is extremely common but causes death in only a minority of men in whom it develops, facts that raise issues regarding screening and treatment morbidity. An elevated PSA level lacks specificity as a test for prostate cancer, but PSA measurements can be useful in combination with clinical risk factors or to measure changes in PSA over time. Screening with theprostate–specific antigen (PSA) blood test has more thandoubled the risk of a prostate cancer diagnosis. although it is not a perfect screening test, it is still the best cancer marker that we have.Rather than relying on PSA screening alone, we should stratify the risk of prostate cancer on the basis of race, age, PSA level, family history, findings on digital rectal examination, whether the patient has ever undergone a prostate biopsy, and whether the patient is taking finasteride (Proscar). A simple online tool is available to do this. There is no PSA level below which the risk of cancer is zero.daily from Between 15men that have PSA test 2-3 men have PSA >4 and 0.1 of them have PSA >20. Finasteride has been found in a randomized trial to decrease the risk of prostate cancer and relative reduction in period prevalence of prostate cancerof approximately 25%, compared with placebo, for men treatedfor up to 7years with the ᵒ-alpha-reductase inhibitor finasteride, but vitamin E and selenium supplements have failed to show a benefit. Prostate cancer screening and use of finasteride can be benefite in decrease the prostate cancer death rates.
Keyword: PSA, Finasteride

421. Investigation the Level of Uric Acid and Magnesium in Patient with Rheumatoid Arthritis
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Background: Recent studies suggested that reactive oxigen metabolites and trace elements play some role in etiology and pathogenesis of rheumatoid arthritis (RA).Magnesium (Mg) is an important trace element for many enzymes activity.Uric acid function as scavenger free radicals and may modulate an important component of rheumatoid autoimmunity.Our purpose was to determine the condition of serum uric asid(SUA) and magnesiumin patients with RA and compaire with control group. Materials and Methods: This case control study we enrolled 43 patients with RA and 43 contol and measured their level of SUA and Mg. Then data was analysed by 2-sample T-test. Results: We obsurved that mean of SUA in patients with RA (4.16±1.6) was decreased compared with control group (5.58±1.5), but this was not significant (Pvalue>0.05). The level of Mg was not different between patients (2.18±0.15) and conrtol group (2.27±0.24). Conclusion: This study showes there is no association between serum Mg and SUA and RA.But more studies need to investigate these factors and other trace elementas in serum and sinovial fluids in RA.
Keywords: Uric Acid, Magnesium, Rheumatoid Arthritis

422. Preparation of Standard Serum and Determination of Normal Range of Complement Components, CH50, CIC in Iranian Population
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Background: The complement system consists of a number of small proteins found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors. This system helps the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is part of the immune system and has important role in host defense against infections. Therefore, evaluation and measurement of complement components in patients sera are important factors to monitor clinical status of patients. One of the main tools for evaluation of complement system at lab tests is the availability of calibrated standard sera for performing the tests. The aim of this study is: 1) preparation of standard serum for laboratories with reproducible and acceptable results, 2) determination of normal range of complement components and related tests in Iranian population. Materials and Methods: The pooled serum was prepared from 100 Iranian normal persons, free of viral infections disease such as HBV, HCV and HIV and the samples were aliqueted and were kept at -80°C as a standard serum. This standard serum was used to draw standard curves for C3, C4, C1q, C1INH, CH50, CIC tests and also to determine normal range of complement components and related lab tests in Iranian population by pooled percent method. Results: Normal range of C3, C4, C1q, C1INH, CH50, CIC tests were found %99±26, %98±35, %95±19, %110± 27, %100±30 and %104±38 respectively. The statistical results were expressed as M±2SD. Conclusion: The results showed that all normal ranges of complement components are around of 100% activity and are acceptable. On the other hand, with regard to high expensive commercial standard serum and unavailability, this method of standard preparation is cost effective and achievable for diagnostic laboratories and also can be applied for measurement of other serum proteins.
Keywords: Standard Serum, CH50, CIC

423. Evaluation of Red Blood Cell Lysing Solutions in the Detection of Peripheral Basophiles of Healthy Normals and SLE Patients by Flow Cytometry
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Background: To evaluate the influence of four widely used red blood cell lysing solutions on counting basophiles and the detection of activation marker of basophiles in peripheral blood of normals and SLE patients by flowcytometric methods. Materials and Methods: Basophiles were measured in whole blood by flowcytometry after lysing by different blood cell lysing solutions, including distilled water, ACK buffer ((0.15M NH4Cl, 1mM KHCO3, and 0.1mM EDTA, pH7.4), RBC Lysis Buffer (Biolegend inc.) and FACS Lysing Solution (BD Pharmigen), staining with antibodies against specific surface makers identified as $FcεRIα^{+}CD203c^{+}CD123^{+}$ cells for gating or validation purposes, then run on FACScan flow cytometers. Results: The light scatter properties including FS and SS value of leukocytes in whole blood are preserved when erythrocytes are lysed in RBC Lysis Buffer and FACS Lysing Solution. In contrast, the light scatter properties of leukocytes are affected when erythrocytes are lysed in distilled water or ACK, especially the FS and SS properties of granulocyte populations. By counting basophiles, RBC Lysis Buffer and FACS Lysing Solution were almost the same level and no significantly difference. In contrast, when erythrocytes are lysed in distilled water or ACK, the absolute numbers of basophiles are significantly lower than RBC Lysis Buffer. The expression of the CD203c on peripheral basophiles of SLE patients was significantly higher than that of normals. Also, FACS Lysing Solution treatment leads to a CD203c down expression significantly, and there were no significant difference of CD203c expression among other three kinds of erythrocyte lysing reagents. Conclusions: We provide a solid description and validation of a novel and rapid method for the flowcytometric enumeration of absolute number of basophiles in whole blood. The widely used repertoire of commercial lysing reagents includes compounds that, by modifying the light scatter properties of leukocytes (including basophiles) can influence the accuracy of quantity of absolute number of the existence of basophiles subsets, also by modifying basophiles plasma membrane integrity, can influence the quantity of staining intensity of cell-activated marker CD203c fluorescence and may well lead to misinterpretations concerning the existence of hyperreactive and less reactive basophiles subsets.
Keywords: Red Blood Cell, Peripheral Basophiles SLE, Flow Cytometry

IMMUNOLOGY & NUTRITION

Oral Presentation

424. Effect of ATRA on Deviated Immune Cells and Restoration of Normal Immune Responses

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Background: An active metabolite of vitamin A, all trans retinoic acid (ATRA) can divert the balance of Th17 cells into regulatory T cells by expression of FoxP3 and suppression of IL-17 in CD4+ T cells. In this study, we evaluated the effect of ATRA on CD4+ T cells of patients with Ankylosing spondylitis (AS) and compared it to the healthy controls. Materials and Methods: Eighteen AS patients and 18 age- and sex-matched controls were included in this study. CD4+ T cells were isolated from peripheral blood mononuclear cells (PBMC) by negative selection. Th1, Th17 and Treg cells were evaluated by intracellular staining of IFN-γ, T-bet, IL-17 and FoxP3. Results: IL-17+CD4+ T cells were significantly higher and FoxP3+CD4+T cells were significantly lower in AS patients compared to healthy controls. IFN-γ+ and T-bet+ CD4+ T cells were not significantly different between the two groups. ATRA could significantly decrease the frequency of IL-17+CD4+ T cells in patients with higher numbers of IL-17+ CD4+ T cells. It could also increase the frequency of FoxP3+CD4+ T cells in patients. It had no significant effect on Th1 cells. Conclusion: This study showed that ATRA can decrease the frequency of Th17 cells while increasing the frequency of regulatory T cells in patients with deviated immune responses. It had no effect on healthy controls or patients with lower numbers of Th17 cells. This effect of ATRA can be beneficial in the treatment of AS patients.
Keywords: ATRA, CD4+ T cells, Ankylosing spondylitis

425. Genetic and Epigenetic Regulatory Mechanisms Involved in Induction of Differential Immune Response by non-Viable Probiotics

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Background: Strain specific properties of probiotics in providing supportive health effects in immune system and gastrointestinal tract have been widely investigated *in vivo* and *in vitro*. Unraveling probiotics-induced responses in complex network of interacting signaling elements including TLRs, NFκB, MAPK as well as miRNAs, newly discovered fine regulators of cellular responses at post transcriptional level, is of particular interest. In this context we have investigated the effects of heat- inactivated *Lactobacillus Rhamnosus GG (LGG)* and *L. delbrueckii subsp. Bulgaricus(L.Bulg)* on expression level of NFκB, IκB as well as expression of post-transcriptional elements including, miRNA 7i, 146a and 155 which target TLR4 and cytokine signalling in NFκB dependent manner, in dendritic cells (DCs). Materials and Methods: Human monocyte-derived DCs were cultured in vitro with heat- inactivated *L.Bulg* and *LGG* for 12 hours. Expression of immune- and inflammatory mediators, Ikbs and miRNAs were analysed by qRT-PCR. Results: Autoclaved strains of *lactobacilli* are still able to influence TLR4 expression and they can directly influence p38 and IKB mRNA expression. However, *LGG's* effect is more significant in down regulating p38 expression (0.47±0.1, *p=0.01*), while *L.Bulg* induces significant inhibitory response in IKB expression (0.37±0.2, *p=0.03*). Our results show for the first time that *LGG* imposes down regulatory effect on expression of miR7i which has been shown to target TLR-4 and also reduces expression of mi146a (0.6±0.01, *p=0.02*), which directly control TLR4 and regulates the maturation process in dendritic cells and pro-inflammatory cytokine signaling through NFκB pathway. Conclusion: These findings could provide genetic and epigenetic mechanistic explanations for the proposed immunomodulatory effect of probiotics. By combining the results of current study with our similar works in other cell lines we can suggest that non-viable probiotics strains posses heat stable components that can address regulatory mechanisms in immune cells by influencing the key immune signaling nodes.
Keywords: *Lactobacillus Rhamnosus GG, L. delbrueckii subsp,* NFκB, IκB,post-transcriptional elements

426. Vitamin D Intake Favors Anti-Inflammatory Adipokine Profile in Type 2 Diabetes Subjects: the First Report of a Randomized Controlled Clinical **Trial**

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Background: Adipokines and their associations with obesity and the related comorbidities including systemic inflammation, insulin resistance and diabetes, are rather a new area of concern. Several adipokines including adiponectin, retinol binding protein (RBP)-4 and lipocalin (LCN)-2 have been demonstrated to affect both insulin activity and inflammatory reactions. To investigate the effects of vitamin D either with or without extra calcium on certain adipokines in the subjects with type 2 diabetes (T2D). Materials and Methods: This was a double-blind, randomized, controlled trial over 12 weeks in 90 T2D subjects aged 30-60 years from both sexes. Subjects were randomly allocated to one of the three groups to receive two 250 mL bottles a day of either plain Persian yogurt drink or *doogh* (PD, containing 150 mg calcium and no detectable vitamin D3/250mL); vitamin D-fortified *doogh* (DD, containing 500IU vitamin D3 and 150mg calcium /250mL); or calcium+vitamin D3- fortified *doogh* (CDD, containing 500 IU vitamin D3 and 250mg calcium/250mL). serum adiponectin, retinol binding protein-4 and lipocalin-2 concentration were measured at baseline and the end of intervention. The changes in these adipokines were evaluated. Results: Compared to the baseline values, retinol binding protein (RBP)-4 concentrations significantly decreased in both DD and CDD groups. There was a significant increase in serum adiponectin in both DD and CDD groups (51.3±65.3 vs. 57.1±33.8 μg/L, $p<0.05$). Mean adiponectin changes in CDD were significantly higher than in PD (p=0.021). Conclusions: Daily intake of vitamin D-fortified *doogh* improved adipokine profile in T2D subjects and extra calcium conferred additional benefit only for the anti-inflammatory adipokine, i.e., adiponectin.
Keywords: vitamin D; calcium; type 2 diabetes; adipokines

427. The Effect of Fasting on Immune System of Athletes During Holly Ramazan in Zahedan City

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Background: During the ninth month (Ramazan) in Islamic calendar (Hejri), millions of mature and obligated muslims fast many hours during a day in all over the world. In many researches, fasting effect on public immune system have been studied. In this study, we are going to evaluate fasting athlete's immune factors such as immunoglobins level and serum complement components at the beginning and the end of fasting during a physical activity. Material and Methods: This temporal study was descriptive analytic one which has been done during Holy Ramazan studied immune system components (C3 and C4) and blood cells (CBC+Diff) in athletes. Obtained data from laboratory findings were analysed by SPSS-17 software and T-test method has been applied for comparing evaluated quantities at the end and at the beginning of holy Ramazan. Results: In this study, athletes within 16-36 years age with average age of 30/4 ±12/1were evaluated. These athletes exercised about 2/0 ±0/3 hours in per day. Laboratory findings along with their analysis showed that IgA amounts at the beginning of Ramazan were calculated as 239/2 ±98/2 while at the end of Ramazan they were 239/2 ±98/2. C4 serum density before involvement was calculated as 258/2 ±150/6 and after it was 330/7 ±127/6 (P<0/001). Neutrophils amount also before involvement obtained as 60/0±4/2 and at the end it was 56/2±8/3 (P=0/003). All three variables had meaningful differences. Other statistical results did not have meaningful differences. Conclusions: Fasting has had positive effects

on C4, IgA levels and neutrophils amount at the beginning and at the end of holy Ramazan. These may have protective effect on athlete's immune system against infection diseases during exercise.
Keywords: Immune system, athletes, immunogolbulins, complements and fasting

Poster Discussion Presentation

428. Effects Of Vitamins D, E on Serum Th_2 Cytokines in Atopic Dermatitis Patients

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Background: Nutritional factors are among the environmental factors in the etiology of atopic dermatitis. Due to its immunological influences, vitamin D has been considered not only as an allergy inducer but also as a curative agent for atopic dermatitis. The immunological effects of vitamin E would be helpful in the treatment of atopic dermatitis. Considering the cytokines and their roles in the pathogenesis of atopic dermatitis, these two vitamins could be effective in reducing risk of atopic dermatitis through influencing cytokines. This study was designed to determine the effects of vitamins E and D individually and in combination on cytokines in atopic dermatitis patients. Materials and Methods: In a randomized, double blind, placebo-controlled clinical trial 45 atopic dermatitis patients 10 to 45 years old were divided into four groups. They received the following daily for 60 days: Group 1 (n=11) vitamins E and D placeboes; Group 2 (n=12) 1600 IU vitamin D_3 plus vitamin E placebo; Group 3 (n=11) 600 IU synthetic all-rac-α tocopherol plus vitamin D placebo; Group 4 (n=11) 1600 IU vitamin D_3 plus 600 IU synthetic all-rac-α tocopherol. After 10 to 12 hours fasting, serum IL-4, IL-5, IL-13, 25 (OH) vitamin D, α-tocopherol, were determined before and after the intervention. Results: The intervention brought about statistically significant changes in the groups as follows: a) increases in serum 25 hydroxy vitamin D in Groups 2 and 4; b) increases in plasma alpha tocopherol in Groups 3 and 4; c) decreases in serum IL-4 in Groups 2, 3 and 4; d) decreases in serum IL-5 in Group 2; e) decreases in serum IL-13 in Groups 2, 3 and 4. Conclusion: Vitamins E and D could decrease the cytokines of Th2 subset with regulatory effects. It is essential to investigate the allergy-inducing properties of vitamin D.
Keywords: Atopic Dermatitis, Vitamin E, Vitamin D, Cytokine

429. Hot Nature Dietary Intervention with Co-Supplemented Herbal Oils in Multiple Sclerosis Patients

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Background: Multiple sclerosis is the most chronic, inflammatory disorder of the CNS in which ensuing demyelization results in physical disability with no cure. Many of the current treatments are expensive, limited in efficacy, possess unpleasant side effects and need several injections. Then, identifying novel therapeutic and protective agents is very important. To determine whether a co-supplemented herbal oils with Hot nature dietary positively affects EDSS in RRMS patients, we plan this study to assess the potential therapeutic and protective effects of our intervention on RRMS patients with EDSS<6. Materials and Methods: In this 6 months long double blind, randomized trial, 100 patients of RRMS (EDSS<6, with Inclusion and exclusion criteria) were randomized to three dietary interventions: the (case) group A: received co-supplemented oils with advising Hot nature dietary, the (control) group B: received the 'Olive Oil'' supplement as placebo C: received co-supplemented oils and the (case) group. Clinically EDSS and functional score at baseline, 3 months and 6 months of intervention evaluated. At base line and 6 months after intervention, blood samples obtained, to determine immunological factors (plasma cytokines of IL-4, IFN-*y* and IL-17), biocimical factors (GGT, SGOT, SGPT, blood cells PUFA,FADS2, Serum sPLA2) tests. Results: 65 RRMS patients were enrolled, with mean follow up over 6+_SD 2.9 months. Interventions in A and C groups protected against disease and significantly prevented from prolonged neurological sequel by decreasing the severity of disease. Co-supplemented oils in dose (20 g /day) prevented from MS in >50% of A and C groups, furthermore it has beneficial effects in reversing the signs and can improve clinical outcome in patients with newly diagnosed MS. Immunological and biocimical assessment confirmed the clinical results. Conclusion: This study suggests that a co-supplemented herbal oils with Hot nature dietary can have moderate benefits in RRMS patients on concurrent disease modifying therapies (DMT).
Keywords: Delta -6-desaturase ,Fatty acids, functional food, Hot nature dietary, Midzaj, herbal oils, Traditional Iranian Medicine(TIM), Relapsing Remitting MS, Nutrigenomics

430. The Immunopathogenesis Role of Casein Genisteinon Experimental Adriamycin Induced Nephropathy

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Background: The Adriamycin induced nephropathy is an experimental model of nephrotic syndrome. Although the etiology of nephrotic syndrom remains to be elucidated, it has been postulated that it is the result of circulating T-cell factors, such as various cytokines that lead to an increased glomerular capillary permeability and proteinuria. Materials and Method: In this study we used 40 male adult Sprague_dawleyrats aged 15 weeks, with average weight of 300 gr. We divided them into 4 ten groups randomly and they were left to be adjusted to the conditions of their place: Negative control; was only received normal chow diet, Positive control; received a single dose of 8 mg/kg Adriamycin IV injection to induce nephrotic syndrome. They received normal chow diet, Casein group; received 8 mg/kg Adriamycin and gavaged daily with 50 mg/kg CMC (Carboxyl methyl cellulos). They received casein diet for 6weeks, Casein genistein group; received 8 mg/kg Adriamycin and gavaged daily with 50 mg/kg genistein. Moreover, they received casein diet for 6 weeks. During this period we collected their urine once aweek for 6 weeks. Rats were sacrificed after 6 weeks and blood samples were taken from the heart and then kidneys, livers, hearts removed for histopathology. In addition, the activity of matrix metalloproteinase-2 on cell culture, WEHI164 was assessed by Zymography for evaluating its anti-inflammatory effects. Theassessment of IL1,IL6, TNFαcytokines by ELISA kitswere proposed for further investigation. Results: Our data show that the casein genisteinis able to reduce the level of proteinuria in patient rats compared with normal control and can prevent disease progression. Conclusion:Our findings show that treatment with casein genistein can diminish disease progression in experimental model of nephrotic syndrome.
Keywords: Casein Genisteinon, Adriamycin, Nephropathy

431. Anticancer and Antioxidant Activity of New Synthetic Chromene Derivatives

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Background: Antioxidant compounds play a key role in human nutrition as well as in industry. Natural and synthetic antioxidants are widely used in cosmetic as well as nutritional preparations. The antioxidant and anticancer activities of new synthetic chromene derivatives were studied. Materials and Methods: The DPPH (2,2-diphenyl-1-picryl-hydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assays were used for determining antioxidant activity. Furthermore, the dose dependent anticancer effects of these compounds were studied on PC3 and HepG2 human cancer cell lines using MTT assay. Results: Results showed that all compounds displayed the concentration dependent of scavenging activity against DPPH and ABTS, but four of them were potent than others with the IC50 values between 12-14 µg/ml for DPPH and 21-40 µg/ml for ABTS tests. A correlation was found between DPPH and ABTS test for antioxidant activity. The compounds did not exhibited

antiproliferative effects against the cancer cells. Conclusion: Therefore, chromene derivatives may be considered promising for the development of new antioxidants agents.
Keywords: Anticancer, Antioxidant, Chromene Derivatives

432. Which Clinical Signs of SCORAD Test is most Affected by Prebiotic in Atopic Dermatitis Infants?
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Background: Atopic dermatitis (AD) is a prevalent disease in children. Recent studies have focused on the effects of prebiotics as a safe supplementary treatment in children with allergy, especially atopic dermatitis. The aim of this study was to determine which clinical sign of AD is most alleviated by prebiotic supplement? Materials and Methods: Seventy 7-24 month old infants with AD were enrolled in a double-blind placebo-controlled trial and were divided into 2 groups. The prebiotic supplemented group (PS) received fructooligosaccharides-inulin and the control supplemented group (CS) received maltodextrin powder as placebo for 3 months. SCORing for Atopic Dermatitis (SCORAD) questionnaire was completed for all the infants before and after the trial. The data were analyzed using SPSS software and P values less than 0.05 considered significant. Result: All clinical parameters of the SCORAD were significantly decreased in both groups at the end of trial. However, the reduction in the PS group was significantly higher than the CS group ($P<0.001$). Subjective score was the most alleviated among all the clinical signs in PS group (before: 14.3 ± 6.0; after: 0.46 ± 1.6; $P<0.001$), whereas the intensity score reduced most significantly between the two groups (PS group before 10 ± 8.1 and after 1 ± 4; CS group before 4.7 ± 3.6 and after 2.1 ± 2.6; $P<0.001$). Conclusion: It seems that prebiotic supplement affects mostly the intensity of SCORAD in AD infants.
Keywords: Atopic dermatitis, Prebiotics, Fructooligosaccharide, Inulin, SCORAD

433. Vitamin C and Apoptosis of Tumor Cells
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Background: Vitamin C is a well-known anti-tumor agent as well as essential nutrients. It was shown that relatively high concentration of vitamin C (10 mM) induces apoptosis of B16 murine melanoma cells. In addition, it interfere the uptake iron into tumor cells, which is essential process for maintenance of the proliferation of tumor cells. There are other reports regarding anti-tumor effect of relatively low concentration vitamin C, less than 1.0 mM. Materials and Methods: Tumor cells were treated with different doses of Vitamin C (from 0.5 mM to 10mM). Staining with Annexin V flous kit was used in order to determine the apoptosis in cells. Results: We found significant deifferences between case and control groups and also between different doses of Vitamin C. Low concentration of Vitamin C was not as efficient as higher doses. Conclusion: Taken together, Vitamin C can increase susceptibility of tumor cells to apoptosis.
Keywords: Vitamin C, Apoptosis, Tumor Cells

434. Different Maturation Pattern of Human Dendritic Cells by Viable and non-Viable Form of Identical Probiotics Strains
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Background: Probiotics are currently defined as micro organisms which have to be viable at time of ingestion to confer health benefits. However, increasing evidence are demonstrating that cell wall components and DNA of these important functional foods can be also sufficient for stimulating measurable effects including immune modulatory responses. One of the putative mechanisms of action of probiotics in provoking immune responses is inducing maturation of dendritic cells (DCs) as key elements of immunologic synapses which their maturation state can delicately orchestrate the fate of further immune cell responses. We aimed to investigate if both viable and heat inactivated form of two well described and extensively used *Lactobacilli*. *Lactobacillus rhamnosus GG (LGG)* and *Lactobacillus delbrueuckii (L.del)* are able to induce comparable immune response.via studying maturation pattern of DCs. Materials and Methods: Human monocyte-derived DCs were cultured in vitro with *L.del* and *LGG* in viable and heat- inactivated forms for 24 hours. The expressions of co-stimulatory molecules involved in DCs maturation as well as extracellular cytokine production were measured by flow cytometry. Results: Similar ratio of viable and inactivated form of *LGG* caused higher up-regulation in expression of CD80. CD86 and CD54 than *L. del*., whereas *L.de*l down regulated DC adhesion receptor CD209 more than *LGG*. In viable state only *LGG* induced CD83 expression but in heat inactivated condition both strains showed enhanced up regulation of CD83. DCs exposed to viable and non-viable strains secreted significantly higher level of IL-1β. TNF-α.IL-12. Of course, inactivated *lacobacilli* were considerably less potent in cytokine production even though increasing the ratio enhanced their cytokine production. Conclusion: Viable and heat killed *lactobacilli* are both able to influence immune modulation via inducing quite similar phenotypic changes in DCs. although inactivated strains were less potent in elevating inflammatory cytokines than their viable form.
Keywords: Dendritic Cells, Probiotics Strains, *Lactobacillus rhamnosus GG,Lactobacillus delbrueuckii*

435. The Effect of Vitamin A on Immunophenotyping of Peripheral Blood Lymphocytes by Flow Cytometry in Ulcerative Colitis
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Background: Ulcerative colitis (UC) is an inflammatory disease of the rectal and colonic mucosa and seems to result from a complex series of interactions between susceptibility genes, the environment and the immune system. There is a paucity of data on the positive effect of vitamin A on intestinal mocusal immunity. We aimed to evaluate the effect of vitamin A supplementation on peripheral Lymphocyte subsets alterations in ulcerative colitis. Materials and Methods: Immunological asssessment was done in 49 patients with ulcerative colitis participating in pre and post test survey. All participants were at clinical remission stages. Vitamin A supplement was injected twice (50000 IU) with two weeks interval. Study period was considered 45 day. Flow cytometric analysis of CD4+ and CD8+ and CD3+ T lymphocytes was done before and after intervention. Results: Vitamin A supplementation had significant effect on decrease of CD8+ T-cells ($P=0.003$) while absolute lymphocyte count ($p<0.05$), CD4+ T-cells ($p=0.03$) and CD3+T-cells ($p= 0.01$) increased significantly. Conclusion: These results suggest that Vitamin A supplementation could suppress inflammation by decrease of cytotoxic T-cells, and had a beneficial effect on control of inflammation, and should be considered as part of the treatment protocol in these patients.
Keywords: Vitamin A, Immunophenotyping, Peripheral Blood Lymphocytes, Flow Cytometry, Ulcerative Colitis

436. The Effect of Probiotic Yogurt on hs-CRP Level in Obese Persons
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Background: One the risk factors in obese people witch secrets from liver in respond to visceral fat´s adipokines, is hs-CRP. Changes in gut micro biota by means of inhibition inflammatory process in body can trigger to reduce this risk factor in people who suffer from high fat percent. Materials and Methods: In a randomized clinical trial 80 persons with BMI higher than 25, divided in 3 groups, one group had weight reduction regimen with consumption of probiotic yogurt, second group without weight reduction regimen but with consumption of probiotic yogurt and another one with weight reduction regimen but consumption of regular yogurt for 2 months. Intervention factor was probiotic yogurt with L.casei,

Lactobacillus acidophilus La5, Bifidobacter bb12. The duration of intervention was 2 month. Serum samples were collected and analyzed for hs-CRP. Results: The mean hs-CRP level was 4.2 mg/L (CI=3.15-5.26), 7.6mg/L (CI=5.43-99) and 8.8mg/L (5.91-11.75) in diet and probiotic, diet and non-probiotic and non-diet with probiotic yogurt group respectively. There was significant different between after and before of intervention for first and third groups (p=0.001) for this variable. The different between three groups after intervention was significant (p=0.0001). Conclusion: In regard to our results it is recommended for prevention of inflammation in vessels witch mainly is happened by cytokine(IL6 from adipocyte) and hs-CRP reactions in obese people´s body , is suggested that these people had better consume food witch included probiotic or supplementary.

Keywords: probiotic, obesity, hs-CRP, adipokine

437. Regular consumption of vitamin D-fortified yogurt drink (*Doogh*) improved endothelial biomarkers in subjects with type 2 diabetes: a randomized double-blind clinical trial

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Background: Endothelial dysfunction has been proposed as the underlying cause of diabetic angiopathy. In this study, the effects of improvement of vitamin D status on glycemic status, lipid profile and endothelial biomarkers in T2D subjects were investigated. Materials and Methods: Subjects with T2D were randomly allocated to one of the two groups to receive either plain yogurt drink (PYD; no vitamin D/250 mL, n1 = 50) or vitamin D3-fortified yogurt drink (FYD; 500 IU/250 mL, n2 = 50) twice a day for 12 weeks. Anthropometric measures, glycemic status, lipid profile, body fat mass (FM) and endothelial biomarkers including serum endothelin-1, E-selectin and matrix metalloproteinase (MMP)-9 were evaluated at the beginning and after the 12-week intervention period. Results:
The intervention resulted in a significant improvement in fasting glucose, the Quantitative Insulin Check Index (QUICKI), glycated hemoglobin (HbA1c), triacylglycerols, high-density lipoprotein cholesterol (HDL-C), endothelin-1, E-selectin and MMP-9 in FYD compared to PYD ($P < 0.05$, for all). Interestingly, difference in changes of endothelin-1, E-selectin and MMP-9 concentrations in FYD compared to PYD (-0.35 ± 0.63 versus -0.03 ± 0.55, $P = 0.028$; -3.8 ± 7.3 versus 0.95 ± 8.3, $P = 0.003$ and -2.3 ± 3.7 versus 0.44 ± 7.1 ng/mL, respectively, $P < 0.05$ for all), even after controlling for changes of QUICKI, FM and waist circumference, remained significant for endothelin-1 and MMP-9 ($P = 0.009$ and $P = 0.005$, respectively) but disappeared for E-selectin ($P = 0.092$). On the contrary, after controlling for serum 25(OH) D, the differences disappeared for endothelin-1($P = 0.066$) and MMP-9 ($P = 0.277$) but still remained significant for E-selectin ($P = 0.011$). Conclusions: Ameliorated vitamin D status was accompanied by improved glycemic status, lipid profile and endothelial biomarkers in T2D subjects. Our findings suggest both direct and indirect ameliorating effects of vitamin D on the endothelial biomarkers.

Keywords: Vitamin D-fortified yogurt drink, endothelial biomarkers, type 2 diabetes

Poster Presentation

438. Effect of Zinc Supplement on Serum Antibody Titers to Heat Shock Protein 27 in Thalassemia Major Patients

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Background: Heat shock proteins (HSPs) are molecular chaperonesthat protect against stress stimuli including heatshock, oxidized LDL, mechanical stress, oxidants, andcytokine stimulation. Heat shock protein 27 over-expressed when cells are exposed to oxidative stress and provides cells with a mechanism of defense against such stressors. An immune response to heat shock proteins appears to be involved in atherogenesisand other oxidative stress diseases such as thalassemia..Increased of oxidative stress and decreased antioxidant statussuch as zinc has been identified in patients with thalassemia. Thus, we have investigated whether zinc supplement in thalassemia major patients is capable of affecting serum antibody titers to heat shock protein 27 levels. Materials and Method: We studied 80 Beta thalassemia major, age 7-22 years old. To double-blinded subjects were randomized into two group for receiving zinc or placebo(30mg/day). The duration of supplement consumption is 12 months. Anti HSP27 were assessment at 3, 6 and 9 months for follow-up by ELIZA method.
Results: At the first time, the mean Anti HSP27 of the patients in zinc and placebo group were 0.430±0.165 and 0.436±0.167 respectively. The mean Anti HSP27 of the patients three months after the beginning of the study in zinc group was 0.419± 0.182, and in placebo group 0.449±0.160 (P= 0.461). Six months later it was 0.390±0.167 in zinc group , and 0.414 ±0.157 in placebo group (P = 0.529). Nine months later in zinc group was 0.392±0.157 and in placebo group 0.454±0.185 (P= 0.191). Conclusion: After a period of 3,6 and 9 month ,anti HSP27 value decreased, but no statistically significant difference in Anti HSP27 between thalassemia received zinc supplements and placebo. Thus ,consumption of zinc supplement may be useful on decrease of serum antibody titers to heat shock protein 27 and atherosclerosis in thalassemia major patients.

Keywords: Zinc Supplement, Heat shock proteins 27, Thalassemia

439. Vitamin D Status and Ulceritive Coilitis Patients in Kerman, Iran

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Background: Ulceritive colitis is one type of inflammatory bowel disease, which occurs in the colon, large intestine, and this factor distinguishing it from Crohn's disease which may affect any portion of the digestive tract. One of the well-studied effects of vitamin D is its regulatory effect on the immune systems and recent studies showing anti-inflammatory activity of vitamin D. In this study for the first time, the association between the vitamin D deficiency and ulcerative colitis was investigated in our patients in Kerman, Iran. Materials and Methods: Vitamin D serum level was detected in 85 UC patients and 85 healthy controls using competitive ELISA technique.
Results: There was a highly significant decrease in vitamin D serum level in UC patients (20.83 ng/ml, SD=11.48) compared to healthy controls (28.44 ng/ml, SD=13.46) with *p value* of less than 0.001. Conclusions: The present study is the first to show that vitamin D serum level is reduced in ulcerative colitis patients in Kerman and also in our knowledge, in Iran. The aetiology of ulcerative colitis is certainly multifactorial. Our data indicate that ulcerative colitis might be associated with vitamin D insufficiency as one of the effective factors. Vitamin D therapy would be valued for further trials in our ulcerative colitis patients.

Keywords: Vitamin D, Ulceritive Coilitis, Serum level

440. Evaluation ofThe Relationship Between the Levels of Pro-Inflammatory and Anti- Inflammatory Cytokines in Mothers' Milk And Risk of Chronic Diarrhea in their Breastfed InfantsUnder 18 Months

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Background: Breast milk is the gold-standard feeding in the infancy period for optimal nutrition in the majority of diseases. Chronic diarrhea is an important worldwide pediatric disease. Although other studies indicated that risk of chronic diarrhea in non-breastfed infants increases, definite influence of breast milk in the prevention of various inflammatory diseases is still not well understood. The objective of this studywas determination ofthe relationship between levels of pro-inflammatory and anti-inflammatory cytokines in mothers' milk and risk of chronic diarrhea in their breastfed infants under 18 months. Materials and Methods: The current work is a case-control study. Forty-five infants with chronic diarrhea were selected from mothers who had breastfed their infants as long as 1-18 months. They were admitted in GastroenterologyandHepatology clinic in Afzalipour hospital; Forty- five infants without chronic diarrhea were selected from motherswho had healthy infants without any other inflammatory disease. In this survey a data collectionform was filled. Cytokine levels (TNF-α , IFN-γ , IL-13 and IL-4) were determined by Enzyme-linked immunosorbent (ELISA) assay in milk samples.Each cytokine was individually tested for determination of means, differences between 2 groups by Independent sample T-test. We compared the effect of the cytokines on decreasing or increasing riskof chronic diarrhea between 2 groups by Logistic regressiontest. Data were analyzed by using SPSS. Results: Levels of IL-4 and IL-13 (anti inflammatory cytokines) were statistically significant (P =0.012, P<0.001 respectively). Only effect of IL-13 on decreasing riskof chronic diarrhea was statistically significant (P =0.001). Conclusion: The results indicate riskof chronic diarrhea by increasing one unit (pg/mL) in level of IL-13 (anti inflammatory cytokine) as 54% decrease. So presence of this cytokine in the breast milk, will probably decrease riskof chronic diarrhea in infants.

Keywords: Pro-Inflammatory and Anti- Inflammatory Cytokines, Mothers' Milk, Chronic Diarrhea, InfantsUnder 18 Months

441. The Effect of Social Stresses on Response of Immune Cells and Serum Concentration of TNF-α, Interleukin-1 and Interleukin-6 in Mice

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Background: Social stress is a factor found to be involved in the etiology of many diseases. Gender differences are as factors affecting the predisposition of individuals for certain diseases. Results from animal and human studies suggest that socially stressed men are more vulnerable to disease and death than those of women. Materials and Methods: The role of chronic social stress and sex differences were examined in present study by implementing food deprivation, food intake inequality and unstable social status (cage-mate change every 3 days) for a period of 14 days in 76 male and female mice, then vital activity of peritoneal macrophages and spleen's lymphocytes by MTT test and the concentration of proinflammatory cytokines (IL-6, IL- 1 and TNF-α) by ELISA technique were assessed. Results: Our results showed cell viability of peritoneal macrophages decreased; however, cell viability of spleen's lymphocytes and proinflammatory cytokines concentration in serum of all female and male stressed animals in comparison with controls increased (P<0.05). Moreover, Sex differences in immune function were also apparent; in female subjects, these changes were prominent as compared to males. Conclusion: These results suggest that social factors have significant effects on immunity and should be considered in studies of sex differences in immunity at both proximate and ultimate levels for evaluating possible mechanisms contribute to that.

Keywords: Social Stresses, Response of Immune Cells, TNF-α, Interleukin-1, Interleukin-6

442. Impact of Iron Deficiency Anaemia on and Immunology of Recurrent Vulvovaginal Candidosis

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Background: Relationship between states of iron deficiency Anemia (IDA) and susceptibility to infections remains controversialA predominant Th1 response leads to resistance against recurrent vulvovaginal candidosis (RVVC) whereas a Th2 response exacerbates the disease through the inactivation of fungicidal effector cells .We investigated whether in RVVC iron deficiency through govering the polarization of Th1/Th2 cytokines could influence the host's susceptibility to disease. Materials and Methods: The study consisted of 92 women in 4 groups based on strict inclusion and exclusioncriteria: The first group, labeled ($RVVC^+$ IDA^+, consisted of 23 women with RVVC and IDA. The second group, labeled ($RVVC^+$ IDA^-), consisted of 23 women with RVVC Without IDA. The third group, labeled ($RVVC^-$ IDA^+), consisted of 23 women without RVVC and with IDA and forth group, labeled ($RVVC^-$ IDA^-), consisted of 23 healthy women without RVVC and IDA. . Blood samples were gained and the key cytokines (IFNγ،IL_{10}،IL_{12}،IL4) were measured. Results: The mean age of the participants was 31±7.9. Comparing IL-4 production between $RVVC^+$IDA+ (12.2±1.3) and $RVVC^+$IDA- (2.4±4.02) groups showed a significant difference (P=0.044) as well as between $RVVC^-$ IDA+(14.6±1.7) and $RVVC^-$IDA- (1.28±3.6) groups (P=0.006). IL-10 was produced significantly more in CAN+ IDA-(5.3±2.3) than CAN+ IDA(3.8±1.9) group (P=0.02).There were notsignificant differences between the levels of other measured cytokines in defined groups. There was a negative correlation between the serum iron and IL4 levels of the $RVVC^-$IDA-group (p=0.041).An association was seen in the $RVVC^+$ IDA- group between the IL10 and Hb levels (p=0.041)and there was another association between IL4 and TIBC in the same group (p=0.046). Conclusion: The results of this study showed that IDA can act as an underlying cause of candidiasis. It seems that in the IDA patients, the increase in IL4 leads to a response from Th2 and this result in the reoccurrence of candidiasis. Therefore IDA may act as a risk factor of RVVC.

Keywords: Recurrent candidiasis - Th1/Th2 – cytokine – cellular immune

443. The Effect of Genistein on Immunopathogenesis of Experimental Model of Glomerulosclerosis

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Background:Glomerulosclerosis is a cause of nephrotic syndrome in children and adolescents, as well as an important cause of kidney failure in adults. One of the effective factors in the etiology of this disease is Free radicals and ROS. Soybean protein contains isoflavones such as Genistein as antioxidantwhich can be proposed in treatment of Glomerulosclerosis. Materials and Methods: 40, adult male (Sprague Dawley) rats with15 weeks age were selected. They were randomly divided into four groups and isolated for 14 days using the selected diet: Normal group; was chow diet for 6 weeks without intervention, Patient groups; was chow diet for 6 weeks and intervention with single dose of 8 mg/kg Adriamycin IV injection to induce Glomerulosclerosis, Soybean group; was soybean protein diet for 6 weeks and Adriamycin injectionalong with daily oral administration of 50mg/kg carboxyl methyl cellulose (CMC), Soybean-genistein group; was soybean protein diet for 6 weeks and Adriamycin injection along with daily oral administration of 50mg/kg genistein. Urine collection was done every week. Rats were sacrificed after 6 weeks and blood samples were taken from the heart and then the kidneys, livers, hearts removed for histopathology. In second stage, the factors of malondialdehyde, protein carbonyl, total antioxidant capacity, and catalase activity were measured. Moreover, the expression of matrix metalloproteinase-2 on cell culture WEHI164 was done in order to evaluate the anti-inflammatory process. Results:Our findings show that the soybean´s genistein withisoflavone Property can reduce the level of proteinuria in patient rats compared with normal group and is able to prevent disease progression. Conclusion:The results of this study show that treatment with soybean´s genistein can reduce disease progression in experimental model of glomerulosclerosis.

Keywords: Genistein, Immunopathogenesis, Experimental Model of Glomerulosclerosis

444. Effect of Vitamin E on Immunity and Inflammation in Adults with Active Rheumatoid Arthritis (RA)

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Background: Little informationon the effects of Vitamin E on inflammation and immune function in RA is available so we decided to investigate the effect of Vitamin E on this autoimmune disease. This study investigated the effects of Vitamin E on the inflammatory mediators and Immunity factors in adults with active RA. Materials and Methods: In a randomized, double-blind, placebo controlled clinical trial 43 RA patients

were randomly divided into 2 groups, each group receiving one of the following daily supplement for 3 months; Group E: 400 mg Vitamin E, , Group P: placebo. Cytokines, Citrolinated Antibody (CCP-A) were measured by Elisa method, Vitamin E measured by HPLC. Results: After study there were no significant differences between groups in cytokines IL2, IL4, TNFα, IL1β, Il2/ IL4, CCP-A, WBC and Neutrophils, Lymphocyte, Monocytes, Eeosinophils numbers. TNFα decreased in 2 groups nonsignificantly, but its reduction was more in group E. IL1β increased in groups P and E (P=0.004, P=0.041respectively). IL4 decreased in groups E and P (P = 0.07, P=0.068 respectively). ESR (P≤0.05) and CRP(P=0.055) decreased in group E . Conclusion: Vitamin E probably can be useful in RA patients.
Keywords: Vitamin E, Immunity, Inflammation,active Rheumatoid Arthritis

445. Effect of Probiotics on Inflammatory Parameters and Oxidative Stress in Patients with Type 2 Diabetes

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Backgraund: Mortality from cardiovascular disease in diabetic patients and the growing incidence of diabetes, indicate the importance of providing of solutions to control and delay complications of diabetes. The present study examines the effect of probiotics on inflammatory parameters and oxidative stressas markers of cardiovascular disease. Materials and Methods: 40 Patients with diabetes type 2referredtotwo clinics in Shirazwere enrolledwithFBS ≥ 126 mgr/dland lessthan15years oftheirdiseasewereover andthe age range was 25to65years. UsingBalanced Block, they weredivided to either a probioticorplacebo groups and fasting blood samples tested for baseline hs-CRP,IL-6 and malondialdehyde (MDA). After sixweeks,fasting blood sampleswere tested again and datatested usingSPSS software. Results: After some detachment, eventually34 patients including 18 patients (16 female, 2 male) in the placebo group and 16 patients(10 female, 6 male) in the treatment group were studied. The mean age in the placebo and treatment groups were 51.8± 10.2 and 55.4±8 respectively.The mean±SEWHR and BMIwere 0.9±0.02 and 27.2±0.64 respectively in the placebo group and 0.94±0.13 and 27.9±0.95 respectively in the treatment group. After therapy with probiotics,MDA and IL-6 levels were reduced and hs-CRP levels were elevated, however all changes were not statistically significant. Conclusion: Overall results of this study indicate a declining trend in the MDA and IL-6 levels after consumption of probiotics. It appears the decrease in these parametersmay have effect on decreasing cardiovascular disease risk factors.
Keywords: Probiotics, Inflammatory Parameters, Oxidative Stress, Type 2 Diabetes

446. Effect of Lactobacillus casei Fermented Milk on Infection Induced by *Escherichia coli* O_{157}:H_7 in Mice

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Background*:* Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is a highly infectious pathogen that causes gastrointestinal illness with potentially serious consequences in human world wild. The organism is known to produce one or more shiga toxins, which may produce diarrhea, hemorrhagic colitis and life-threatening haemolytic uremic syndrome in human and animals. Recent reports have focused on novel biotherapeutic agents including probiotics , defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host. The aim of this study was to determine effect of consumption of fermented milk by lactobacillus casei as a probiotic on infection induced by *E. coli* O_{157}:H_7 in mice. Materials and Methods*:* In this study 45 mice of 6-8 weeks age were randomly divided into 5 groups, each containing 9 mice. These groups consisted of control group (A), Infected group (B), non-infected group fed by probiotic (C), pre-infected group fed by probiotic (D) and post-infected group fed by probiotic (E). Each of the mouse in groups (B, D) and E received 1.5×10^8 CFU/ml of *E. coli* O_{157}: H_7 throughintra gastric tube (gavage). Group (C) mice fed with 0.5ml of lactobacillus casei fermented milk daily for 14 days, group (D) mice fed with 0.5ml of lactobacillus casei fermented milk daily for 7 days post-infected and group (E) mice fed as mentioned above for 7 days prior to be infected. Stool of mice studied for recovery of *E. coli* O_{157}:H_7 before getting infected and on days 2,4and 7 after getting infected with the test organism. For identification of *E. coli* O_{157}:H_7 MacConkey sorbitol agar was used and for confirmation of the diagnosis specific antiserum against *E. coli* O_{157} was employed . Rate of food and water uptake were measured daily and weight of the mice were recorded in the beginning and last of the survey. Results*:* Alive *E. c*oli O_{157}:H_7 was not isolated in mice of Control group (A) and non-infected group fed with the probiotic (C) . Statistical analysis showed meaningful differences between group (E)and groups (B) and (D) ($p<0.01$) . Statistical differences in mean of gaining weight was not significant in groups studied. Conclusion*:* Consumption of fermented milk by lactobacillus casei can shorten the duration of illness and reduces the severity of the illness. This report shows that mice fed with probiotic L.casei have lower signs of infection as indicated by lower feces *E .coli* counts. Our results suggested that the lower feces *E .coli* counts and falling in duration and severity of illness may have a significant relation with enhancing immunological response to the pathogen. Further more studies are needed on human.
Keywords: Lactobacillus casei, *Escherichia coli* O_{157}:H_7, Mice

447. The Effects of Omega- 3 Fatty Acids on the Apoptosis of MCF-7 Cells

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Background: Essential fatty acids are molecules that cannot be synthesized by the human body but are vital for normal metabolism. One of the two families of these essential fatty acids is the omega-3 fatty acids. Although omega-3 fatty acids have been known as essential to normal growth and health since the 1930s, awareness of their health benefits has dramatically increased since the 1990s. The health benefits of the long-chain omega-3 fatty acids -DHA and EPA omega-3 - are the best known. Several studies report possible anti-cancer effects of omega−3 fatty acids (particularly breast, colon, and prostate cancer). Omega-3 fatty acids reduced prostate tumor growth, slowed histopathological progression, and increased survival. So, the aim of this study was to investigate the effects of DHA and EPA on the *MCF-7* cells (human breast adenocarcinoma cell line). Materials and Methods: For this purpose MCF-7 cell lines were treated with different concentrations of DHA and EPA (100, 150, 200 and 250) and evaluated the effects of DHA and EPA on apoptosis of MCF-7 cells after 4 and 24 hrs.by staining them with Annexin V Flous kit. Statistical analysis were made by non-parametric statistical tests (Kruskal Wallis and Mann Whitney U tests). Results: There was not any significant difference regarding the percentage of apoptotic cells between different concentration of DHA and EPA after 4 hrs. After 24 hrs. higher concentrations showed significant increase in inducing of MCF-7 apoptosis ($P<0.05$). Conclusion: It is concluded that probably DHA and EPA can help the immune defense against cancers. Of course more studied are needed in order to define their exact roles.
Keywords: Omega- 3 Fatty Acids, Apoptosis, MCF-7 Cells

448. Dietary Effect of Pomegranate Seed Oil Rich on Serum Levels of Lipids and Lipoproteins in Cholesterol-Fed Male Rats

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Background: hypercholesterolemia is one of the risk factors of cardiovascular diseases. Diet with high cholesterol increases LDL and decrease LDL receptors activity in liver. Oxidation of vessel wall lipoproteins increases development of inflammatory atherosclerosis. This study evaluates dietary effect of pomegranate seed oil rich on blood lipid levels in high cholesterol-fed rats. Materials and Methods : in this experimental study, three groups of male rats (n=10 for each group) were used. The control group received basic diet and one of the other two groups received a diet containing one percent cholesterol and while the other received the same diet plus1% pomegranate seed oil for one month. Results: after determining the values of TC, LDL, VLDL, HDL and TG the results indicated that in rats fed with 1% cholesterol apart from HDL and VLDL

the other lipids had increased significantly compared with the control group ($p<0.01$) .Supplementation with pomegranate seed oil decreased the TC and LDL levels and increased HDL level in comparison with the 1% cholesterol fed group ($p<0.01$). Conclusion: this effect of pomegranate seed oil may be related to its antioxidant and anti-inflammatory effects. The validity of these points in humans needs further investigations.
Keywords: Pomegranate Seed Oil Rich, Serum Levels, Cholesterol-Fed Male Rats

449. Improvement of Vitamin D Status via Daily Intake of Fortified Yogurt Drink Either With or without Extra Calcium Ameliorates Systemic Inflammatory Biomarkers in The Subjects with Type 2 Diabetes

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Background: Systemic inflammation is thought to have a central role in diabetic long-term complications. To investigate the effects of vitamin D either with or without extra calcium on certain inflammatory biomarkers in the subjects with type 2 diabetes (T2D). Materials and Methods: This was a double-blind, randomized, controlled trial over 12 weeks in 90 T2D subjects aged 30-60 years from both sexes. Subjects were randomly allocated to one of the three groups to receive two 250 mL bottles a day of either plain Persian yogurt drink or *doogh* (PD, containing 150 mg calcium and no detectable vitamin D3/250mL); vitamin D-fortified *doogh* (DD, containing 500IU vitamin D3 and 150mg calcium /250mL); or calcium+vitamin D3- fortified *doogh* (CDD, containing 500 IU vitamin D3 and 250mg calcium/250mL). Serum hsCRP concentration and cytokines (IL-1β, IL-6,TNF-α and IFN-γ) in the peripheral blood mononuclear cells (PBMCs) culture supernatants were measured and the changes in inflammatory markers were evaluated. Results: Compared to the baseline values, highly sensitive C-reactive protein (hsCRP), interleukin (IL)-1β, IL-6, concentrations significantly decreased in both DD and CDD groups. Though the decrement in hsCRP was more in CDD compared to DD (-4.0±8.5 vs. -1.3±2.8 mg/L), the differences was not significant. Conclusions: Daily intake of vitamin D-fortified *doogh* improved inflammatory markers in T2D subjects and extra calcium has no additional benefits.
Keywords: vitamin D; calcium; type 2 diabetes; cytokines; systemic inflammation

450. Effect of Vitamin A on Interleukin 17 in Patients with Ulcerative Colitis

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Background: Ulcerative colitis (UC) is an inflammatory disease of the rectal and colonic mucosa and seems to result from a complex series of interactions between susceptibility genes, the environment and the immune system. There is a paucity of data on the positive effect of vitamin A on intestinal mocusal immunity. We aimed to evaluate the effect of vitamin A supplementation on proinflammatory interlukin 17 (IL17) and CRP in ulcerative colitis. Materials and Methods: Immunological asssessment was done in 49 patients with ulcerative colitis participating in a before and after interventional survey. All participants were at clinical remission stages. Disease activity was assessed by Truelove- Witt Index. Vitamin A supplement was injected twice (50000 IU) with two weeks interval. Study period was considered 45 day. Serum IL17 and CRP levels were measured by ELISA and turbidimetric methods, respectively. Results: There was a positive correlation between IL17 levels and disease severity. Vitamin A supplementation had significant effect on decrease of IL17 ($P=0.003$) and clinical symptoms severity ($P= 0.001$), and CRP decreased ($P=0.002$) significantly too. Conclusion: These results suggest that Vitamin A supplementation could suppress inflammatory factors and be beneficial in reduction of clinical symptoms severity of disease in ulcerative colitis and should be considered as part of the treatment protocol.
Keywords: Vitamin A, Interleukin 17, Ulcerative Colitis

451. Effect of Vitamin A on Serum Resistin Levels in Ulcerative Colitis

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Background: Adipocytokines are involved in inflammatory and metabolic pathways in human beings. Human metabolism dramatically changes in ulcerative colitis (UC) and chronic inflammation is the hallmark of the disease. Resistin is a recently discovered cysteine-rich adipokine that has emerged during this decade as a promising inflammatory marker in various diseases. It is synthesized either from adipocytes or from immune cells, and exerts a pro-inflammatory profile in a variety of different experimental settings. According to recent experimental studies, vitamin A may downregulate resistin expression. The aim of this study was to investigate the effect of vitamin A supplementation on serum levels of resistin in ulcerative colitis patients. Materials and Methods: Resistin serum levels were measured in 48 UC patients before and after vitamin A supplementation, using commercially available enzyme-linked immunosorbent assays. Vitamin A supplement was injected twice (50000 IU) with two weeks interval . Study period was considered 45 days. C- reactive protein (CRP) was measured by turbidimetric immunoassay. Clinical disease activity was assessed by Truelove and Witts' score. Also body mass index of patients were calculated. Results: There was significant correlation between serum resistin with Clinical disease activity and CRP levels ($P = 0.02$ and $P = 0.04$), respectively. Vitamin A supplementation had significant effect on decrease of serum resistin ($P=0.003$) and clinical disease activity ($P=0.001$). While CRP changes were not significant. Conclusion: Serum-resistin levels are significantly decreased after vitamin A supplementation in UC patients, suggesting a possible pro-inflammatory status for resistin in ulcerative colitis and a role as a marker of successful therapy.
Keywords: ulcerative colitis, resistin, Vitamin A

452. The Effect of Probiotics on Gene Expression of Cytokines in Mucosal Tissue

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Background: Probiotics exert several immunomodulatory functions performing on cellular and humoral reactions. Also they can stimulate Type 1 Helper T Cells (L. Casei), mononuclear cells leading to increased clearance of circulating pathogenic bacteria (L. Acidophilus), macrophages to create TNF-alpha, IL-6 and NO production, increase clearance of circulating pathogenic bacteria by Kupffer Cells and they can harvest anti rotaviral IgA and antinfluenza IgG antibodies. Materials and Methods: Mucosal gene expression of the pleiotropic proinflammatory cytokines (interleukin(IL)-1beta, IL-6), TH1 cytokines (IFN-gamma, TNF-alpha, IL-12), regulatory cytokines (IL-10,TGF-beta), and the chemokine IL-8 were measured. Adding to measure the cytokines gene expression, the presence of polymorphonuclear cells in the mucosal tissue was assessed. Results: Patients who are treated with probiotics have significant lower mucosal mRNA expression levels of IL-1beta, IL-8, and IFN-gamma compared with placebo-treated.patients. A lower number of polymorphonuclear cells is present in the tissue of patients within the probiotic group compared with the number of polymorphonuclear cells in the tissue of patients receiving placebo and patients having an period of pouchitis. probiotic treatment is able to regulate the mucosal immune response reducing mucosal levels of neutrophilchemoattractant IL-8 and tissue influx of polymorphonuclear cells, and may further act by inhibit T cells activation, reinforce the barrier function and keep a tight control of the potent proinflammatory cytokine IL-1beta. Conclusion: Changes in the composition of the gut microbiota have been implicated in the pathogenesis of allergic disorders suggesting beneficial interactions between the intestinal immune system and specific bacterial strains.
Keywords: probiotic, gene expression , inflammation, cytokine

453. Improvement of Vitamin D Status Resulted in Amelioration of Biomarkers of Systemic Inflammation in the Subjects with Type 2 Diabetes

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Background: Both vitamin D deficiency and inflammation have been linked to cardiovascular disease (CVD), the major cause of death in diabetes. In this study, the effects of daily intake of vitamin D-fortified yogurt drink (*doogh*) on systemic inflammatory biomarkers in subjects with type 2 diabetes (T2D) were investigated. Materials and Methods: In this 12-week randomized controlled clinical trial (RCT), T2D subjects received either plain *doogh* (PD; containing 170mg calcium and no detectable vitamin D/250mL, n_1=50) or vitamin D3-fortified *doogh* (FD; containing 170 mg calcium and 500 IU/250mL, n_2=50) twice a day. Glycemic status, body fat mass (FM) and systemic inflammatory biomarkers including serum hsCRP, serum amyloid A (SAA), interleukin(IL)-2, IL-6, IL-10 and tumor necrosis factor (TNF)-α were evaluated at the beginning and after the intervention. Data were expressed as either mean±SD or median (interquartile range [IQR]) whenever they had either normal or non-normal distribution, respectively. Results: In the FD group, compared to the PD group, a significant increase in serum 25(OH)D was accompanied by significant changes in TNF-α (-57.9 (-264.6) vs. +106.3 (683.2), p=0.044), IL-6 (-6.3 (-69.2), p=0.002), hsCRP (-0.39 (-1.50) vs. +0.8 (1.52), p<0.001), SAA (-14.2±44.5 vs. +5.6±37.5 mg/L, p=0.022) and IL-10 (+38.7±157.0 vs. -51.9±165.2 ng/L, p=0.013). The between-group differences of hsCRP, SAA and IL-6 changes remained significant even after controlling for changes QUICKI (p<0.001, p<0.001 and p=0.009 respectively). Conclusions: Improvement of vitamin D status of T2D subjects resulted in amelioration of the systemic inflammatory markers. This may have preventive implications against CVD and other diabetic complications.
Keywords: Vitamin D, Systemic Inflammatory Biomarkers, Type 2 Diabetes

454. Evaluation of Serum Levels of IFN-α, IFN- γ, TNF-α,TGF-β IL-1α, Cortisol and Immunoglobulins in Islamic Ramadan Fasting

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Background: This search aimed determining the levels of cortisol, immunogloubulins and major serum cytokines, following Ramadan fasting, as Immune response indexes. Materials and Methods: For this before and after Ramadan fasting study, twenty five students residing at the dormitory of the Tehran university of medical sciences were selected. All cytokines level (Il-1 α, IFN-α, IFN- γ, TNF-α,TGF-β) were determined by ELISA method, Immnoglubolins were determined by the Redial immunodiffiusion (SRID) technique and cortisol levels were measured by EIA too. Results: While IFN- γ, a showed a considerable increase after Ramadan Fasting (p<0.01), IFN-α and TNF-α had decreased(p=0.5 and,0.02 respectively)Serum level of TGF-β increased but not significantly(p.0.3), cortisol also increased after one month of fasting (p<0.05). Serum Il-1 α had a non significant decrease (p>=0.5). Although IgG & IgM levels showed no significant increase, IgA levels showed significant decrease (p<0.5). Conclusion: Our data revealed that Ramadan Fasting increase IFN- γ, while decreasing TNF-α and IgA levels, Ramadan Fasting can probably increase T-cell function, lower the inflammatory appreance rate and effect immunoglobulin production.
Keywords: Islamic Rmadan Fasting, Cytokine, Cortisol, Immunoglobuline

IMMUNOLOGY of BACTERIAL DISEASE

Oral Presentation

455. Sensivity and Specificity of Antibodies against Recombinant Biofilm Associated Protein to Detect *Acinetobacter Baumannii* Infection

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Background*: Acinetobacter baumannii* is an emerging nosocomial pathogen resistant to many antibiotics. The treatment of infections caused by *A. baumannii* has become increasingly complicated. Nevertheless no vaccines or antibody-based treatments have been developed for A. baumannii infections. Serological methods based on the detection of elevated levels of antibody to microbial antigens offer rapid, non-invasive detection of infection. Hence, a fast, sensitive and specific test for rapid diagnosis is needed. In this study we have focused on diagnosis of *Acinetobacter baumannii* infection by serum antibody produced against recombinant protein from the conserved region (G1E2F2G2G3) of bacterial Bap gene. Materials and Methods: Recombinant Bap subunit (539 acid amine) was expressed, purified and injected to mice. The immunized mice showed significant rise of IgG. The antibody specificity was evaluated using Bioinformatics tools and ELISA. Other bacteria such as Salmonella and Staphylococcus were taken as negative control. Animals received adjuvant and PBS had no Bap-specific antibodies in serum.Resultsand Conclusion: Bap is suggested as a candidate for development of a diagnostic test for the presence of *A. baumannii*. Our recombinant protein is a potential candidate for use as a diagnostic biomarker.
Keywords: Sensivity, Specificity, Recombinant Biofilm, *Acinetobacter Baumannii*

456. Production of *Mycobacterium tuberculosis* ESAT-6 Recombinant Protein and Use of this in Skin Test

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Background: Tuberculosis (TB) is the leading infectious disease in the developing world. In the 1882, Robert Koch has identified *Mycobacterium tuberculosis* and then in 1920 a delayed-type hypersensitivity skin test reaction has introduced based on tuberculin purified protein derivative (PPD). Unfortunately, this test is incapable of distinguishing *Mycobacterium tuberculosis* (MTB) infection from bacille Calmette-Guérin (BCG) vaccination or infection with non-tuberculous mycobacteria. Thus, there is an urgent need to develop a prefect and sensitive test for detection of tuberculosis. For introducing a more specific diagnostic tool for TB detection, this study was performed for cloning and expression and skin test reaction of early secreted antigen target 6 (rESAT6), a secretory protein found only in MTB, *M. bovis*, and few other mycobacterial species. Materials and Methods: After amplification of esat-6 gene from *M. tuberculosis* H37Rv genome, it was cloned in expression vector (PQE60) and followed for expression in *E.coli* M15 and purified with Ni-NTA agarose affinity chromatography. The expressed protein was confirmed with electrophoresis and western blotting. For skin test, different groups of guinea pigs were sensitized with *M. tuberclusis*, *M. avium* and BCG vaccine and two months later skin test was performed with ESAT-6 and PPD.Results: Our results showed that recombinant protein of ESAT-6 (rESAT-6) was successfully expressed and purified in prokaryotic system. Skin test data show that, unlike PPD skin tests, purified rESAT6 antigen elicited a positive skin response in animals exposed only to MTB and no skin responses were observed in the guinea pigs sensitized with BCG vaccine, or with *M. avium*. In compare of PPD, The sensitivity of rESAT-6 was reported as 114 in potency test. Conclusion: This could hypothesize that ESAT6 is more specific to MTB infection than PPD and could be replaced as a more specific skin test for detection of tuberculosis in large animals or human.
Keywords: *Mycobacterium tuberculosis*, Skin test, PPD, ESAT-6,rESAT-6, PQE60 vector

457. Evaluation of the Antigenicity Properties of the Vibrio Cholera Recombinant Flagellin Protein A

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Background: Flagellum is a major virulence factor in vibrio cholera pathgenicity. Flagellum consists of peptide units which were known as flagellin. V. cholera genome contains five genes that encoded flagellin (FlaA, FlaB, FlaC, FlaD, FlaE). Among these, FlaA gene is necessary for assembling and function of flagellin. In spite this role, it has been poorly studied. The main aim of this study was expression and production of the recombinant flaA protein in *E.coli* by expression Vector PGEX4T1. Materials and Methods: The FlaA gene was provided by polymerase chain reaction method with using the specific primers and further cloned into the DH5a easy vector. The cloned FlaA gene was cut out again with two restriction enzymes and was inserted into the prokaryotic expression vector pGEX-4T-1. The cloned vector was transformed to *Escherichia coli* BL21-DE3 and successfully expressed by induction of IPTG. Expressed protein was purified by GST affinity resin. For preparation of primary antibody purified recombinant protein was injected to mice. Western blot assay method was used for determining the antigenicity of recombinant flaA. Results and Conclusion: Results of this study demonstrate that FlaA protein is immunogenic and could be evaluated as vaccine designing and a diagnostic tool for detection of cholera infection.
Keywords: Vibrio cholera , FlaA , flagellin, cloning

458. The Assessment of Cytokines Responses against *Bordetella pertussis* Antigens in Animal Model

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Background: Cytokines are non-antigenic, specific low-molecular weight proteins that mediate cellular interactions involving humoral and cell mediated immunity. Colonization of the respiratory tract by the gram-negative coccobacillus *Bordetella pertussis* results in whooping cough, a significant cause of morbidity and mortality in human infants. Since the causative agent has an intricate nature and survives in the host both as an intracellular and extracellular parasite, therefore the study of immunological responses to this organism on base of cellular and humoral immunity as well as induction of cytokines is very important. In this study we have demonstrated thatinduction of cytokines as a parameter of immune responses,couldbe a valuable tool for the evaluation of the immunityagainst *B. pertussis* antigens. MaterialS and Methods: In order to investigate the immunological responses against two main extracellular antigens of *B. pertussis* consist of detoxified pertussis toxin (PTd)and filamentous hemagglutinin (FHA) were studied. The above antigens were used as a singular or combined and with or without following adjuvants; aluminum phosphate, saponin and BCG. Several groups of animals were injected at days 0, 30, 60 injected with the antigens and compared with the control group injected with killed *B. pertussis* suspension. The following cytokines IL-4, IL-10 and IFN- γ in all of the groups were measured by ELISA. Results: Our results indicated that the highest amount of IL-10 and IL-4 were related to the groups were immunized with PTd alone and together with FHA combined with saponin adjuvant,also the group injected with killed *B. pertussis* suspension showed the highest amount of above cytokines. In addition the highest amount of IFN - γ was produced by the group which were injected with killed suspension of *B. pertussis*. Furthermore, simultaneous injection of PTd alone and combined PTd, FHA together with BCG increased production of IFN - γ in mouse spleen lymphocytes. Conclussion: previousstudies have demonstrated protective immunity against *B. pertussis* infection induced by Th1 cells. Cytokines which are associated with Th1 are involved in protection in various *B. pertussis*infection models, and in particular in humans. The induction consist of axis IL-12-IFN-γ for protectionfrom pertussis, our above results emphasized the importance of Th1 cytokines production and its cooperation with cellular immunity against pertussis infection and suggest that measurement of the induction of cytokines as a parameter of immune responses, couldbe an valuable tool for the evaluation of the immune response to*B. pertussis*. Furthermore the evaluation of T-cell responses to pertussis antigens may provide information on the protective immunity by acellular pertussis vaccines in animal models.
Keywords: Pertussis antigen, Cytokine assay, Protective immunity

Poster Discussion Presentation

459. Detection of *Helicobacter pylori* in Infective Nasal Polyp and Sinus Mucosal Specimens of Patients with Nasal Polyp and Comparison with Sinus Biopsy Specimens of Healthy Control Group by Immunologic and PCR Methods

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Background: There are several studies evaluating *Helicobacter pylori* (*H. pylori*) in nasal and sinus mucosa in chronic rhinosinusitis; however studies dealing with the direct evaluation of *H. pylori* in nasal polyps are limited. So the objective of this research was to evaluate the frequency of *H. pylori* nasal polyp and sinus mucosa of patients with nasal polyp in comparison to sinus biopsy specimens of healthy control group. Materials and Methods: In this case-control study 62 patients with nasal polyp and 25 healthy individuals with nasal bone fracture, older than 12 years and without any chronic systemic disease were enrolled by nonrandom consecutive sampling method. Serum anti-H.pylori IgA and IgG were evaluated by ELISA and the antigen was evaluated by PCR in nasal polyp and sinus mucosa specimens of patients and controls, respectively. To compare the study variables between the two groups Chi-square analysis was performed. Results: Median age of patients and controls was 38 years (range: 12-65 yrs) and 26 years (range: 18-54 yrs), respectively. Male percentage was 63% in patients and 40% in controls. IgA positives were similar in both patients and controls (14.5% vs. 4%, p-value=0.27), but significant difference was observed in case of IgG (71% vs. 32%, p-value=0.001). Also the PCR results were different between groups (32.3% vs. 4%, p-value=0.005). There were more cases of both IgG/PCR positive results in patients group (29% vs. 4%, p-value=0.01). Conclusion: Based on the molecular study and variation in IgG concentration for *H. pylori*, there is a correlation between *H. pylori*, as a potential etiologic agent, and nasal polyp in our study.
Keywords: Nasal polyp, *Helicobacter pylori*, ELISA, PCR

460. Pertussis Toxin Engages Toll like Receptors 2 and 4 to Activate the Innate Immune System

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Background: Pertussis Toxin (PT) is the major virulence factor of Bordetella pertussis (BP), the causative agent of whooping cough, and also the main component of acellular vaccines against this disease. There are lots of reports in the literature regarding PT interaction with different immune cells and molecules, but we have less understanding about its effects on different pattern recognition receptors such as Toll-like receptor (TLR) molecules. Here PT was purified from BP culture supernatant and its interaction with TLR2, TLR4 and TLR5 was studied. Materials and Methods: Purification of PT was performed using antibody-based affinity chromatography. The purified antigen was characterized by SDS-PAGE and Western blot analysis. TLR interaction and signaling were investigated using HEK-293 cell lines cotransfected with different TLR molecules and NF-kB reporter gene. Stimulation of these cell lines was monitored with measurement of both secreted alkaline phosphotase and IL8 in the supernatant. Results: Our results indicate that PT was successfully purified from culture supernatant at a high purity. Stimulation

conditions of different TLR transfected HEK-293 cells were optimized by their commercial ligands including heat-killed Staphylococcus aureus Cowan I (SAC), Lipopolysaccharide (LPS) and Flagellin for TLR2, TLR4 and TLR5, respectively. Stimulation studies showed that PT can trigger TLR2 and TLR4 molecules, but has no effect on TLR5 molecule. Conclusion: Our data has demonstrated interaction of PT with TLR2 and TLR4 receptors suggesting its implication in induction of the innate immune system against BP through the TLR molecules.
Keywords: Bordetella pertussis, Pertussis Toxin, Toll like Receptor, HEK293

461. Expression and Immunogenicity of *Salmonella Typhi* Outer Membrane Protein A

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Background: Typhoid fever remains a major public health problem in developing countries. As yet, little is known about the microbial factors that determine pathogenicity and those that elicit a protective immune response in humans with typhoid infection. The current study was undertaken to assess the ability of the outer membrane protein A (OmpA) of *Salmonella typhi* to induce a humoral immune response in humans with typhoid fever by using enzyme-linked immunosorbent assay techniques(ELISA). Several investigators have shown that C-terminal domain of the OmpA contains an important protective epitope therefore OmpA, like porins and LPS,is also a target of the host immune response. Materials and Methods: OmpA protein of *S.typhi* PTCC 1609 was cloned, expressed and purified. Mice were immunized with 10mg of recombinant protein on days 0,15,30 and 45, by injecting 0.1ml of antigen/adjuvant mixture.Blood samples were collected 10 days post-injection. An additional 10 BALB/c mice served as a control group. Quantitation of antibodies raised against recombinant OmpA was performed by ELISA. Results: Comparison of high titer anti OmpA IgG in control and test groups shows that the recombinant OmpA produced a high titer ($P < 0.001$) of IgG in BALB/c in response to 3 injection routes of intraperitoneal, subcutaneous and intramuscular with intraperitoneal injection leading to higher antibody titer.
Conclusion: Marked IgG responses in mice immunized with OmpA support the immunogenic nature of recombinant OmpA suggestive of its application as a good immunogen for vaccine studies.
Keywords: Immunogenicity, *Salmonella Typhi*, OmpA

462. Comparison between Expression Severity of TLR4 and TLR2 in Biopsy Specimens of Antrum and Corpus Zone of the Stomach in *H. Pylori* Infection

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Background: Toll like receptors are transmembrane proteins on the surface of the host cells that initiate intracytoplasmic reactions cascade through sending signals after diagnosis of *H. Pylori* lipopolysacarides.These signals end in increase of expression in many pro-inflammatory cytokine and chemokine genes. Products of these genes cause inflammation and destruction in gastric ulcers. It's doubtful that which of TLR molecules have role in bacteria recognition. Comparing the expression severity of TLR4 and TLR2 molecules in biopsy specimens of *H. Pylori* infected patients. Materials and Methods: 38 patients with gastrointestinal disorders classified into four groups entered this study. A group with *H. Pylori* infection and gastric ulcer (n=15), another with gastric ulcer without *H. Pylori* infection (n=5), the other one with *H. Pylori* infection without gastric ulcer(n=10) and a normal group (n=8). Biopsy specimens from antrum and corpus site of redness or atrophic mucosa in patients with gastritis were derived separately.RNA samples from antrum and corpus biopsies were prepared separately and cDNA of each specimen was made. Results: Our data showed that there is a significant increase in expression severity of TLR4 analogous with TLR2 in the antrum zone of H.Pylori infected patients with gastric ulcer(3.9±3.6 (0.6-14.3) vs 2.5±2.8 (0.6-9.6), P=0.05).But there isn't any significant differentiation in corpus zone between these tow receptors. There isn't any significant differentiation in expression severity of TLR4 and TLR2 between the antrum and corpus of *H. Pylori* infected patients stomach with gastric ulcer .Also no meaningful difference was seen between these 2 zones in *H. Pylori* infected patients without gastric ulcer for TLR4 and TLR2. No remarkable difference was seen in the normal group. Conclusion: We believe that TLR4 has more interfere in *H. Pylori* diagnosis in comparison with TLR2. Also we believe that both TLR4 and TLR2 molecules in antrum and corpus of the stomach express with the same severity.
Keywords: TLR4, TLR2, Antrum, Corpus Zone of the Stomach, *H. Pylori*

463. Development of Heat-Killed *Salmonellae Thyphimurium* Vaccine, Evaluation of it's Protectivity and Immune Responses in Mice Model

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Background: Salmonellosis, which is caused by *Salmonellaespp*, remains a major health concern in man and livestock. *Salmonellae* can stay alive in macrophages so cellular immunity plays an important role in defending against these bacteria. Vaccination is widely suggested as the most reliable approach to control the invasive serovars. Here, we develop formalin – heat killed *Salmonella Thyphimurium* vaccine containing Iranian common serotypes and evaluated it's immune responses and protectivity in mice model. Materials and Methods: Interapretoneal injection of 0.5 ml of suspension with different doses (36, 72, and 150 cfu/ml bacteria) in nine groups of Balb/c mice for LD50 determination was done. For vaccination of Balb/C mice, formalin–heat killed *Salmonella Thyphimurium* was subcutaneously injected in three times with 2 week intervals. Blood samples were collected on days 0,7,14,21,28,35 and serums were used for study of specific antibody assay by ELISA. One week after last immunization live *Salmonellae Thyphimurium* injected for protectivity assay (challenge). On day 42 mice were sacrificed and spleen cells were used for cell culture and cytokine assay. Results: LD50 of *Salmonella typhimurium* was 36 cfu/ml live bacteria in i.p injection. After 1 month of challenge about 75% of control mice (PBS injected) died but all vaccinated mice survived. Vaccine received mice had a greater titer of antibody in compare to control groups. We are collecting information on production of IL4 and IFN-γ of this vaccine, so we will give these data after additional analysis in future for congress day. Conclusion: These results suggest that our inactivated vaccine can be useful for induction of specific immune responses and preventing of salmonellosis in mice model. This could be considered as a preliminary result for future study of national salmonaella vaccine.
Keywords: *Salmonellae Thyphimurium* Vaccine, Immune Responses, Mice

464. Specificity of Serum Antibody against a Recombinant Conserved Region of *Acinetobacter baumannii* Bap Gene

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Background: *Acinetobacter baumannii* is an emerging nosocomial pathogen that is resistant to many types of antibiotics, and hence, a fast, sensitive, specific, and economical test for its rapid diagnosis is needed. Presence of organism is still detected using conventionalculture methods and itsidentification is by biochemical means. The conventional method is time consuming and may take at least 2–5 days toproduce results. Late detection of the bacteria in patientscauses problems in disease control and can result in severecomplications in patient care and management. . Development of rapid test requires a specific antigen, and outer membrane proteins (OMPs) are the prime candidates. Matrials and Methods: A biofilm-associated-protein epitope of 371 amino acid, was expressed and purified. After bioinformatic analysis, *Salmonella enteritidis* and *Staphyloccocus aureus* surface proteins showed high similarities to recombinant Bap and were selected for cross reaction tests. *Pseudomonas aeruginosa* was selected as a bacterium with high biofilm formation ability. Total of twenty four BALB/c mice were divided in six groups. Group

one to four were injected with *A.baumannii*, *S.aureus*, *S.enteritidis*, *P.aeroginosa* whole cells respectively. Negative control group was injected with PBS and recombinant Bap immunized mice served as positive control. For whole cell immunizations, LD_{50} were determined and all injections were under LD_{50}. Each group was immunized with three injections at 15 days interval. Blood was collected before each injection. Indirect ELISA was performed using Bap recombinant protein as an antigen. Results: Immunization of each group was confirmed with whole-cell ELISA with injected bacterium serving as an antigen. Results of indirect ELISA with recombinant Bap protein indicated that recombinant protein is specific to *A.baumannii* showing no interaction with sera collected from groups immunized with *S.aureus*, *S.enteritidis* and *P.aeruginosa*. Conclusion: recombinant Bap could be a good candidate for rapid and specific diagnosis of *A.baumannii* infection.
Keywords: *Acinetobacter baumannii*, Bap Gene, biofilm

465. Evaluation of Expression of IL-23 Gene in the Pathogenesis of Tuberculosis

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Background: Tuberculosis is one of the most important infectious diseases with high mortality rate in the world especially among developing countries. Interleukin-23 (IL-23), a member of the IL-12 family, is a heterodimeric cytokine that is composed of the p40 subunit of IL-12 plus a unique p19 subunit. Interleukin 23 (IL-23) has an important role in the development of chronic inflammation and early cessation of bacterial growth. IL-23 reduces the bacterial burden and promotes granuloma formation when IL-12 is absent. Furthermore it is essential for establishment of an IL-17-producing CD4+ T cell population in the lung. In order to quantify the level of IL-23 gene expression in peripheral blood mononuclear cells (PBMCs), a real time RT-PCR TaqMan method was developed in our lab for each one and then the validity were assessed. Materials and Methods: PBMCs were isolated from peripheral blood of PPD skin test positive healthy donors and newly diagnosed Tuberculosis patients by using Ficoll-hypaque density centrifugation. PBMCs were cultured in RPMI 1640 complete culture media. Cells were activated with purified protein derivative (PPD) (5 TU/0.1 ml) for 72 hours and then after harvesting the cells, total RNA was extracted and cDNA was synthesized. A real-time PCR Taqman method was designed and optimized for evaluation of IL-23 human gene expression. The results for activated and unactivated cells in active TB patients and positive healthy donors were analysed. Results: Using our optimised TaqMan real-time PCR (R> 0.95 , Efficiency > 0.9),the analysed data for this study have been showed that Expresion of IL-23 in PBMCs of active TB patients is significantly lower compared to positive healthy donors .Conclusion: This study demonstrates that IL-23 in active TB patients is significantly lower than positive healty donors and may play a critical role in the regulation of mycobacteria-induced inflammation and suggests that IL-23 could be a potential target for immunotherapy to treat airway inflammation in tuberculosis.
Keywords: IL-23, Pathogenesis, Tuberculosis, PBMCs, Real time PCR

466. Evaluation of Expression of Foxp3 Gene in the Pathogenesis of Tuberculosis

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Background: Tuberculosis (TB) remains a major cause of morbidity and mortality around the world. Regulatory T cells (Treg) constitute key components of peripheral tolerance suppressing potentially autoreactive T cells and preventing autoimmune diseases. *Forkhead box P3 (FoxP3) is a* transcription factor whose expression characterizes regulatory T cells (Treg). The number of FoxP3-expressing cells increased in the blood of patients with tuberculosis. Therefore, CD4+CD25+FoxP3+ Treg expanded in TB patients suppress M. tuberculosis immunity and may therefore contribute to the pathogenesis of human TB. In order to quantify the level of Foxp3 gene expression in peripheral blood mononuclear cells (PBMCs), a real time RT-PCR TaqMan method was developed in our lab for each one and then the validity were assessed. Materials and Methods: PBMCs were isolated from peripheral blood of PPD skin test positive healthy donors and newly diagnosed Tuberculosis by using Ficoll-hypaque density centrifugation. PBMCs were cultured in RPMI 1640 complete culture media. Cells were activated with purified protein derivative (PPD) (5 TU/0.1 ml) for 72 hours. Activated cells were harvested and RNA was extracted for making cDNA. A real-time Taqman methode was designed and optimized for evaluation of Foxp3 human gene expression. The results for activated and unactivated cells in active TB patients and positive healthy donors were analysed. Results: Using our optimised TaqMan real-time PCR (R> 0.95, Efficiency > 0.9), the analysed data in this study indicated that there is a significant increasing in foxp3 gene expresionof active TB patients in comparison with positive healthy donors after PBMCs activation with PPD. Conclusion: In the present study, we demonstrate that CD4+CD25+FoxP3+ Treg increase in the peripheral blood of PTB patients compared to healthy donors. CD4+CD25+FoxP3+ T cells that increased during TB infection were functionally characterized by their ability to suppress M. tuberculosis specific immunity that may foster the chronicity of MTB infection.
Keywords: Foxp3, Pathogenesis, Tuberculosis, PBMCs, Real Time PCR

467. A Modified ELISA System to Identify Natural Immunity to *Haemophilus influenza* type b Anti Capsular Antibody

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Background: *Haemophilus influenza*e type b (Hib) is one of the most important causes of meningitis and other bacterial infections, especially in children less than 6 years of age. Among *Heamophilus influenzae* serotypes, serotype b is the most pathogen that can be isolate from patient's samples. It is essential to screen the carriers and patients by a simple, fast, low cost and specific method that can assay the antibody's titration especially in children and infants. Materials and Methods: A modified ELISA system was developed to detect antibodies against capsular polysaccharide of *Haemophilus influenza*e type b in serum samples by use of Bovine Serum Albumin. Eighty clinical serum samples were collected from three groups of children less than 6 years of age and examined by this system to identify their natural immunity to Hib. Sensitivity and specificity of this modified ELISA system compared with a commercial kit produced by The Binding Site (VaccZyme ™ Human Anti *Haemophilus influenzae* EIA Kit). Results: In the present study 29.45% of children did not have the minimum level required for protection. Titers expected to be protective for immediate but short-term periods (0.15 - 0.99 µg/ml) were observed in 30.58% of children. About twenty two percent of children had long-term protective anti-CP antibody titers of 1 - 5 µg/ml. Anti capsular antibody were detected by optimized method for each sample. Optical densities were similar to commercial kit. False-negative or false-positive didn't observe. No background reactions have been seen. Conclusion: Comparison between results of modified ELISA procedure and the commercial kit's result suggest that the method is reliable. We found that the protective titer of antibody against Hib is low in children under 1 year old and gradually increases with age as it shown in other study.
Keyword: ELISA, Natural Immunity, *Haemophilus influenza* Type b, Anti Capsular Antibody

Poster Presentation

468. The Role of Group A Beta Hemolytic Streptococcal Infections in Patientswith tic and tourett's Disorders

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Background*:* Recently, many cases diagnosed as pediatric autoimmune neuropsychiatric disorders associated with group A beta hemolytic streptococcus infection (PANDAS) due to production of autoimmune antibodies. Object of this study was comparison the titer of antibodies

against group A beta hemolytic streptococcus (ASOT, Anti-DNase B, and Anti streptokinase) between children with movement disorders (tic and tourett's disorders; pediatric autoimmune psychiatric disorders) and healthy control. Materials and Methods*:* A cross sectional/ cases control study in pediatric neuropsychology ward and clinics in two referral hospitals (Rasoul & Aliasghar) affiliated by IUMS had done in Tehran, Iran (2008-2010). We selected 53 children with tic disorder and 76 healthy controls (age matched children). The antibody titers (IU/ml) in their area were compared and analyzed statistically. The area under ROC, sensitivity, specificity and positive predictive value of tests calculated. Results*:* Age of cases was between 4-16 years. All antibody titers had significant difference between two groups (p<0.0001; p=0.05; p=0.002 for ASOT, Anti-DNase and Antihyaloronidase respectively). ASOT (cut off level> 200IU/ml) had 75% sensitivity;
84% specificity and 80% PPV; Anti- streptokinase (cut off level> 332IU/ml) had 34% sensitivity; 85% specificity, and 90% PPV; Anti-DNase (cut off level> 140IU/ml) had 70% sensitivity; 99% specificity and PPV 90%. *Conclusion:* Patients with tic disorder had a significant high antibody titer against streptococcal infection in comparison with healthy children. It presents possible role for streptococcal infection in tic disorders. Treatment of streptococcal infection is achievable by using of long acting Penicillin in our country. Use of aggressive treatment like plasmaphresis etc needs future RCT studies.
Keywords: Beta Hemolytic Streptococcal Infections, tic and tourett's Disorders

469. Comparison of Gamma Interferon Produced by Peripheral Mononeuclear Cells and Tuberculin Skin Test in Active Pulmonary Tuberculosis

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Mycobacterium Tuberculosis induces infection in 1/3 of world population. It causes 8 million new infections and 2 million deaths in each year. Rapid diagnosis and effective treatment of patients are the best way of TB control. Tuberculin skin test is the oldest diagnostic test for TB infection that is evaluated with intradermal injection of PPD solution. In-vitro T-cell reaction to different mycobacterial antigen with Gamma interferon release is used now. Purified Protein Derivative (PPD), Early Secretory Antigen Target-6(ESAT-6), and Culture Filtrate Protein-10(CFP-10) are some of them. Gamma interferon is measured by Enzyme-linked Immunospot Assay (ELISPOT)orEnzyme-linked Immunosorbent assay (QuantiFERON). These new methods have some advantages such as: 1- quick result in 1 day, 2- no need to revist the patient, 3- no interfer with anergy and immunosupression, 4- specific for M. Tuberculosis, 5- without any booster effect. Accuracy of these tests for diagnosis of tuberculosis is mainly dependent to the prevalence of infection in the society, so we decided to evaluate QuantiFERON in Iran and compare it with PPD. This survey was a cross-sectional study. Patients with pulmonary TB and positive sputum smear or culture from March 2008 to March 2009 that were admitted in Hazrat Rasoul Akram Hospital have evaluated. TST and QuantiFERON test were done for them. Mean ± SD for quantitative variables and percentile for qualitative variables were used. Mc-nemar and Kappa test were used for evaluation of agreement between TST and QuantiFERON. We had 34 patients with 20 (58.8%) female and 14 (41.2%) male patients. Their mean age was 20.77±51.35 years. Their PPD mean was 5.9±7.4mm and 70.6% of patients had lower than 10mm and 17.6% had between 10 to 15 mm and 11.6% had more than 15 mm PPD size. Mean age of these groups had no statistical differences (T test, CI95%, p=0.9). 8 patients had positive Quantiferon test with mean age of 47.12±26.45 years. 26 patients had negative test with mean age of 52.65±19.13 years. Mean age of these groups had no significant differences (T test, CI95%, p=0.5). 10 out of these 26 patients had indeterminate result. In this study according to McNemar test, Quantiferon and TST test had significant differences (P= ۰.۰۰, K=0.05). In this study there is no correlation between TST and Quantiferon and Quantiferon was not a good diagnostic test for diagnosis of active TB.

Keywords: Gamma Interferon, Peripheral Mononeuclear Cells, Tuberculin Skin Test, Active Pulmonary Tuberculosis

470. New Skin Test in the Diagnosis of Tuberculosis

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Tuberculosis is an infectious disease. Up to one-third of the world's population is infected with latent TB. Active tuberculosis may develop in 5 to 10 percent of people with latent infection. Detection of latent TB is therefore important in controlling the disease. The TB skin test (PPD) is the most commonly used test for diagnosing TB. Unfortunately, this test is incapable of distinguishing *Mycobacterium tuberculosis* (MTB) infection from BCG vaccination or infection with non-tuberculous mycobacteria. Thus, there is an urgent need to develop a prefect and sensitive test for detection of tuberculosis. Aim of this research was based on purification of lowmolecular weightproteins of MTB for develope a specific mantoux test for diagnosis of tuberculosis. Materials and Methods: At this current study, we extracted protein purified derivatives from short term culture filtrate ofMTB as antigenic cocktail and protein concentrations were determined by using the lowry protein assay. Lowmolecular weightproteinswerepurifiedbySephadex-G75 gel chromatography. For skin test, different groups of guinea pigs were sensitized with MTB and BCG and skin test were performed with purified proteins and PPD.Results: Skin test data showed that, unlike PPD, purified proteins elicited a positive skin response in animals exposed only to MTB and no skin responses were observed in the guinea pigs sensitized with BCG. Conclusion: This study demonstrated that new test is specific to MTB infection than PPD and could be replaced as a more specific skin test for detection of tuberculosis in large animals or human.
Keywords: New Skin Test, Diagnosis, Tuberculosis

471. Characterization of Low Molecular Weight Proteins from *Mycobacterium tuberculosis*

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Background: Tuberculosis is a disease caused by a bacterium called *Mycobacteriumtuberculosis*. Low molecular weight proteins secreted by *Mycobacteriumtuberculosis* induce strong immune responses in tuberculosis and constitute prime candidates for development of novel vaccines against tuberculosis as well as for immunodiagnostic assays. The purpose of the study was to determine melting temperature (The temperature at which 50% of the protein is unfolded, T_m) and pH stability of Low molecular weight proteins at different temperatures.Materials and Methods: After isolation and purification of low molecular weight proteins from *Mycobacteriumtuberculosis* by chromatography, the Purified proteins were investigated at different conditions including different pH values and different temperature. Furthermore, melting temperature was determined by denaturation curve.
By taking help from absorption curve of temperature changes the value of melting temperature was found to be 62˚C . On the other hand the protein with low molecular weight resistance to pH at 40˚Cas compared to the temperature of 20˚C and 60˚C. Results and Conclusion: The value of T_m = 62˚Cindicating high temperature resistance of this complex protein. Also negligible changes in absorbance with different pH at 40˚C indicating high resistance of this complex protein than pHChanges at near body temperature.
Keywords: Characterization, Low Molecular Weight Proteins, *Mycobacterium tuberculosis*

472. Isolation and Purification of High Molecular Weight Proteins from Short Term Culture Filtrate of *Mycobacteriumtuberculosis* by Chromatography

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Background: Tuberculosis (TB) is the leading infectious disease in the developing world. A TB vaccine (called Bacillus Calmette-Guerin, or BCG) is given in many countries to prevent infection with TB. BCG protects against miliary tuberculosis in children but fails to consistently protect against pulmonary tuberculosis in adults, the most prevalent form of disease, with a variability of 0% - 80%. high molecular weightproteins secreted into the culture medium by *Mycobacterium tuberculosis* are thought to play an important role in the development a new vaccines against tuberculosis.In this report, we describe Isolation and purification ofhigh molecular weight proteins secreted by *Mycobacterium tuberculosis*. Materials and Methods: Initially *Mycobacterium tuberculosis*transferredtoDorset− Henley Liquidmedium.After6 weeksofgrowth, the bacteriawith a0,22 micron filters ofliquidmediumcontainingsecretedproteinswere isolated andthe secretedproteinswasprecipitatedbyammonium sulfateand protein was confirmed by using kjeldahl method. We used gel cromatoghraphy, to purify high molecular weight protein andpurificationof high molecular weight proteins confirmed byCoomassie- Blue stained SDS-PAGE. Results: The resultsshowed thathighmolecularweightsecretedproteinspurifiedfrom*Mycobacteriumtuberculosis*. Also, high molecular weight proteins made up approximately 18.7% of total proteins. Conclusion: This study demonstrated that we can Isolation and purification of high molecular weight proteins withoutbreak down of bacteriabodies from short term culture filtrate of *Mycobacteriumtuberculosis*.
Keywords: *Mycobacteriumtuberculosis*, Chromatography

473. Identification of Mycobacterium Tuberculosis Culture FiltrateProteinsandComparisonwith Human Tuberculin Purified Protein Derivative byElectrophoreticMethod

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Background: Tuberculin is containing a purified protein derivative of the culture mediumtuberculosis bacterium which wereprecipitationbytrichloroaceticacid(TCA)orammoniumsulfate and used for diagnosis of Tuberculosis. The aim of this study is to comparison between the human tuberculin produced by Razi Institute and Mycobacterium tuberculosis Culture Filtrate Protein. Materials and Methods: Initially Bybiphasicmedium, BacteriafromLowenstein–Jensen solid medium transferredto a Dorset−Henley Liquidmedium and After6 weeksofgrowth, the bacteriawith a 0,22 micron filterofliquidmediumcontainingsecretedproteinswere isolated. Thenthesolution containingsecretedproteinswasprecipitatedbyTCAandammonium sulfate, separately. Thenbyspectrophotometerandkjeldahl protein assay, proteins existence in solution was confirmed. At leastprotein sampleswere comparedwiththe humantuberculinbyCoomassie-Blue stained SDS-PAGE. Results: Theprotein samplesprecipitatedwithTCAhadmorebandshigherthan 20kDa but the protein samplesprecipitatedwithammoniumsulfate hadmorebandsless than20kDa. Human tuberculin purified protein derivative was like smear and itsweightwasless than 16kDa. Conclusion: It seemsthatammonium sulfateismore suitable forlowmolecular weightproteins than TCA for precipitation.
Keywords:Mycobacterium Tuberculosis,Culture Filtrate Protein, ElectrophoreticMethod

474. Characterization of Immunological Differences of Two Recombinant Vaccine-Candidates against Botulinum Neurotoxin Type E

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Background*: Clostridium botulinum* neurotoxins are the most toxic proteins which are composed of a heavy chain (consist of translocation and binding domains) and a light chain. These neurotoxins cause botulism syndrome and have seven different serotypes (A-G). Recently, recombinant proteins have been noted by researchers as vaccine. Considering botulinum neurotoxin type E (BoNT/E), here we studied on two of these binding domain-based recombinant proteins: a multivalent chimer protein (187 amino acid residues) which is composed of botulinum neurotoxin serotypes A, B and E binding subdomains and a monovalent recombinant protein (259 amino acid) consist of 93 amino acid residues of C-terminal heavy chain of BoNT/E (named "recombinant botulinum neurotoxin type E heavy chain-C-terminal" or "rBoNT/E-HCC ") in order to compare their efficiency in antibody production. Materials and Methods: In this study *Escherichia coli* strains BL21 (DE3) which had been previously transformed by pET expression vectors, were used for recombinant gene expression. Ultracentrifugation and NTA-Ni affinity chromatography were applied to purify recombinant proteins. Purified recombinant proteins were injected into rabbits and Enzyme-linked immunosorbent assay were used for Immunology studies. Results: The results showed that antibody yields against rBoNT/E-HCC were higher comparing with chimer protein. Cross Enzyme-linked immunosorbent assay (cross ELISA) confirmed that antibodies against chimer protein (anti-chimer Abs) recognize rBoNT/E-HCC more efficiently, although both antibody groups (anti-chimer and anti-rBoNT/E-HCC antibodies) were able to recognize the opposite protein. Conclusion: In conclusion the results suggested that BoNT/E-epitope in rBoNT/E-HCC is more exposed, thus lower antibody production of chimer protein and therefore less efficiency of the multivalent vaccine, refer to epitope position in these two proteins.
Keywords: Botulinum neurotoxin type E, Cross Enzyme-linked immunosorbent assay, recombinant vaccine-candidates, ultracentrifugation, NTA-Ni affinity chromatography

475. The Role of Chlamydia trachomatis IgG Antibody Testing in Predicting Tubal Factor Infertility in Northern Iran

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Background: The purpose of this study was to investigate the role of Chlamydia serology as a screening test for tubal infertility and to compare the results with hysterosalpingography (HSG) and laparoscopic findings.Materials and Methods: This was a cross-sectional study undertaken on 110 infertile women treated in the IVF Ward, at Emam Khomeini Hospital, Sari, Iran who underwent laparoscopy and HSG as part of their infertility workup. Prior to laparoscopy, 5 ml of venous blood was drawn for measurement of serum Chlamydia IgG antibody titer (CAT). Patients' tubal status and pelvic findings were compared with CAT, as measured by microimmunofluorescence.Results: Tuboperitoneal abnormalities were seen in 81.4% of seropositive patients versus 13.2% of women who were seronegative. In women with tubal damage, the numbers of positive CATs (≥1:32) were significantly more than in those who had a normal pelvis (66.6% vs. 6.5%, $p<0.001$). CAT levels were higher in patients who had bilateral hydrosalpinges, bilateral tubal occlusion and pelvic adhesions (severe damage), than those with tubal distortion and unilateral occlusion (mild damage) ($p<0.05$). The positive likelihood ratio for C. trachomatis antibody testing was 10.28 as compared with HSG, which had a positive likelihood ratio of 3.03. Conclusion: The results of this study revealed that C. trachomatis serology is an inexpensive and non-invasive test for tubal factor infertility screening.
Keywords: Infertility, Chlamydia, Antibody, Laparoscopy, Hysterosalpingography

476. Comparison of Serological Methods for Diagnosis of Brucellosis

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Background: Brucellosis is one of the most prevalent infectious diseases in Iran. Clinical signs are not specific and laboratory methods are not necessary for definite diagnosis. Isolation of microorganism from clinical samples is the most definitive methods, but its succession depends on many factors that cannot be used in all cases. Standard agglutination test (SAT) and recently Enzyme Linked Immunosorbant Assay (ELISA) are

the most important serological tests for diagnosis of brucellosis. In this study we compared these two diagnostic methods in patients suspected of brucellosis in Sari. Materials and Methods: In this descriptive study, all patients suspected of brucellosis who referred to health centers of Sari city were chosen regardless of age, sex and condition. Their sera were collected and tested by SAT, 2ME (according to WHO standard methods with Pasteur institute antigen) and ELISA (IBL Hamborg). 1.8 titer in SAT consider as positive and 2 dilution difference between 2ME and SAT consider as positive IgM. Results: Overall the sera of 276 patients (183 female and 93 male) were tested from IgG and IgM antibodies against brucella. 12 samples were positive for IgG+ IgM with both SAT and ELISA methods. IgG detected in 98 samples by ELISA method while 27 samples were positive for IgG by SAT. ELISA detected IgM in 6 samples while SAT and 2ME were negative and SAT and 2ME detected IgM in 3 samples while ELISA was negative. Conclusion: In diagnosis of acute brucellosis (IgM+IgG) both technique were the same but in diagnosis of subacute and chronic disease (IgG without IgM or visevera) two methods were different.
Keywords: Agglutination, Brucellosis, Enzyme Linked Immunosorben

477. Evaluation of ELISA for the Diagnosis of *Helicobacter pylori* Infection in Children and adult: Comparison of serological test with Stool Antigen test

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Background:*Helicobacter pylori* infection causes chronic gastritis that is related to duodenal ulcer, gastric ulcer, and possibly gastric adenocarcinoma. Noninvasive diagnostic tests consist of the urea breath test, serology, and stool antigen testing. Serodiagnosis of *H.pylori* infection is inaccurate for children. In order to investigate the immune response to H.pylori in children and adult, we compared anti-*H. pylori*IgG and IgA antibodies with H. pylori antigen (HpSA) in the stool. Materials and Methods: Serum and stool samples were obtained from 512 children and adult patients with clinical symptom in the range of 4 to 77 years old. Paired results of *H. pylori* serology (IgG and IgA) and HpSA were analyzed by enzyme immunoassay method. Results: There were 512 paired serology and HpSA results for 105 children (≤17 years) and 407 adult (≥18 years). The positivity rate of HpSA (26%) was significantly lower ($P < 0.001$) than those for *H. pylori* IgG (54.5%) and IgA (29%). Moreover in child patients with positive HpSA and clinical symptoms, the range of positive titers was lower than adult. Conclusion: In this study, HpSA was sensitive and specific as a clinical and epidemiological tool to evaluate H. pylori infection. IgG correlated better with HpSA than IgA, and also IgG was much more specific in children than adults; confirm the fact that adults are more possible to have been exposed to *H. pylori* in the past. Using HpSA as the gold standard, we found that the performances of IgG and IgA serology tests differ significantly by age because immature immune response or tolerance to *H. pylori* is present in childhood and serodiagnosis of *H. pylori* infection is less useful. Hence, we recommend that laboratories reevaluate reference serologic titers based on age and further clinical correlation is needed to establish the optimal ranges.
Keywords: *Helicobacter pylori*, serology tests, HpSA

478. Induction of Protective Immunity in Mice against Brucellosis by Vaccinationwith Combination of Naloxone, Alum and Heat Killed of *Brucella melitensis*

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Background: Cellular immune response is effective in protection against infection of intracellular bacteria, including *Brucella*. The studies have indicated that Naloxone (NLX) can promote immune response to cellular immunity. This study was carried out to evaluate the effect of naloxone together with whole killed *Brucella melitensis* (HKB) on induction of immunity against the bacteria in mice. Materials and Methods: BALB/c mice were categorized into five groups, and received intraperitoneal vaccination at days 0 and 7. The groups received the following combinations; first group, combination of (HKB + Alum + NLX); the second group (HKB + Alum); the third group (HKB + NLX); the fourth group, HKB; and the fifth group as the PBS. Two weeks after the last vaccination, mice were intraperitoneally challenged with 2×10^4 CFU living *B.melitensis* 16M. 48 h after the challenge, serum samples were obtained and levels of IFN-γ and IL-4 were measures with sandwich ELISA method. Bacterial load in spleen of mice were measures with Real-time PCR method. Two weeks after the last vaccination, five mice from each group were intraperitoneally challenged with 5×10^9 living *Brucella melitensis* 16M as the LD_{100}. Two weeks after the challenge, the survival rate of mice was determined. Results: According to the results, the serum levels of IFN-γ, IL-4, and IgG in the NLX + Alum + HKB group significantly increased ($P < 0.05$). Furthermore, the lowest bacterial load after challenge was observed in the NLX + Alum + HKB group. The survival rate in groups vaccinated with combinations containing adjuvants was 100%. Conclusion: The study indicated that combination of naloxone and alum enhanced the immunogenicity of whole killed *Brucella melitensis*, which can be used in vaccination of animals and men at risk of the disease.
Keywords: Brucellosis, Vaccination, Naloxone, Alum, Heat Killed of *Brucella melitensis*

479. Prevalence of *homB* Positive *Helicobacter pylori* Isolated from Iranian Gastric Cancer Patients

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Background: Distinct virulence factors of Helicobacter pylori have been associated with clinical outcome of the infection. However, the exact role of H. pylori in gastric carcinogenesis is still being investigated. This study was aimed to determine the distribution of the *cagA* and *homA* and *homB* genotypes in strains isolated from gastroduodenal diseases and to evaluate relationship between genotype and disease status. Materials and methods: DNA was extracted from *H. pylori* positive cultures taken from 138 dyspeptic patients including, aendoscopic and histopathological confirmed, 62 duodenal ulcers (DU), 41 gastric cancers (GC) and 35 gastritis. Genotyping was carried out by PCR using specific primers for *cagA*, *homA* and *homB* genes. Results: In overall, the prevalence of *cagA* , *homA* and *homB* were 58%, 55.6% and 44.4%, respectively. Stratification of patients showed a significant difference in prevalence of *H. pylori* virulent genes among gastritis, duodenal ulcer (DU) and gastric ulcer. Among *cagA* positive strains, the frequency distribution of *homB* was higher in GC patients than that DU and gastritis (88% vs. 35.3% and 50%, P<0.0001). Likewise, in *cagA* negative strains, the prevalence of *homB* was also increased significantly than that DU (76.9% vs. 3.6%; P<0.0001). cagA+/homA+ strains were significantly higher in DU than that GC (64.7% vs. 12.%, P<0.0001). Conclusion*:* These findings are suggesting that *homB* status may be an independent predictor in distinguishing higher pathogenic *H. pylori* strains.
Keywords: *H. pylori*, homA, homB, cagA, Gastric cancer, Duodenal ulcer

480. Emergence of Multi Drug Resistant strains of *Escherichia coli* Isolated from Urinary Tract Infection in Girl Childrens

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Background: A current phenomenon of great concern in the medical community in developing countries is raising multi-drug resistant organisms, and their problems with curing the infections in children. Most urinary tract infection (UTIs) in children are monomicrobic, often caused by *Escherichia coli* (60 to 80 percent of cases), Proteus (more common in boys and in children with renal stones), *Klebsiella*, *Enterococcus*, and coagulase-negative *staphylococci*. Infected children need more care because they threaten by death and for treatment of them expensive drugs should be used, so in this study we evaluated the multi drug resistant strains of *Escherichia coli* isolated from urinary tract infection in girl children. Materials and Methods: The search was laboratory and performed in DEY laboratory during of 2009-2010 years in Isfahan. Totally 1027 samples collected from urine samples of girl children, aged from 1 month to 10 years. After confirmation of urine culture which is

expressive of urinary infection, gram stain and biochemical differential test was done for the isolates and then susceptibility of all of them to nine different antibiotics was determined by standard disk diffusion method. Results: The antibiogram patterns of isolates showed a high percentage of multi drug resistant phenotype among the *E coli* strains. From 1027 study sample, frequency of UTIs in girl children's was 55 (5.35%). Seventy nine percent of the isolates were resistant to tow or tree antibiotic. The predominant pattern among these strains included resistance to Nitrofurantoion (90.90%), Ceftizoxime (66.66%), Ciprofloxacin (70.069%) and Gentamicin (61.90%). Conclusion: With regard to the present data and high percentage of multi drug resistant strains of *E. coli*, regular monitoring antimicrobial drug resistance in the different areas is necessary to prevent unsuitable utilization of drugs which is the most important cause of emerging multi drug resistant strains.
Keywords: Multi Drug Resistant Organisms, *Escherichia coli*, Urinary Tract Infection, Children

481. Expression of *Salmonella Typhi* Outer Membrane Proteins and Antibody Titer in Typhoid Patients against them

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Background: *Salmonella typhi* is a Gram-negative bacteria and the causative agent of human typhoid fever. Diagnosis of typhoid fever is carried out by haemoculture or by the detection of antibodies in the patient's serum. Isolation and biochemical characterization is a lengthy process. The porin proteins of S. *Typhi* can be exploited in studies related to typhoid diagnostics and vaccination. Here, we report a expression of three outer membrane proteins viz, OmpA, ompC and OmpF of *S. Typhi* and the antibody titer raised specifically against each protein in the sera of patients with typhoid fever. Materials and Methods: OmpA OmpC and OmpF proteins of *S.typhi* were expressed and purified.Sera samples were collected from individuals with acute typhoid fever. The antibody levels to three porins IgG were measured by ELISA, using paired sera from the typhoid patients, sera from the control group, and antibody conjugated Humans. Results: Comparison of high titer anti porins IgG in control and test groups shows that anti porins were excellent antigens that may be of diagnostic value in typhoid patients. Conclusion: Outer membrane proteins, play a role in pathogenesis and induce both humoral and cell mediated immunity in human models. These can be useful tools for diagnostic purposes.
Keywords: *Salmonella Typhi*, Outer Membrane Proteins, Antibody Titer, Titer in Typhoid Patients

482. Detection of Legionella pneumophila in Cooling Water Systems of Hospitals and Nursing Homes as Immuno-compromised Cases of Kerman City, Iran by Semi-Nested PCR

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Background*: Legionella pneumophila*is involved in more than 95% cases of severe atypical pneumonia. Infection is mainly by inhalation the indoor aerosols through the water-coolant systems. Because some *Legionella* strains may be viable but not culturable, therefore, *Taq*polymerase, DNA amplification and semi-nested-PCR were carried out to detect *Legionella*-specific 16S-rDNA sequence. Materials and Methods: For this purpose, 1.5 litter of water samples from 77 water-coolant system were collected from four different hospitals, two nursing homes and one student hostel in Kerman city of Iran, each in a brand new plastic bottle during summer season of 2006 (from April to August). The samples were filtered in the sterile condition through the Millipore Membrane Filter. DNA was extracted from membrane and used for PCR to detect *Legionella* spp. The PCR product was then subjected to semi-nested PCR for detection of *L. pneumophila*. Results: Out of 77 water samples that were tested by PCR, 30 (39%) were positive for most species of *Legionella*. However, *L. pneumophila* Was detected from 14 (18.2%) water samples by semi-nested PCR. Conclusion: From the above results it can be concluded that water coolant Systems of different hospitals and nursing homes in Kerman city of Iran are highly contaminated with *L. pneumophila*spp. and pose Serious concern, especially in the immuno-compromised people. So, we recommend avoiding such type of coolant system in the hospitals and nursing homes.
Keywords: *Legionella pneumophila*, water-coolant system, immuno-compromised, semi-nested –PCR

483. *Bordetella Pertussis* IgG and IgA Antibodies Seroprevalence Among 1–35 y-old Population: The Role of Subclinical Pertussis Infection

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Background: To determine age-dependent pertussis specific IgG and IgA antibodies seroprevalence in apparently healthy subjects. Materials and Methods: A total of 595 healthy 1-35-y-old individuals divided into 5 different age groups were selected from Sari district. Antipertussis IgG and IgA antibodies levels were measured quantitatively by ELISA method. Positive sera for IgA and also IgG titer ≥150 were considered for recent pertussis infection. Results: High seroprevelence levels (72% and 71%) were observed among preschool (<7 y) children. After decreasing the seroprevelence rates significally to lowest level (54.4%) among school aged (7–11 y), the rates increased again to the highest levels of 60% and 73% at adulthood (P=0.03 and P=0.003). In total, 1.55% of study subjects were IgA positive, and 5.7% showed high IgG titers. Conclusion: The present study reveals, vaccine induced immunity has decreased among school-aged children and natural pertussis infection is common among adolescent and young adults. Also, asymptomatic/sub-clinical recent pertussis infection was prevalent among studied population. These findings necessitate developing new strategies to reduce and control pertussis infection in Iran.
Keywords: *Bordetella Pertussis*, IgG, IgA, Seroprevalence

484. HFE gene Expression and tuberculosis

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Background: Tuberculosis is the most important disease in the world that leads to the death of approximately 2 million people every year. Iron is one of the critical factors for intracellular pathogens and hosts that necessary for growth and metabolism. HFE proteins adjust cellular iron balance via interaction transferrin receptor. In addition, mutation of HFE gene resulted to decrease iron storage in macrophages and resistant to tuberculosis. The purpose of this study was to investigate the role of HFE as iron regulator in the TB patient and control groups. Materials and Methods: In a case control study, 62 patients with tuberculosis and 74 normal individuals were selected. We determined genotypes with PCR-RFLP and then after isolation of macrophages, we studied HLA-H gene expression by Real time PCR. Statistical analysis was conducted with SPSS16.0 software. Results: The results of this study showed that the polymorphism of HFE in H63D situation did not show significant difference in the groups, but in situation C282Y either genotypic or allelic was significant ($p<0.05$). Evaluation of HFE gene expression showed that the highest expression related to genotype GG C282Y (homozygous normal) that, the frequency in the patients group was 1.5 fold more than control group. Also the lowest expression was related to genotypeAA (C282Y) that the frequency in the control group was 9 fold less than patients group ($p=0.009$). Conclusion:Our results suggest that macrophages of individuals with mutation HFE acquire significantly less Fe from Tf that lead to resistant to tuberculosis. Therefore, it is concluded that subjects with mutation HFE have a protection against intra-macrophage pathogens such as mycobacterium.
Keywords: Tuberculosis, HFE, mutation

485. Isolation and Purification of Lactoferrin from Bovine Colostrums and Evaluation Antibacterial Effect on *P. aeruginosa*

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Background: Lactoferrin is an iron-binding glycoprotein involves a diverse range of biological activities. Lf is a major component of milk and is present in exocrine secretions such as tears, salvia, bile, and neutrophil granules. Lf has more potent antimicrobial activities with various range including both of gram negative and positive bacteria as well as antivirus activities.Materials and Methods: In this study, antibacterial activity of Lactoferrin has been scrutinized after isolation and purification from cow´s colostrums against *pseudomonas aeroginos* . After taking casein of milk, purification of lactoferrin was performed during two steps by ammonium sulfate precipitation and using cation exchange chromatography, CM-Sephadex C-50 resulting purified protein with 80 KDa molecular weight. Bactericidal samples were isolated from scald patients (Shahid Zare Hospital) then microbial activity confirmed by biochemical tests like oxidase, catalase and growth on TSI medium. Four concentration 400,500,600,700 µg/ml of lactoferrin were assayed.*Pseudomonas* colonies counted and compared with the control (without lactoferrin) as well as *E. coli (DH5α, JM2163)* as positive control was considerd. Results: our results suggest that 400µg/ml concentration of lactoferrin has the least inhibitory effect with 35%growth inhibitory on *Pseudomonas and* 700µg/ml concentration of lactoferrin has the highest inhibitory effect with 86%. Therefore lactoferrin can effectively reduce the growth of *Pseudomonas aeroginosa.*Concultion: Our result showed that all of lactoferrin concentrations have more effective inhibitory activity against *Pseudomonas aeroginosa.*
Keywords: lactoferrin,isolation, purification,*Pseudomonas aeroginosa*

486. Seroepidemiology of Brucellosis in Persons with High-risk and No Risk Occupations in Jahrom

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Background: Brucellosis is a zoonotic disease that transmitted to humans through contact with infected animals or animal products. It remains a major public health problem in many developing countries. Cases occur among livestock producers, livestock markets employees, and veterinarians. The main goal of this study is to evaluate of Wright and 2-mercaptoethanol tests (2-ME) for diagnosis of Brucellosis in persons with high-risk and no risk occupations. Materials and Method: Three hundred sera (150 at high risk and 150 at no risk) were tested using Wright and 2-ME tests by conventional methods. The results accessed by statistical analysis. Results:In 150 samples of high risk individuals, only 9 sample (6%) were positive with Wright test (4 with 1/80 titer, 3 with 1/160 titer and 2 with 1/320 titer). Also results showed that 6 sample (4%) were positive with 2-ME test (3 with 1/40 titer and 3 with 1/80 titer). Wright and 2-ME tests were negative in 150 samples of no risk individuals. The acute brucellosis approved in at least 3 sample (2%of the cases) from positive samples that Wright and 2-ME titers were 2 with 1/80 and 1/40 titers, 1 with 1/160 and 1/80 titers respectively. Conclusion: Results showed that both two tests were negative in no-risk group but in high-risk group titers more than 1/160 in Wright and 1/80 in 2-ME are not indicated of brucellosis. However it is recommended to adhere biosafety and consider precautions in persons with high-risk occupations.
Keywords: Seroepidemiology, Brucellosis, Occupations, Jahrom

487. Immunophenotypic Characterization of Peripheral T Lymphocytes in Tuberculosis Patients before and after Chemotherapy

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Background: Tuberculosis is a infectious disease caused by mycobacterium tuberculosis.Groth population, increase in incidence of AIDS, poverty and drug resistance are factors which are responsible for an increase in incidence of tuberculosis.Cell mediated immunity plays a pivotal role against Mycobacterium tuberculosis Better understanding of different aspect of the disease, for example immunology and immunopathogenesis is fundamental for the development of new immunogenic vaccines and control of disease. In this study quantative of T-lymphocytes and subpopulations in patients with tuberculosis before and after chemotherapy has been evaluated. Materials and Methods: In this study 20 patients and 20 healthy control have been selected. Flow cytometry was done for TCD3+, TCD4+ and TCD8+ lymphocytes by monoclonal antibody in patients and healthy control. Results: The result of this study demonstrate that mean percentage of TCD3+ and TCD4+ lymphocytes in the patients group in compared with healthy control was decreased. But mean percentage of TCD8+ was increased. After two month treatment mean percentage of TCD3+ and TCD4+ lymphocytes in the patients group was increased. But the mean percentage of TCD8+ decreased slightly which was insignificant. Conclusion: The result of this study shows that the cellular immunity is supressed, as demonestarated by a diminish in the mean percentage of TCD3+ and TCD4+ lymphocytes. Drug therapy is effective to abolish this disorder. The discrepancy in the finding of this study as compared to other studies is likely due to different criteria in patient selection, involved area and different methods for cell counting.
Keywordes: Tuberculosis, Cell mediated immunity, TCD3+, TCD4+,TCD8+ Flowcytometry

488. Enhanced Expression of mRNA Interleukin-18 in Gastric Mucosa of Patient Infected with *Helicobacter pylori*

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Background:*Helicobacter pylori* infection is associated with gastritis and marked infiltration of the gastric mucosa by inflammatory cells secreting of several cytokines that contribute to maintain and expand the local inflammation. Different clinical expressions of the infection may reflect different patterns of cytokine expression. Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-17, IL-23, and IL-18 have been reported to be involved in H. pylori-induced gastric mucosal inflammation, but the details and relation to different patterns of inflammation remain unclear. Materials and Methods: Analysis of IL-18 RNA transcripts was performed by real-time PCR. Total RNA was extracted from gastric biopsies of 20 Hp-infected patients, 20 Hp-negative patients (we used for rapid urease test, PCR 16srRNA and histological examination) with gastritis, by biozol reagent according to the manufacturer's instructions. cDNA was synthesized from 1 mg of total RNA using First Strand cDNA Synthesis Kit(fermentas) and 2 µL cDNA was amplified by PCR using the 2x Rotor-Gene Probe PCR Master Mix (QIAGEN) and specific primers for each cytokine and β-actin. Results: To confirm that IL-18 is produced in excess in Hp infected gastric mucosa, we first analyzed IL-18 RNA transcripts in gastric biopsies of Hp-infected patients and uninfected patients by real-time PCR. IL-18 RNA was detectable in all samples regardless of whether biopsies were taken from Hp-infected or uninfected patients. However IL-18 RNA expression was significantly increased in biopsies of Hp-infected patients compared to Hp-uninfected patients. Conclusions: IL-18 may play an important role in the inflammatory response and promoting gastric Th1 responses to *H. pylori* colonization, and may ultimately influence the outcome of H pylori-associated diseases that arise within the context of gastritis.
Keywords: *Helicobacter pylori*, Gastric, Interleukin-18

489. CD4+CD25+ and CD4+FoxP3+ Treg Cells in Peripheral Blood of Patients with Acute and Chronic Brucellosis

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Background: Brucellosis remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually. In humans, Brucellosis can be a serious, debilitating and sometimes chronic disease. Different mechanisms can be postulated as to the basis for the induction of the chronic status of infectious diseases that T regulatory cells are one of the most important related mechanisms. For the first time, the current study was designed to determine whether percentage of CD4+CD25+ and CD4+FoxP3+Treg cells in peripheral blood is changed in human Brucellosis samples (Acute form:n=16, Chronic form: n=10) in comparison to control group(n=15). Materials and Methods: Heparinized venous blood was obtained from both patients and healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque density gradient centrifugation. For surface staining, cells were incubated with the respective mAbs for CD4, CD25 and FoxP3 and finally CD4+CD25+ and CD4+FoxP3+Treg cells are evaluated by FACS. Results: The results obtained in this study have revealed a new finding in relation to Treg cells and human Brucellosis. Our study indicates that the number of $CD4^+CD25^+$ and CD4+FoxP3+ Treg increases in the peripheral blood of acute and chronic forms of Brucellosis samples compared with healthy groups and this increase in chronic group is further. Conclusion: $CD4^+CD25^+$ regulatory T cells (Treg) play a central role in the prevention of autoimmunity and in the control of immune responses to transplants, allergens, tumors, and infectious microbes by down-regulating the function of effector $CD4^+$ or $CD8^+$ T cells. In conclusion, it seems to be a correlation between increase of CD4+CD25+ and CD4+FoxP3+Treg cells and the disease progress from healthy state to acute and chronic brucellosis.

Keywords: $CD4^+CD25^+$ regulatory T cells, CD4+FoxP3+Treg cells, acute brucellosis, chronic brucellosis

490. Reactivation of Latent Tuberculosis Infection in Markazi Province–Iran

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Backgrounds: Tuberculosis is an old problem that is the great challenge now, neighbourhood of Iran with Afghanistan and Pakistan ,that they are among 22 high burden countries around the world , it notice the necessity of more attention to this disease. Therefore, the present study was conducted in order to analyze the current molecular epidemiology of TB and survey of genetic diversity of *Mycobacterium tuberculosis* strains in the Markazi province. Materials and Methods: During this research 65 sputum specimens collected from smear positive patients that admitted of health centers from Markazi province in the period of Februery 2010- April 2011 were cultured on Lowenstein-Jensen media. Totally,57 mycobacterial isolates were grown, genomic DNAs was extracted (by Iso amil alcohol- chloroform method) and digested with *PvuII* and *AluI* ,then performed electrophoresis and DNA fragments were transfered to positively charge nylon membrane by southern blotting and performed hybridization by PGRS , DR and IS6110 probes, subsequently the signals were detected.
ResultsGenotyping of the isolates by PGRS-RFLP, DR-RFLP and IS6110-RFLP with *Pvu* II and AluI displayed a wide range of genetic diversity as 50, 44 and 48 genotypes were identified by PGRS, DR and IS6110 respectively with PvuII. forand 45 genotypes were identified by PGRS with AluI .Conclusion: Generally speaking, despite the relatively limited number of isolates in the study, high age of patients (>60 years) and also large heterogeneity found in this study, Based on the obtained data it appears that tuberculosis among the study population in Markazi province mainly from reactivation of latent infection.

Keywords: reactivation, tuberculosis, latent infection, molecular epidemiology

491. Western Blot Analysis of Polyclonal Antibodies against Whole Proteins of *Mycobacterium avium paratuberculosis*

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Background: Paratuberculosis or Johne's disease caused by *Mycobacterium avium subsp. paratuberculosis* (MAP), is chronic granulomatous enteritis in cattle. It is characterized by intermittent diarrhea, weight loss and eventual death. Recently reported suggest that Map is associated with Crohn's disease in humans. Up to now differential microbiological, serological and molecular methods have been used for detection of Map infection but most tests were neither sensitive enough for effective control. In recent years researchers focus on identification Map antigens to use in diagnosis test and preparation of effective vaccine. A whole range of new antigens has been identified and characterized. These include the cell wall derived glycolipid lipoarabinomannan and protein antigen with molecular weights of 12 kDa to 70 kDa. Some of these antigens are now being evaluated for the development of new and more sensitive diagnostics for paratuberculosis. The goal of this study was Western Blot Analysis of polyclonal antibodies against whole proteins of *Mycobacterium avium paratuberculosis* and investigated antibodies response to antigens. Materials and Methods: For produced polyclonal antibodies against MAP proteins a New Zealand white rabbit immunized at a certain time period with antigens. After immunization of the animal, serum was obtained from rabbit blood. Antibodies were purified from serum with ion exchange chromatography (DEAE-celloluse). Western blotting tests were used. Results: western blotting analysis has shown several bands. That has shown positive interaction between antigens and purified polyclonal antibodies. Conclusion: Polyclonal antibodies are relatively inexpensive and easy to produce in large quantities and can connect to the more connective sites resulting in better sensitivity. Identification of polyclonal antibodies employing western blot analysis is of importance in studying the MAP disorder. Polyclonal rabbit anti-MAP from immunized rabbit serum was observed that MAP proteins reacted with polyclonal antibodies.

Keywords:Western Blot, Polyclonal Antibodies, *Mycobacterium avium paratuberculosis*

492. Comparison of Immunological Parameters of Tuberculin Staff Workers with PPD Positive Reaction and Normal Controls

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Background: According to the occupationally risk of infection in staff workers who have direct contact with mycobacterium species, we investigated their immunological parameters and compare with healthy PPD negative volunteers. Materialand Methods: We investigated 20 PPD positive volunteers working at tuberculin unit of Razi vaccine and serum research Institute and PPD negative healthy controls with no exposure or history of active tuberculosis. The percentages of circulating lymphocyte subpopulations were detected by flowcytometry. IL-4 and IFN-γ production levels were measured by ELISA in supernatants of PPD-stimulated peripheral blood mononuclear cells (PBMCs) culture. Results: Tuberculin workers showed an increase in IFN-γ level and significant decrease of CD4+ T cells percentage and CD4/CD8 ratio in compare to PPD negative normal individuals. However the IL-4 production and percentage of other lymphocyte population has been unchanged. Conclusion: These observations suggest that the immunological parameters of tuberculin workers with PPD positive reaction, who have occupationally exposed to mycobacterium antigens, could be changed. Future studies will be directed towards cytokine networking and regulatory lymphocytes, which will help us to validate the valuable data presented in this study.

Keywords: Tuberculin unit workers, Lymphocyte subpopulation, IFNγ, IL-4

493. Comparison of Serological and Histopathological Diagnostic Method in the Diagnosis of *Helicobacter pylori*

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Background: *Helicobacter pylori* (HP) are one of the important causes of chronic stomach infection, chronic gastritis, peptic ulcers and stomach cancer. Designing rapid, simple and less expensive techniques for its diagnosis lead to early treatment and preventing several complications due to *H. Pylori* infection .The aim of this research was to compare invasive (histology) and non invasive (serology) methods in HP detection in patients with digestive problems. Materials and Methods: This type of cross-sectional descriptive study was conducted on 100 patients with digestiveproblems. All of the patients underwent endoscopy and biopsy was taken from gastric antrum. For detecting *helicobacter pylori* histological examination by Giemsa staining was done in biopsy specimens and for serologic study serum levels of IgG were measured by ELISA. Then the data were statistically analyzed. Results: Of 100 patients with digestive problems, serology in 67 (67%) and histologyin 65 (65%) was positive. Histology In men %60 and in women 70% was positive. Serology in men 66% and in women 68% was positive. In our studySensitivity%90.8, accuracy 77.1%, false negative 9.2%, false-positive 22.9%, positive predictive value 88.1%,negative predictive value 81.8%, the coefficient Kappaagreement between two methods was 0.69.(P value<0.001). positive Histologyl in cases under 50 years old was 55.2% and in cases more than 50years old was 84.8%. Positive serology in cases under the age of 50 years was 59.49% and in cases more than 50years old was 81.8%.Conclusion: According to the high sensitivity, simplicity, rapidity and lower cost of serologic examination in comparison with histological evaluation for Helicobacter pylori detection, serology can be applied as the best major technique in screening and epidemiological evaluation of *H. pylori* detection.
Keywords: *Helicobacterpylori*, endoscopy, serology, histopathology

494. Evaluation of Human Brucellosis in Contact with Cattle Vaccinated by Rev1 and RB51 Vaccines in Jahrom 1389

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Background: Brucellosis is a zoonotic disease that transmitted to humans through contact with infected animals or animal products. It remains a major public health problem in many developing countries. Attenuated strains such as B.melitensis Rev1 and B. abortus S19 and RB.51 are being used to control brucellosis in domestic animals. However, no safe and effective vaccine is available for human use. This study was designed to evaluate the immunogenicity and the protective efficacy of RB 51 and Rev1 vaccine for cow and sheep in Jahrom. Materials and Methods: This study reviewed the cases of people testing positive wright and 2ME in all laboratories Jahrom, during the 1389. These people who were related with animals vaccinated by Rev1 and RB51.Vaccine prepared from Razi Institute. Results: In this study, 39 cases were positive with Wright and the 2ME tests that of these cases, 36 patients(92.3%) had history of contact with animals and 3 cases (7.69%) consumed ofunpasteurize dairy products. Conclusion: Note that despite being vaccinated animals still often associated with people who had brucellosis and according to research done on 100% of vaccine immunogenicity and the high prevalence of brucellosis in iran to producte new and effective vaccine against brucellosis is a serious need.
Keywords: Brucellosis, Rev1, RB51, Vaccines, Jahrom

495. Evaluation of an Interferon-Gamma Release Assay in Young Contacts of Active Tuberculosis Cases

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In a cross-sectional study in a hospital in Tehran in 2008-08 the QuantiFERON-TB interferon-gamma release assay (QTB) was compared with the tuberculin skin test (TST) in 59 young people (aged<20) with close contact with immunocompetent pulmonary tuberculosis. After 1 year follow-up 10 subjects had progressed to tuberculosis disease and received treatment; TST was positive in 30% and QTB in 100%. Of the 49 non-progressive subjects, TST was positive in 10.4% and QTB in 16.3%. The agreement between TST and QTB assay in non-progressive subjects was poor (ƙ=0.43). False positive and false negative rates for TST were 40.0% and 9.3% respectively; positive and predictive values were 60.0% and 90.7%. We suggest adding the interferon assay to the skin test in the decision to perform chest X-ray or to stars chemoprophylaxis at least in younger subjects (aged<20).
Keywords: Interferon-Gamma, Active Tuberculosis Cases

496. The Incidence of Chlamydia and Mycoplasma Infections in Nasal Polyp Tissues using the PCR Method and Its Correlation to Serum Antibody Level

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Background: The etiopathogenic mechanism of nasal polyposis isn't clearly defined, although initiation of chronic inflammatory process due to stimulation by an environmental factors including infectious agent, in genetically susceptible individuals is of great concern. Among the infectious agents, respiratory pathogens with low virulence are more reasonable candidate, therefore the evaluation of Mycoplasma and Chlamydia involvement both as local agents and for systemic infection are mandatory. Materials and Methods: A cross sectional study designed and 51 patients with nasal polyps candidated for polypectomy and 19 normal individuals selected. Nasal polyps excised during surgery and a mucosal biopsy takes from control group which were admitted for rhinoplasty. Blood samples also takes from all group under study for antibody measurement. Results: 9.8 And 47.1% of patients had a significant IgM, IgG autoantibody against Chlamydia respectively and Chlamydia DNA was positive in 7.8% of patient's tissues. No IgM antibody and Chlamydia DNA detected in control group while 47.7% of these individual had IgG antibodies. Chlamydia infection is significantly associated in polyposis concerning the specific tissue DNA. The antibody level against Mycoplasma was higher in patients, 15.7% IgM, 68.6% IgG and 19.6% DNA positive Vs 15.8% IgM, 47.7% IgG and No DNA in normal individuals. Conclusion: Both Chlamydia and Mycoplasma infections may contribute in polyp development. Also Mycoplasma might be considered as a more participating agent.
Keywords: Chlamydia, Mycoplasma, Nasal Polyp Tissues, Serum Antibody Level, PCR

497. Prevalence of Common Serotypes of *Streptococcus pneumoniae* in Iran to Get the Appropriate Combination of Pneumococcal Vaccine

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Background: *Streptococcus pneumoniae* is the most common cause of invasive infection. The introduction of pneumococcal conjugate vaccines has led to a decline in the serotypes covered by the vaccines, but common serotypes causing invasive disease and the emergence of carriers of *Streptococcus pneumoniae* is unknown yet in Iran. Past-vaccine surveillance studies of serotype prevalence patterns in Iran are necessary to monitor the epidemiology of *Streptococcus pneumoniae* in order to evaluate the appropriate formulation for future vaccines. Because of variation of pneumococcal serotypes in different geographical regions, in this study we evaluated common serotypes causing pneumococcal infections in Iran by Multiplex PCR as a part of surveillance studies to get the appropriate combination of pneumococcal vaccine in Iran. Materials and Methods: A total of 500 nasopharyngeal swabs were collected from patients in Tehran hospitals between December 2004 and February 2011. Identification was performed by biochemical and molecular tests. Serotyping was done by multiplex PCR. We designed primers based on the

sequences available for the capsular types 1, 3, 4, 6A, 6B, 9V, 14, 18C, 19F, 19A, and 23F and combined them into seven multiplex PCR. Results: From 500 nasopharyngeal swabs, 60 isolates of *Streptococcus pneumoniae* identified after identification tests. Five serotypes (3, 6A, 6B, 14, 19A) of *Streptococcus pneumoniae* accounted for 81% of isolates. Other serotypes accounted only for 12%, and 7% of isolates could not be typed by multiplex PCR test. Serotype 19A was the most common serotype, followed by 6A and 6B serotypes. Conclusion: The multiplex PCR approach was successfully adapted to identify serotypes from more than 90% of the isolates tested. Result showed that 20% of the pneumococcal serotypes were from serotypes of pneumococcal conjugate 7-valent vaccine (4, 6B, 9V, 14, 18C, 19F, 23F). Continued monitoring of common serotypes of *Streptococcus pneumoniae* is essential for future vaccine formulation in Iran.
Keywords: *Streptococcus pneumonia*, Pneumococcal Vaccine, Iran

498. Syphilis and HIV Infections in Blood Donors of Bushehr in 2011

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Background: The numerous transfusion-transmitted infectious diseases such as HIV, HBV, HCV and syphilis affect blood safety for transfusion recipients. This study evaluated the prevalence and incidence of serologic markers for syphilis and HIV among blood donors of Bushehr during 2011. Materials and Methods: Serum from 16755 blood donors was tested for the presence of treponemal antibodies using rapid plasma reagin test (RPR) following the manufacturer's instructions. The screening for HIV was done by ELISA kits. Results and Conclusion: Five donors (0.02%) were positive for Syphilis and no HIV marker was detected in blood donors. Blood transfusion is an important mode of transmission of infections to recipients. Strict selection of blood donors and comprehensive screening of donors' blood using standard methods are highly recommended to ensure the safety of blood for recipient.
Keywords: Syphilis, HIV, Blood Donors, Bushehr

499. Typing of Typhoidal and Non-typhoidal Salmonella Species by Serology Method and Electrophoresis of Proteins Soluble in Water by SDS-PAGE Technique

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Background:The genus of Salmonella is one of the most important of Enterobacteriacea family can cause serious diseases in human and animals. The aim of this study was extraction of whole-cell proteins of typhoidal and non-typhoidal Salmonella from patients with systemic infections by polyacrylamide gel electrophoresis SDS-PAGE method and comparing with serotyping of species.
Materials and Methods: In this study, 100 cases of Salmonella strains were collected from patients who referred to cilinical centers in Hamadan and 4 reference strains of Salmonella species were also included in study. Serotyping of strains were performed by Biomerix and diffco monovalent antisera. Whole-cell proteins of strains were also detected by 10% polyacrylamide gel. Gels were stained by coomassie Brilliant Blue and photogarphed through an orange filter. Rate flow (RF) of each protein band was also determined. The density of protein bands were analysed by densitometery. Results: Of 100 cases of Salmonella species isolated from patients,: 43 cases (43%) were *S. typhy*, 20 cases (20%) *S. typhimurium*, 12 cases (12%) *S. paratyphy B*, 10 cases (10%) *S. paratyphy C* and one case was also *S. paratyphy A*. The results of serotyping were compared with the results obtained by SDS-PAGE. Many protein bands from major protein 220 KD_a to minor protein 18.5 KD_a were detected by SDS-PAGE and differentiated the strains well. Protein profiles of clinical strains were compared and showed some variations with results of serotyping and could divided *S. typhy* to 5 subgroups and *S. paratyphy B* and *S. paratyphy C*each to 3 subgroups. Conclusion : The our results showedthat extraction of whole-cell proteins of typhoidal and non-typhoidal Salmonella speceis by polyacrylamide gel electrophoresis SDS-PAGE technique could be used more reliable than serotyping methods for identification and typing of these microorganisms. However, it is needed more researchs for other organisms to demonostrate this sugestion
Keywords : Serotyping,Salmonella, Typhoid, SDS-PAGE

500. Evaluation of Humural Immunity in Infection with *Borrelia* in the Blood Serum of Patients with Gout in Ardabil Province

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Background: The main area of Iran affected by relapsing fever (RF) is Ardabil Province, for which *Borreliapersica* is the most common cause in this country. And According to current statistics gout is also in this province that we will examine the relationship between these two diseases. Materials and Methods: Presence of antibodies against Borrelia persica disease using ELISA test in Serum of 68 patients with gout and 130 controls who were matched for age and sex of the experimental group was tested. Results: The data obtained suggest that the 18 subjects studied and from 14 controls in their blood serum contains antibodies against *Borrelia* is some of the protein band. T-test showed significant differences between two groups. Conclusion: According to the Infection with *Borrelia* is a multiorgan infection, and disrupts cell metabolism in different organs Can be concluded that the risk of getting Infection with *Borrelia* increases the incidence of gout.
Keywords: *Borrelia*, gout, Ardabil Province

501. Effectiveness of Combination Therapy with Colostrum in *H. Pyloi*Eradication in Ali Asghar Pediatrics Medical Centre

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Background: There are several million new cases of peptic disease annually. The disease has a various range of presentations. Gram negative *helicobacter pylori* bacilli is considered as an etiologic factor in this disease. Goal of treatment in peptic disease is eradication of the *helicobacter pylori* (HP). Combination therapy has been implemented in the treatment of this disease. Different modalities have been recommended up to now. In order to lower adverse effects, cost and drug resistance, researchers have introduced a new combination therapy in which Colostrum is substituted for metronidazole. Materials and Methods: A step II of clinical trial was designed. The sample size was 21 children. Diagnosis of HP infection was confirmed with histopathology. Treatment regimen consisted of omeprazole, amoxicillin, bismuth and Colostrum. After a 3-4 week follow-up,eradication was evaluated. Results: 21 children completed the follow- up period. Mean age of patients was 9.7 years. Treatment effectiveness was 77 percent. Conclusion: Combination therapy with 3 drugs along with Colostrum has significant effectiveness on HP eradication.
Keywords: *Helicobacter pylori*, peptic disease, clinical trial, Colostrum

502. Inhibition of enterotoxigenic *Escherichia coli* Attachment to Erythrocyte by Antibody against Colonization Factor I Major Subunit (CfaB)

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Background: Enterotoxigenic*Escherichia coli* (ETEC) is the most important bacterium causes childhood diarrhea. This bacterium is the cause of 380 thousands of death in children under five years of age. The Colonization factor B (CfaB) as major subunit of fimberiae has a critical role in bacterial attachment to small intestine epithelium. In this work, we produced recombinant CfaB in *E. coli* with the aim of studying its

immunogenicity. Materials and Methods: The cfaB gene was cloned into pET28a and regarding the presence of rare codons in cfaB gene, it was not expressed. Therefore, an optimized gene with codon preferences was synthesized and cloned into pET28a vector and subsequently expressed. The recombinant protein was purified with Ni-NTA column and used as an antigen for mice immunization and in ELISA test. Microplate agglutination inhibition test was utilized to show antibody blocking activity. Results and Conclusion: codon optimization is a useful approach for obtaining large quantities of a desired protein. Relying on agglutination inhibition experiment, anti-CfaB serum was able to inhibit the binding of CFA/I fimbriated ETEC to erythrocytes.

Keywords: Enterotoxigenic *Escherichia coli*, CfaB, Erythrocyte, ELISA

503. Antibody against Recombinant Heat Labile Enterotoxin B Subunit (rLTB) Could Block LT Binding to Ganglioside M1 Receptor

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Background: Enterotoxigenic *Escherichia coli* (ETEC) is one of the most common agents of diarrhea among other bacterial agents. Regarding to presence of diverse clones of ETEC strains in the world, the use of global vaccines for ETEC infection is controversial. B subunit of heat labile toxin (LTB) was introduced as a vaccine candidate molecule by several investigators. The expression of LTB gene isolated from a local bacterial strain and investigation of its immunological property was the objective of this study. Materials and Methods: LTB gene was isolated from ETEC, cloned and expressed in pET28a expression vector. For LTB gene expression, the three main expression parameters (IPTG concentration, time and temperature of induction) were investigated. The recombinant protein was purified (> 95%) with Ni-NTA column using 6XHis-tag and used as an antigen in ELISA test. Results: The immunological analyses showed production of high titer of specific antibody in immunized mice. Anti LTB Antibody could bind to whole toxin and neutralize the toxin through inhibition of its binding to the Ganglioside M1 receptor.Conclusion: Heat labile enterotoxin (LT) B subunit is considered as a vaccine candidate molecule because: a) it is nontoxic subunit of LT molecule that play an important role in ETEC virulence, b) most clinical ETEC isolate can produce LT and c) LTB is a potent immunogen and possess adjuvant properties.

Keywords: ETEC, rLTB, Ganglioside M1 Receptor

504. Levels of Interleukin-6 (IL6) as Predictors of Early Term Neonatal Sepsis during 2010-11

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Background: Neonatal sepsis is an important cause of neonatal mortality and morbidity. Its prevalence is 1 to 10 cases per 1000 live births, with mortality rate of 10 to 50 percent. But the rate of hospital admission of neonates suspicious to systemic infections are more than sepsis prevalence, due to nonspecific sign and symptoms of neonatal sepsis. This study was conducted interleukin 6(Il6) plasma level as a marker for early detection of neonatal infection. This can help us to decrease the rate of unnecessary hospital admission and treatment of patients with similar sign and symptom as sepsis. Materials and Methods: Level of plasma Il6 and blood culture were checked in all term neonates who were admitted with suspicious of sepsis in neonatal intensive care unite of kashan Shahid beheshti hospital during 2010-11. Positive blood culture was considered as definit sepsis and negative blood culture as suspicious sepsis. Then level of Il6 compare with blood culture states. Patients with prematurity, low APGAR score, more than 7 days age, and the history of previous admission were excluded from the study due to probable effect on plasma Il6 level. Results: A total of 142 hospitalized neonates were checked for plasma level of Il6 .10 cases of them were healthy neonate who were admitted due to physiologic jaundice for phototherapy treatment (control group). Others had signs and symptoms of infection and Bactec blood culture was obtaind. 74 cases were male and 68 cases female. The prevalence of early neonatal sepsis was 7% .The most etiologic bacterial agents were Streptococcus in Group B (GBS) and staphylococcus Epidermis (SE). The most common symptoms of patients was tachypnea (35.9%). The average level of Il6 in first group (admitted with sepsis signs and symptoms and positive blood culture)was 1545.65pg/ml and in second group(admitted with sepsis signs and symptoms and negative blood culture)was 14.79pg/ml and in control group was 11.04pg/ml. Conclusion: Plasma level of Il6 as an inflammatory factor is a good predictive marker for detection of sepsis in neonates who are admitted with nonspecific sign and symptom of infections and sepsis.

Keywords: Interleukin-6, Predictors of Early Term Neonatal

505. Seropositivity of *Helicobacter Pylori* Infection in Patients Suffering from Gastritis in a Tertiary Hospital

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Background: *Helicobacter pylori* is a worldwide infection and is causally related to chronic active gastritis, peptic ulcer disease, primary low-grade B-cell gastric lymphoma, and is also a risk factor for gastric cancer. The most common symptom of is gastritis associated with burning pain in the epigastrium. The aim of this study is to determine the seroprevalence of *H. pylori* infection among subjects with gastritis problem within six months in a tertiary hospital. Materials and Methods: We carried out a cross sectional study in a sample of persons who met the following criteria: older than 15 years old, suffering from gastritis and has been referred to the laboratory in University Hospital Center of Arak, during the period of the study. Sera from of 454 patients and were examined for the presence of anti-H. pylori (IgG) antibody with commercial kit. Results: H. pylori seroprevalence was 39.2% [84 (18.5%) male, 94(20.5%) female].While Seropositivity increased with age; no correlation was detected between H. pylori seropositivity and gender. Conclusion: Considerable rate of antibody among children and adolescents can be considered as a possible factor of gastritis and ulcer rate increase in these age groups.

Keywords: Seropositivity, *Helicobacter Pylori*, Gastritis

506. Extraction of *H. pylori* Alkylhydroperoxide Reductase from Stool

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Background: Alkylhydroperoxide reductase (AhpC) from *H. pylori* not only has an important bifunctional role in pathogenesis of *H. pylori* but alsois a species–specific protein. It can be used as an antigen in detection of *H.pylori* infection, thusextraction of fecal AhpC antigen of H. pylori is necessary to investigation ofits alterations while passing through gastrointestinal tract. This study was performed with the objective of developing a practical and simple system for extraction AhpC from stool. Materials and Methods: The method consisted of preparation of fecal extracts from positive stools, performance of preparative SDS- PAGE on the fecal extracts, detection of proteins with Zinc sulphate staining, and electroelution by utilizing a horizontal electrophoresis apparatus, two small dialysis bags and a plastic tube filled with a mixture of gel pieces containing the protein and agarose gel. For desalting and SDS elimination, dialysis was performed against distilled water (pH 4.5, three times) and then PBS (pH 7.2). Results: The protein was extracted from stool with a high purity and its immunoreactivity was shown by immunoblotting. Conclusion: This practical and simple method is applicable to extract Alkylhydroperoxide reductase from positive stools.

Keywords: Alkylhydroperoxide reductase(AhpC), Electroelution, *Helicobacter pylori*, Fecal extract, Zinc sulphate staining, Protein purification

IMMUNOLOGY of CHEMICAL VICTIMS

Oral Presentation

507. Fibrinogen and Inflammatory Cytokines in Spontaneous Sputum of Sulfur Mustard Exposed Veterans

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Background: Sulfur mustard (SM) causes delayed complications respiratory system of exposed individuals. Inflammatory cytokines are considered as the main regulators of pulmonary complications and fibrinogen which is regarded as a biomarker in certain pulmonary difficulties is important in SM-exposed victims as well. Materials and Methods: In this pilot study, the sputum level of IL-1α and β, TNF, IL-1Ra, IL-6 and fibrinogen in SM-exposed veterans having spontaneous sputum, was examined and their correlation with pulmonary function was studied. The participants were categorized in two major subgroups (hospitalized and non-hospitalized) based on the severity of the clinical problems at the time of exposure.All participants were visited by clinicians and their respiratory functions were measured by spirometry. The classification of severity of pulmonary involvement was also done according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD). ELISA assay was performed to measure levels of cytokines in sputum and serum samples. Results: The mean value of TNF, IL-1α and IL-1β was 524.15, 115.15, 1951.33 pg/ml respectively, IL-1Ra and IL-6 are 6410.52 and 124.44 pg/ml respectively; fibrinogen is 71.59 ng/ml and index of IL-Ra/IL-1β is 7.78. There is more TNF-α and IL-1β and less IL-1Ra and fibrinogen in the sputum of hospitalized ones, but there is no statistically significant difference between IL-1α and IL-6 amounts in the two groups. TNF-α and IL-1β are also increased in moderate and severe conditions of pulmonary status and fibrinogen is decreased significantly in problematic persons. TNF has negative and IL-1Ra has positive correlation with FEV1. IL-1β and fibrinogen have strong correlation with spirometry GOLD. Conclusion: The study demonstrated significant correlations between fibrinogen, TNF and pulmonary function. It was also concluded that sputum is valuable for evaluating respiratory system status.
Keywords: Sulfur mustard, Sputum, Fibrinogen, TNF, IL-1, Pulmonary Function

508. NFκB Gene Expression Survey in Peripheral Blood Cell of Sardasht Cemical Victims 20 Years after Exposure to Sulfur Mustard

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Background: Transcription factor NFκB is responsible for a large number of genes expression including inflammatory cytokines, chemokine, immune receptors, enzymes and other preinflammatory molecules. NFκB deviation is one of the mechanisms of some diseases especially those that are associated with inflammation or apoptosis. Sulfur mustard is an alkylating agent that can damage enzymes, DNA and other macromolecules, also induces oxidative stress responses. Results obtained from recent studies on Sardasht sulfur mustard victims 20 years after exposure showed alterations on immune and inflammatory responses. Regard to NFκB significance in inflammatory and its related cytokines in this research we assessed NFκB level in these victims. Materials and Methods:Population study was 189 people of Sardasht sulfur mustard victims and control group include 32 people of Rabat civil. Sampling procedure was systematic random. The result analyzed by SPSS and X^2 and T-test static procedures. For nonparametric analysis Mann-Witney and Kruskall-Wallis were used. NFκB expression levels were evaluated by standard PCR in peripheral white blood cells. Results: Result assessment in two groups showed that NFκB median in exposure group were 188.75 ngr/µl and in control group were 142.84 ngr/µl which NFκB expression level in exposure group was upregulated. This increasing was significant (P= 0.009). Conclusion: NFκB factor involved in many cellular functions, so it's increasing or decreasing has its own results. Due to reduction of inflammatory factors in these victims, its decline was expected but the results of this study showed its increment which likely was for compensates the reduction of inflammatory factors.
Keyword: NFκB, Sulfur mustard, Sardasht, cytokine, inflammatory, PCR,

509. Long Term Impact of Sulfur Mustard Exposure on Leukocyte Subpopulations; Sardasht-Iran Cohort Study (SICS)

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Background: The most important long-term morbidity problem of SM toxicity is pulmonary complications but the pathogenesis of these complications is not clearly understood. This study evaluates the leukocyte sub-sets and their correlation with pulmonary function in SM exposed civilian cases 20 years post-exposure as gathered in the context of the Sardasht-Iran Cohort Study (SICS). Materials and Methods: Samples were randomly selected from two groups, SM-exposed (n=372) and control (n=128), with the same ethnicity, culture, and demography. Three color flow cytometry (BD Biosciences) was applied for leukocyte sub-population detection. Results: It was found that absolute numbers of NK cells are highly increased in peripheral blood of exposed cases but no changes were seen in any other peripheral blood sub-populations. Conclusion: We have proposed that NK cells are involved in the pathogenesis of long term SM-induced pulmonary complications.
Keywords: Sulfur Mustard, Leukocyte Subpopulations, Pulmonary function

Poster Discussion Presentation

510. Studies on the Correlation between Genetic Features and Dosage of Used Drugs in Chronic Myelogenous Leukemia Patients Exposed to Sulfur Mustard

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Background: Chronic Myelogenous Leukemia (CML) is the abnormal growth of myeloid stem cells with or without forming of Philadelphia chromosome or/and gene. Researchers believe that sulfur mustard (SM) as chemical warfare which used in Iraq-Iran war by Iraq can increase the incidence of CML in exposed people. Materials and Methods: In this study we considered correlation of genetic feature and dosage of use drugs in four CML patients exposed to SM and comparison of findings to eleven CML patients non-exposed to SM. Results and conclusion: Our findings show that administration of imatinib in exposed patients was 35 units more than non-exposed patients. In exposed patients Philadelphia chromosome was formed more than in comparison to non-exposed patients (Pv=0.0475). Exposed and non-exposed to SM didn't affect dose of IFN-α used by patients in two groups. More dosage of imatinib used by exposed patients indicates more severely clinical complications which

results from more incidence of Philadelphia chromosome. One of our suggestions is following up the exposed patients with checking genetics features more times to prodiagnose before changing chronic phase of disease to accelerated stage.
Keywords: CML, SM, Philadelphia chromosome

511. Studies on the Cytogenetic Features in Chronic Myelogenous Leukemia Patients Exposed to Sulfur Mustard and Comparison to CML Patients Not Exposed to SM

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Background: Studies have shown that Sulfur Mustard (SM) can suppress the immune system specially $CD8^+$ cytotoxic cells. Also reports indicated that people exposed to SM have high incidence of tumor specially Chronic Myelogenous Leukemia (CML). The involving in CML is correlated with incidence of Philadelphia chromosome with 95%. Materials and Methods: We took 10 cc of peripheral blood and 2 cc of bone marrow from patients. Samples divided in two groups; one is CML patients exposed to SM and other was CML patients non-exposed to SM. To find the cytogenetic variations of CML patients exposed to SM, we determined the fusion of bcr/abl by real time reverse transcriptase (RT) PCR examination. The Philadelphia chromosome formation will examined by karyotype and fluorescent in situ hybridization (FISH) and expression of the bcr/abl gene was evaluated by western blot technique. The finding data were assessed by SPSS ver.16 software including: Generalized Estimation Equation (GEE) and Wald Chi-square tests if the data distribution is not normal population. Results: Data showed that the bcr/abl formation in CML patients exposed to SM is 1.778 times more than that in non-exposed CML patients. Subsequently, the anemia is expected to be severe in CML patients exposed to SM in comparison to other group. In spite of few cases of CML exposed patients, we found some evidences we expect. Conclusion: For predicting progression of CML we suppose to research on immunologic characteristics to prodiagnose and find real results.
Keywords; CML, SM, Philadelphia chromosome, real time RT-PCR

Poster Presentation

512. Study of Apoptosis of Peripheral Blood Mononuclear Cells of Chemical Victims 25 Years after Sulfur Mustard Exposure

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Background: Sulfur mustard (SM) is one of the most important chemical warfare agents that used in World War I and the Iran–Iraq War. SM causes the short and long term complications on different organs especially lung, eyes and skin. It is reported to inducing DNA damaged, disturbance of cell metabolism and causing apoptosis and necrosis. Although the short term effects of sulfur mustard was more studied, the mechanisms of its long term toxicity is unclear up to now. The aim of this article is the study of the apoptosis of peripheral blood mononuclear cells (PBMC) of chemical victims 25 years after SM exposure in compared to the control group. Materials and Methods: Blood samples were obtained from 15 male sulfur mustard exposed patients and 15 age and sex matched healthy individuals. The PBMCs were cultured and lysed with lysis buffer and the apoptosis was measured by cell death detection ELISA kit. Results and Conclusion: Results will be presented in the congress.
Keywords: Apoptosis, Peripheral Blood Mononuclear Cells, Chemical Victims, Sulfur Mustard

513. Assess of Stromal Cell-Derived Factor-1 (SDF-1) Polymorphism in Sardasht Chemical Victims 25 Years after Exposure to Sulfur Mustard

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Background: Sulfur mustard (SM) is one of the widely chemical weapons that used during the Iraq- Iran war. SM causes the short and long term complications on different organs. Although the acute effects of SM were more studied, the mechanisms of its long term toxicity are unclear to now. Stromal cell-derivedfactor-1(SDF-1) produced by stromal cell and play important roles at repair and inflammation of different tissues.The aim of this study is the assessment of SDF-1 genpolymorfism in Sardasht chemical victim 25 years after SM exposure in compared to healthy sex-matched controls. Materials and Methods: Genomic DNA was extracted by DNAzol from Sardasht chemical victim and health control group peripheral blood. SDF-1 gene was amplification by PCR with specific primers. Polymorphism was evaluated by PCR-RFLP for all PCR products with MSP-I enzyme. Digested products were run in 2% agarose gel and analysis by transilluminator. Results and Conclusion: Genotype frequency of SDF-1 gene of 100 SM exposed group were compared with 100 control individuals.
Keywords: SDF-1, Polymorphism, Sulfur Mustard

514. Evaluating of Relationship between Serum Levels of MMP-9 and TIMPs-MMP-9 complex and Ocular Injuries 20 years after Sulfur Mustard Exposure (Sardasht-Iran Cohort Study)

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Background: Sulfur mustard (SM), is an alkylating agent which induces short and long term effect against various organs including the eyes but the damaging mechanisms have not clearly been defined. MMP-9 has been known as the gelatinase B, it is particulary important in the pathogenesis of inflammatory, infectious, and neoplastic disease including the eyes injuries. In the present study the relationship between serum levels of MMP-9 and TIMPs and ocular injuries induced by SM was evaluated. Materials and Methods: In this historical cohort study 372 male SM exposed subjects and 128 age matched unexposed controls were studied. A complete ophthalmologic assessment including ocular history, visual acuity changes, and ocular examination using Slit lamp biomicroscope was carried out for all participants. Final ophthalmologic assessments were recorded using the criteria verified by the Medical Committee of the Foundation of Martyr and Veterans Affairs. Serum concentration of MMp-9, TIMP-1, TIMP-2, TIMP-4 were measured by a sandwich ELISA technique using DuoSet ELISA Development kits. Results and Conclusion: The results show a significant difference between TIPM-1-MMP-9 complex of exposed participants who had slit lamp findings with those exposed who didn't have slit lamp findings. There were not significant differences between control and exposed group who had slit lamp findings.
Keywords: Sulfur Mustard, MMp-9, TIMP-1, TIMP-2, TIMP-4, ELISA

IMMUNOLOGY of FUNGAL DISEASE

Oral Presentation

515. Evaluation of the Correlation between Tissue Reaction and Cytokines Patterns Induced by *Alternaria alternate* in Mice

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Background: *Alternaria alternata* (*A. alternata*) is well-known as a source of allergenic components in the cell wall and cytoplasm of conidia and hyphae that cause respiratory allergic disorders. The purpose of this study was to evaluate tissue reaction and Th_2 cytokines in mice exposed to *A. alternata*.Materials and Methods: *A. alternata* was cultured and fungal extract was prepared by freeze-defreeze and sonication methods. BALB/c mice in one group were sensitized by two intraperitoneal injections of *A. alternata* extract and then intra-nasally challenged with spores suspended in sterile normal saline solution and in another group, mice only received spores intra-nasally. Blood sampling and necropsy were performed at 1 and 72 hours after spore inhalation. Results: Histopathology demonstrated an inflammatory response with cells including lymphocytes, macrophages, neutrophils and eosinophils present and mucus hypersecretion in the lungs and airway epithelial cell hyperplasia and necrosis observed in sensitized and non-sensitized mice. Sera were analyzed by ELISA to determine serum levels of IL-4 and IL-13 in immediate response and late-phase reaction, respectively. Increasing Th_2 cytokine (IL-4 and IL-13) levels in the sera was also observed in the sensitized and challenged mice. Conclusion: The results showed that exposure to extract and then spores of *A. alternata* induced rapid and highly elevated production of IL-4 and IL-3. These cytokines were associated with respiratory histopathological changes.
Keywords: *Alternaria alternata*, IL-4, IL-13, Lung, Macrophage

516. Immediate Hypersensitivity and Serum IgE Antibody Responses in Patients with Drmatophytosis

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Background: Infections by dermatophytes induce a specific immune response, with humoral and cellular components. The purpose of this study was to determine skin reactivity and serum immunoglobulin E (IgE) antibody responses in patients with chronic and acute dermatophytosis. Materials and Methods: One hundred and sixty-three patients with chronic dermatophytosis, 35 patients with acute dermatophytosis, 41 atopic patients and 49 healthy subjects were enrolled with sequential trials. Sensitization to *Trichophytonmentagrophytes* (*T. mentagrophytes*), *Candida albicans* and *Aspergillus fumigatus*antigens has been evaluated in patients by skin prick test (SPT) and by the presence of specific IgE antibody in enzyme-linked immuno-sorbent assay (ELISA). Results: Positive immediate hypersensitivity (IH) were obtained in 23.9% of the atopic patients with chronic infection for *T. mentagrophytes*, representing significant differences with other patient groups ($P<0.05$). Specific anti-*T. mentagrophytes*IgE antibodies were detected in atopic patients with chronic (16.6%) and acute (2.9%) infections, while none of the atopic subjects had positive IgE reactions to *T. mentagrophytes*. Conclusion: Our findings suggest that there is a particular predisposition to develop chronic dermatophytosis in atopic patients.
Keywords: Atopy, Dermatophytosis, Immunologic responses

517. Propolis Efficacy on IFN-γ Cytokine Production in Old Mice with Systemic Candidiasis

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Background: Propolis is a natural product and its immunomodulatory efficacy has been demonstrated; however, there is little information concerning its effect on the innate and the adaptive immunity of old mice. The purpose of this study was to investigate the effect of propolis on cytokine level of IFN-γ in old healthy mice and old mice with systemic candidiasis. Materials and Methods: Fifty Balb/C mice aged 8 months were divided into 5 groups. Mice group 1 received ethanolic extract of propolis (100 mg/kg/day) by gavage for 7 days, mice group 2 received both *Candida albicans* (*C. albicans*) intravenously (2×10^5 cell) and propolis, and mice group 3 received only *C. albicans* (intravenously, 2×10^5 cell). After 8 days of experiments, all mice were euthanized, blood samples were collected, and the spleens were excised. Splenocytes were isolated immediately and cultured in RPMI-1640 medium without stimulation and/or stimulated in the presence of Concanavalin A (Con A) for 48 h. Supernatants of splenocytes cultures and serum of mice were tested for cytokine of IFN-γ by Enzyme-linked immunosorbent assay (ELISA). Results: Orally-administered propolis treatments showed that it alone suppressed production of IFN-γ in the serum of mice when compared to controls, whereas the cytokine production was strongly stimulated in old mice receiving propolis altogether with *C. albicans*. Conclusion: Our results indicated that propolis alone does not induce cytokine production in old mice, but it may have a beneficial effect on pathogenesis of systemic candidiasis by modulating levels of cytokines including IFN-γ.
Keywords: Propolis, Systemic candidiasis, Immunity, Cytokines

518. Anti Candida activity & Immunostimulatory effect of Spirulina in BALB/c Mice with systemic Candidiasis

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Background: The purpose of this study was to evaluate the immunostimulatory effects of oral administration of *Spirulina platensis*, blue-green algae, on resistance to systemic candidiasis in Balb/C mice. Materials and Methods: There were separate experiments: first we used 4 groups, 10 mice per group for cytokines assay. Animals were inoculated intravenously (I.V) with 1×10^6*Candida albicans*after four days oral administration with 800mg/kg.b.w of *Spirulina*. Control groups received 200µl&100µl normal saline for prophylaxis and inoculation, respectively. Five mice from each group were euthanized after 24 and 72 hours and the levels of IFN-γ and TNF-α in their serum were measured by ELISA method. In second experiment, two infected groups, 11mice per group were included to evaluate survival rate. Animas were monitored 30 days for survival and kidney, liver, lung and spleen were analyzed for fungal invasion. Results: *Spirulina*-treated mice produced more IFN-γ and TNF-α than their control groups. The mean survival time (28.86±2.7) was significantly ($P=0.033$) higher than control group (13.9±3.34), and also exhibited fungal clearance in target organs at death time, significant differences were observed in spleen and liver ($P<0.05$). Conclusion: Prophylaxis with *Spirulina* had synergistic effect on production of IFN-γ and TNF-α and in consequence, these data provides important information for the potential application of *Spirulina* in the therapy and affect host resistance to systemic Candidiasis in BALB/c mice.
Keywords: Spirulina platensis, Systemic candidiasis, Cytokine production

519. *Aspergillus* Allergen: Environmental Distribution and Clinical Importance

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Fungi are ubiquitous organisms that are widely distributed in nature. Several fungal genera are associated with a number of allergic diseases in humans. The prevalence of respiratory allergy to fungi is estimated to be 20% to 30% of atopic individuals and up to 6% in the general population. *Aspergillus* is one of the most prevalent airborne fungi both in indoor and outdoor environments. Several species of *Aspergillus* have been shown to be allergenic, including *A. fumigatus, A. niger, A. nidulans, A. terreus, A. flavus*, and *A. oryzae*. It has been demonstrated previously that individuals can and do become sensitized to *Aspergillus* allergens. The major allergic manifestations induced by *Aspergillus* are allergic asthma, allergic bronchopulmonary aspergillosis (ABPA), rhinoconjunctivitis and hypersensitivity pneumonitis. These diseases result from exposure to spores, vegetative cells, or metabolites of the *Aspergillus*. *Aspergillus* is a major source of allergen among different fungi. Based on the official website of the systematic allergen nomenclature that is approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee, over 20 allergens have been characterized in *A. fumigatus*, one from *A. flavus*, three from *A. niger*, one from *A. versicolor* and two from *A. oryzae*. Our previous studies that conducted in different environments including indoor and outdoor of work place and asthmatic patients houses revealed that the genus of *Aspergillus* is one of the most prevalent fungi in these sampling sites. The accurate in vivo and in vitro diagnoses of fungal allergies depend on the availability of well-characterized allergen preparations. The diagnosis of allergy is based on clinical history, skin test reactivity to the offending allergen and in vitro determination of allergen- specific IgE-antibodies in serum. Large scale serological studies showed that specific IgE responses to at least four *A. fumigatus* allergens (Asp f 2, 4, 6, and 8) are highly specific for sera of patients suffering from ABPA.

Keywords: *Aspergillus,* allergen

Poster Discussion Presentation

520. A Study of the Effects of *Ochratoxin A* on Th_1 Cytokines Profile

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Background*:* Molds secreted over 400 differenced mycotoxins, all which can toxic for all humans. There are four mechanisms by which fungal toxins cause pathology. *Ochratoxin A (OA)* is a secondary metabolite, mostly produced by *Aspergillusochraceus* and Penicillium genera and contaminated animals feeds and humans foods. The metabolism of *Ochratoxin A* and its toxicity different from organ to organ. The aim of this study was to assess the OA effect on cytokines IL-2, TNF-α, IFN-γ and IL-12. Materials and Methods*:* 12 adult female balb/c mice were orally administrated OA, 3mg/kg/daily for months. Control group received normal feed. The animals were scarified and the levels of the cytokines were assessed by ELISA, Kit. Results*:* Based on the results the mean of IFN-γ, IL-12 were significantly reduced in compared with control group ($p<0.05$), but in respect to IL-2& TNFα.These reducing were not significant. Conclusion: Our data approval that OA involved some important the cytokines during ochratoxicosis in animals & probably humans.

Keywords: *Ochratoxin A*, IL-2, TNFα, IFN-γ and IL-12

521. Esterase Activity of the *Candida albicans* Inhibits IL-12 Production by Human Monocytes

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Background: Enzymeactivityof C*andidaalbicans*isknownasanimportantvirulencefactorin candidiasis.Asit'sdemonstrated, regulatinginflammatorycytokineproductionofmacrophagesinteractswithdifferentfungi. Materials and Methods: Inthisstudy,weobtainedmonocytesfromhealthyhuman.Monocytescollectedfromperipheralblood.Cellswerethenharvested,purified,andcultured.Asweplanned,IntracellularesteraseenzymesobtainedbyaspecificmethodanddeterminedbyELISA. Weestablishedamonocyte-candidainvitromodel to assessthepotentialactivityofesterasetoinhibitIL-12productionbymonocytes. Results: Theresultsshowedthatesterasesignificantlyinhibitstheproductionof IL-12bymonocytes.Basedonstudiesmentionedabove,itwasrelatedtoadose-dependentmanner. Conclusion: Overall,based on the resultsofthisstudy,esteraseisanimportantagentinthat*Candidaalbicans*caninvadedeeporgans.

Keywords: Esterase Activity, *Candida albicans,* IL-12, Monocytes

522. A study on the Effect of Fumonisin B1 on Immune System

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Fumonisin B1 is a worldwide mycotoxin produced by several species of Fusarium specially F. verticillioides. It is found mainly in corn. It inhibits ceramide synthase action and therefore affects sphingolipids synthesis. Limited information on immunological properties of FB1 is available. Effect of fumonisin B1 on nonspecific immunity has been studied and low doses of fumonisin were cytotoxic for peritoneal macrophage while high doses led to morphological alterations like blebbing, nuclear disintegration and decreased phagocytosis in them. Fumonisin B1 consumption decreased antibody levels in chickens dose related as well as reduced viability in lymphocytes. In BAlB/C mice both immunostimulatory and immunosuppression effects were observed based on exposure duration and dose. Accumulation of membranous materials in pulmonary macrophages and kupffer cells was detected as a result of immunosuppression in mice. Immunostimulatory effect included increase in B lymphocytes response to T-dependant antigen. Fumonisin may also have role in Delayed Type Hypersensitivity (DTH). Impaired lymphocyte blastogenesis was observed in calves feeding fumonisin. Turkey lymphocytes showed cytoplasmic vacuolization and therefore no proliferation after exposure to fumonisin B1. In rats after IP administration of FB1 reduced thymus weight, thymus necrosis and elevated IgM levels was shown. Both sphingomyelin cycle products and FB1 affect the T lymphocyte surface antigen expression, disrupt balance between different subpopulation of lymphocytes, inhibit DNA synthesis in normal lymphocytes, and suppress an immune response to T-dependent antigens in vivo. Cytokine production has been shown to be modified by exposure to fumonisin. For example, serum tumor necrosis factor-α (TNF-α)-like activity was increased in pigs fed culture material containing 150 mg/kg fumonisins. Fumonisin-induced changes in the TNF pathway have also been seen in lipopolysaccharide (LPS)-stimulated macrophages collected from BALB/c mice dosed with pure FB1. Mechanism of action of the above described fumonisin B1 will be discussed during the lecture presentation.

Keywords: Fumonisin B1, Immune System

523. Evaluation of Direct Agglutination Test & Conventional Methods in the Diagnosis of Vulvovaginal Candidiasis

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Background: We compared the current diagnostic methods (clinical examination, microscopy and mycological culture) with a direct agglutination (DA) test in the diagnosis of vulvovaginal candidiasis (VVC). Materials and Methods: One hundred fifty women with suspected VVC were examined by microscopy and culture methods. The patients' sera were tested for the presence of anti-*Candida* antibody and iron and zinc

concentration. Results: Of the 150 patients studied, 120 (80%) were found to be *Candida* positive using both microscopy and culture methods whilst 30 cases (20%) were infected with non-*Candida* agents. Forty nine (32.7%) and 71 (47.3%) were positive in microscopy and culture, respectively. VVC was mostly observed in patients ranging from 20 and 29 years of age (42.5%). *Candida albicans* (65.3%) was found in significantly higher proportion of patients compared to other isolated yeasts ($P < 0.05$). The iron and zinc concentrations were similar in the two patient groups. The DA test was positive in 114 (76%) of patients, indicating the incidence of a positive value increasing in direct proportion to the amount of yeast isolated. A titre of 1:16 was the most frequent value in both groups with positive and negative cultures. Conclusion: This study offers the DA test as a useful alternative to conventional methods for the diagnosis of VVC.
Keywords: *Candida*, conventional methods, direct agglutination test, vulvovaginal candidiasis

524. Detecting the *Aspergillus spp.* in (BAL) Fluid Samples by Nested PCR, Culture and Direct Smear

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Aspergillosis are among the most prevalent cause of the respiratory infections. These fungi show invasive aspergillosis (IA) in immunocompromised patients. The number of immunocompromised patients are increasing due to immunodisorder illness, grafts and immunosuppressor drugs, so, rapid identification methods are very important. Conventional detection methods such as culture and direct smear are unsensitive and time consuming. Some methods such as immunodetecting methods have high false positive and are unreliable. Today, molecular methods and PCR are very helpful. These methods are both sensitive and reliable and very rapid . In this study, we used Nested PCR, culture and direct smear to detect Aspergillus spp in BAL fluid samples. We found that positive results by PCR were more effective and sensitive than two other methods.
Keyword: Aspergillus spp, Direct smear, Nested PCR, Fungal culture

525. Evaluation of the TCD4 Count & TCD4 Percentage in Iranian HIV Patients with Oropharyngeal Candidiasis

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Background: Oropharyngeal candidiasis (OPC) is the most common human fungal infection that is known as common opportunistic oral cavity infection in immunocompromised patients. Risk factors include smoking, dentures, diabetes, salivary gland dysfunction, age, broad spectrum antibiotics and corticosteroids therapy, malignancies, Sjögren's syndrome, adrenal suppression and AIDS. In this study we evaluated role of TCD4 count/ percentage in OPC. Materials and Methods: The patients for this study were composed of 150 Iranian HIV positive men and women in Iranian Research Center for HIV/ AIDS, Imam Khomeini Hospital, Tehran, Iran (IRCHA). Oral lesions were clinically diagnosed for each individual and oral samples were obtained and cultured on CHROMagarTM and Sabouraud's dextrose agar. A wet mount with 10% Potassium hydroxide (KOH) was used as microscopic examination for confirmation of OPC. TCD4 was measured by Flow Cytometry method. Results: Sixty percent of patients presented an oropharyngeal candidiasis. Patients were suffering from thrush (38%), perleche (20%), erythematous (4.7%), and esophagitis (12%). A broad range of TCD4 was observed between 4-1800 cells/ml. The means for TCD4 count was 289 cells/ml. The means of TCD4 for patient with sign was 251 cells/ml.
TCD4 count was categorized as less than 200 cells/ml, 200- 400 cells/ml and greater than 400 cells/ml. Forty-three percent of patients had TCD4 inferior to 200 (< 15%) cells/ml, 32% TCD4 200-400 (15-25%) cells/ml and 25% TCD4 greater than 400 (> 25%) cells/ml. No significant difference in TCD4 percentage between groups in the study was observed. A considerable increase in Candida colonization was observed in patients with TCD4 inferior to 200 compared to other patients. The differences in TCD4 count were significant between patients with sign and without sign. Conclusion: The relationship between TCD4 count/percentage and appearance of OPC is controversial. It was shown that the occurrence of OPC was significantly associated with TCD4 count in this study.
Keywords: TCD4, TCD4, HIV Patients, Oropharyngeal Candidiasis

Poster Presentation

526. Comparison of Immunosuppressive Effects of *Cyclosporine A* and *Dexamethasone* on Invasive Aspergillosis in Experimental Animals

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Background: Aspergillus species are globally ubiquitous saprophytes found in a variety of ecological niches. Almost 200 species of aspergilli have been identified , less than 20 of which known to cause human disease .Among them *aspergillus fumigatus* is the most prevalent and is largely responsible for the increased incidence of invasive aspergillosis (IA) in immunocompromsed patient papulation. Aspergillus flavus is the second most common species, particularly in invasive disease of immunosuppressed patients. Invasive aspergillosis is used generally to imply histopathologically demonstrated invasion of tissues. Risk factors predisposing patients to IA include corticosteroid therapy, cytotoxic chemotherapy, transplantation and chronic granulomatous disease. Material and Methods: 60 femal new zealand white rabbits were chosen and divided in to 3 groups. The animals in group 1 and 2 were immunosuppressed with Dexamethasone and Cyclosporine A (CY-A) respectively. The animal in group 3 (control animals) were not immunosuppresed. In each group, half of the rabbits were infected intravenously with 4×10^5 conidia of Aspergillus fumigates. Results: All the rabbits in group 1 and 2 with were infected intravenously , developed clinical signs of invasive aspergillosis. However, of these 2 groups the rabbit which were given the conidia intratrachealy, only 7 of 10 animals in group 1 demonstrated clinical signs of aspergillosis. At autopsy there was Macroscopic and Microscopic evidence of invasive aspergillosis. Histopathological examination revealed hyphae invasion in liver, lung, brain and spleen. Conclusion: We conclude, in natural conditions that aspergillosis is initiated by inhalation of airborn conidia , cortisone-treated patients are more susceptible to invasive aspergillosis than individuals who received CY-A .
Keywords: Aspergillus fumigatus, Cyclosporin A, Dexamethasone

527. Development and Standardization of an Indirect ELISA for the Serological Diagnosis of Aspergilloma

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Background: *Aspergillus* spp are ubiquitous opportunistic moulds that cause invasive syndromes. Glactomannoprotein (GMP) is an essential molecule in pathogenic *Aspergillus fumigatus.* GMP is also produced in the host and is a well-known *A.fumigatus* antigen. The main aim of this study was identification of GMP and standardization of an indirect ELISA for the serological diagnosis of Aspergilloma. Materials and Methods: *A. fumigatus* was cultivated. After purification of GMP, samples were analyzed by SDS-PAGE. Antigen, conjugate, serum dilutions and timing of the test were optimized based on the dilutions where the distinction between positive and negative sera was most evident. ELISA microplates were coated with antigen (dilution 1/100). Subsequently, serum (dilution 1:20) was added. After an incubation period, the plates were washed and a conjugate (1/1000) was added to each well. Following further incubation, TMB was added. After 15 minutes, the reaction was stopped. Plates were read on an ELISA reader. 500 sera samples were used through this study. Sera were analyzed for the presence of antibody to *A. fumigatus* GMP. An immunoblotting test was performed as a validation test. Results: The cut-off point was calculated with basis on the mean of the OD of

the 466 sera found negative for *A. fumigatus* galactomannoprotein, plus three standard deviations. Between 500 probable patient sera, 34 were confirmed cases. The same results were obtained, due to comparing with a commercial ELISA kit. The sensitivity and specificity of our ELISA were 97.1% and 99.7% respectively. Conclusion: Here, we described the development of an indirect ELISA for the serological diagnosis of Aspergilloma infections. The need for less handlingand shortened time required for completion of the test (approximately 1.30 hours for our ELISA, as opposed to 2.30 hours with IBL ELISA kit). This may be a substantial benefit when large numbers of samples are to be tested.
Keywords: Indirect ELISA, Serological Diagnosis, Aspergilloma

528. *Penicillium marneffei* Infection is a Major Problem in HIV-Positive Patients

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p*enicillium marneffei* is the only thermally dimorphic species of the genus *Penicillium*; the aim of this study is a review of the papers that shows *Penicillium marneffei* is an opportunistic pathogen which has emerged to become an AIDS-defining illness in the endemic areas. *P. marneffei* infection is endemic among HIV-positive patients in many areas in Southeast Asia. A lot is still unknown about the natural reservoir and route of transmission of P. *marneffei*. The pathology of *penicilliosis* in different organs varies depending on the host immunity. Anergic and necrotizing tissue reaction are often observed in AIDS patients. The most frequent sites of involvement are liver and lungs but lymph node, bone marrow, skin and intestines are also affected. *Penicilliosis* is mostly seen in late HIV infection with CD4+ count less than 100/uL. Up to 80% or more of the cases have CD4+ count below 50/uL. The disease course was rapidly progressive with a high mortality. The diagnosis of penicilliosis may be suspected or made through examination of cytology or biopsy specimens. Various types of antigen and antibody testing specific to *P. marneffei* have been described but they are not widely available.The mortality rate of untreated penicilliosis is 100%.Any delay in the initiation of antifungal therapy is associated with poor outcome whereas the therapeutic response is good with early institution of treatment. The recommended initial treatment for penicilliosis in HIV-positive patients is intravenous amphotericin B (0.6mg/kg) for 2 weeks followed by oral itraconazole 400 mg per day for 10 weeks. For prevention all patients who have completed treatment for penicilliosis should be put on secondary prophylaxis with itraconazole 200 mg/day.
Keywords: *Penicillium marneffei*, HIV-Positive Patients

529. Effects of *Aspergillus fumigatus* on Cellular Immune Response

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Aspergillusfumigatus (AF) is a ubiquitous mold that most common cause of invasive *Aspergillosis*, important agent of morbidity and mortality in immunocompromised hosts. In the absence of intact innate immunity, inhaled *A. fumigates* spores (conidia) germinate in the lung, forming hyphae that invade blood vessels and disseminate to other tissues.The various elements of the pulmonary innate immune system-physical and cellular barriers and soluble mediators-are involved in the recognition and elimination of pathogens, thereby preventing colonization of the respiratory system and it is accepted now that cell-mediated immunity is the main mechanism of defiance. *Gliotoxin(GT)*, a secondary metabolite that product with *Aspergillus fumigatus*, that is an immunosuppressive mycotoxins long suspected to be a potential virulence factor of *Aspergillus fumigatus*. According to the recent studies, *GT* inhibits T-cell activation primarily by inhibiting antigen presentation and inhibits its function in a dose-dependent manner. *GT* induces apoptosis in monocytesand dendritic cells, resulting in the suppression of AF-specific T cell responses. *GT* effects on important neutrophil functions, including phagocytic function, degranulation, myeloperoxidaseactivity, and the production of reactive oxygen species (ROS). Although GT may suppress the adaptive immuneresponse, *GT* may also serve to increase polymorph nuclear leukocytes (PMN)-mediated inflammation, which is likely to play an important role in tissue destruction in the setting of IA. Another study showed that administration of *fumagillin*, an *Aspergillus fumigatus* toxin, to *Galleria mellonella* larvae suppresses its immune response by inhibiting the action of haemocytes and thus renders the larvae susceptible to infection.
Keyword: *Aspergillus fumigates*, cellular immune

530. The Role of TLRs in the Host Defense against *Candida* Infections

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Background: *Candidiasis* is a fungal infection caused by any of the *Candida* species,commonly *Candida albicans*. Infact, host defense mechanisms against *Candida* species and pathogenesis of *Candidiasis* are not completely understood yet.Despite the importanceof *C. albicans* in human diseases, little is known aboutthemechanisms through which *Candida* is recognized by the cellsand how it triggers the host defense. Multiple host defense systems play roles in the control of *Candida* infection depending on the type and site of infection. Recent data have suggested the important role of Toll-like receptors (TLRs) in the innate immunity to pathogens such as *Candida*. Materials and Methods:Thishypothesis that s Toll-like receptor can recognize Candidawas proposedbecause previous research had shown that TLR2/TLR6 heterodimers recognize zymosan, a structure derived from the fungus *Saccharomyces cerevisiae* and in other hand the cell-wall structure of *Saccharomyces* greatly resembles to structure of *C. albicans*. Any suggestion for a role of TLRs in anticandidal host defense was examined in animal model (TLR-defective mice). Since first observation, additional studies have confirmed the important role of TLRs (TLR2 and TLR4) in the Recognition of *C. albicans*. Results: Recognition of microbial components by TLRs initiates signaling transduction pathways that induce gene expression. These gene products regulate innate immune responses and further develop an antigen-specific acquired immunity.In addition, recent studies have showed the role of TLRs in the development of an acquired immune response and resistance to re infection. Many studies reported that both TLR2 and TLR4 are involved in the development of resistance to reinfection. Conclusion: TLRs are one of the major classes of pathogen recognition receptors, and the recent studies reviewed here demonstrate that they recognize PAMPs from *Candida*, leading to production of cytokines, activation of the microbicidal mechanisms of leukocytes, and resistance to infection and reinfection.
Keywords: TLRs, *Candida*, immunity

531. Humoral Immunity against Malassezia globosa in Patients with Pityriasis versicolor

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Background: Pityriasis versicolor (PV) is a common disease in human caused by Malassezia spp. Recently, Malassezia globosa (Mg) has been known as one of the etiology of PV. the presence of the different species of Malassezia in clinical lesions of PV and seborrhoeic dermatitis (SD), as well as in normal skin of patients and healthy controls, was analysed, showing that M. globosa was the predominant species in the PV lesions, while M. sympodialis was found mainly in the normal skin of the trunk and M. restricta in the face and scalp lesions of SD. Materials and Methods: From the present stady, M.globosa obtained from mycology center and according to previous stady antigens were fractinated and F1 was used in this research. 45 patients sera with PV were included to evaluate humoral immunity by using ELISA and immunoblotting. Results: Sera from PV patients showed higer IgG. Anti Malassezia globosa titeres in rang of 1/200 to 1/600 than the control sera ($P < 0.001$). In immunoblotting, using mouse anti-sera consentration with HRP, %85 of the patients serum reacted with 52 KD, 68KD and 74 KD Mg antigens. In control group only 2 serums showed reaction only with 68 KD proteins.
Keywords: Pityriasis versicolor, Malassezia globosa, ELISA, Immunoblotting

532. Experimental Protection against Cryptococcosis by using Glucuronoxylomannan (GXM) and Propolis Extract

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Background: Cryptococcos neoformans Var neoformans occurs in environment especially in pigeon dropping . The capsule plays a significant role in pathogenicity. It has been approved that GXM has immunosuppressive effects to MNS cells. MaterialS and Methods: In this study, 3groups of female rat, 10 rats for each group, were selected. Group 1 received 5µgr GXM, IV, for 8 days. Group 2 administrated GXM plus propolis (5µgr plus 4 µgr) for 8 days. Group 2 injected just by distilled water as control. After 2 days of the last injection the animals were sacrified & the spleens were removed. The mononuclear cells were purified & they stimulated by concavalinA (Con A). Results: In group 1, there were no any stimulation responses to conA, whereas, high prolifrative responses were observed in group 2. No significant differences to the above mentioned response between group 2&3 were observed.
Conclusion: With attention to this investigation, propolis can inhibit some immunosuppressive activity of GXM and it may be act as adjuvant.
Keywords: Cryptococcosis, GXM, Propolis Extract

533. Evaluation of the Effect of *Zataria multiflora*, *Geranium pelargonium*, *Myrth* and *Lemon* essences on Immune System Function in Systemic Candidiasis

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Background: Herbal essences have been noticed to treatment of infections as will used for improving immune functions against microbes. Materials and Methods: White swithzerland femal rabits were chosen in this study. They were divided into 5 groups as follows: Group1: received Zataria multiflora essence 6 times with 6 days of interval, Group2: received Geranium plargonium, Group3: received Myrth essence, Group4: administrated Lemon essence, Group5: administrated normal salin as control group. Five days after the last injection of the essences, candida albicans (1×10^6 cells) were injected in vein tail of all animals. Phagocytosis, killing assay and LTT were carried out using blood and splenocytes of animals. Result: The cellular immunity was significantly stimulated against C.albicans antigen and con-canavalin A (con-A) in group 1 and 2 in compare with group 5, whereas myrth essence (group3) had no consideral effect and lemon essence suppressed cellular response. Except Geranium pelargonium the other essences could stimulated macrophage killing and phagocytosis. Conclusion: It is concluded that Zataria multiflora, Geranium pelargonium and Myrth can be used as CMI stimulation and lemon essence decreased cellular function. Also, it is suggested that essences under study will be used as modulator agent on innate immunity in the future.
Keywords: *Zataria multiflora*, *Geranium pelargonium*, *Myrth*,*Lemon* essences, Candidiasis

534. Induce Sensitivity to Stachybotrys Chartarum in BALB/c Mice

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Background: Stachybotrys Chartarum is a damp building mold that can be found in water damaged building. The severity of human responses to S. Chartarum in both occupational and home settings varies widely. This study was carried out to examine the effects of this fungus to induce pulmonary allergic reaction in experimental animals. Materials and Methods: Twelve BALB/c mice were divided into two groups of six mice each: controlled and treated group. we intratracheally instilled BALB/c mice of treated group with S.Chartarum spores suspended in saline. Bronchoalveolar fluids of each mouse (12 mice) was collected. Results: Eosinophils and neutrophils were significantly increased in BAL compared with control group. In histological sections, sever inflammatory cells in peribronchial and alveolar sites were observed. Conclusion: This concluded that exposure to S.Chartarum stimulates the inflammatory reaction and the people who living in exact houses should be controlled to pulmonary allergic symptoms.
Keywords: Stachybotrys Chartarum, BAL, allergic symptoms

535. Reduction of Phagocytic Activity in Atopic Patients with Immediate Hypersensitivity to *Candida albicans*

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Background: Atopic dermatitis (AD) is a chronic or recurrent disease with a complex etiology. Immidiate hypersensitivity (HI) to *candida albicans* antigens has been known as one of the etiologic signs of AD. Materials and Methods: In this study, 43 patiants, 21 male and 22 female with an average age of 21.37, were selected to examine their phagocytic activity. The occurrence of IH was confirmed in all patiaents. Neutrophiles and mononuclear phagocytes were separated and phagocytic ingestion of *candida albicans* was investigated. Results: Among the studied patients, 44.1% (19 out of 43) had a reduced phagocytic activity and the others showed normal phagocytic function as compared by the normal phagocytes. Conclusion: This study revealed that sensitivity to candida antigens can involve phagocytic activity, so this patiaents are in risk to exact infections.
Keywords: Phagocytic Activity, Atopic dermatitis, Hypersensitivity, *Candida albicans*

536. Exprimental Allergic Pulmonary Aspergillosis (APA) in Mouse

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Background: Aspergillus fumigatus is an opportunistic mold with worldwide distribution. Inhalation of Aspergillus conidia is the main rout of infection. Allergic Broncho_Pulmonary Aspergillosis (APA) is an important disease among atopic patients. Materials and Methods: In this study, we induced APA in Balb/c mice, 4 to 6 weeks old, by using purified A.fumigatus antigens. Results: The results of this study showed that total and specific IgE and eosinophils were significantly increased, comparison with control group. The level of IL4, IL 10 and IL13 were increased as well. But the levels of Th1 cytokines such as IFN-gamma, IL12 and IL17 were significantly decreased.
Conclusion: Regarding to our results, it is concluded that the A.fumigatus antigens understudy play as important role to induce allergic reactions in experimental animals. The characterization of the antigens will be discussed at the paper presentation.
Keywords: Allergic Pulmonary Aspergillosis, Balb/c mice

537. Evaluation of Skin Test using Trichophytin in Patients with Chronic Dermatophytosis

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Background: Dermatophytosis is a skin fungal infection in which caused by different dermatophytosis.Inrespect to clinical signs, these infections divided 2 groups:acute and chronic dermatophytosis.some predisposing factors, such as, atopy, asthma, cushing syndrome have been known to relate with chronic dermatophyte infection. Materials and Methods: In this study, 49 patients with prover chronic dermatophytosis were selected. Skin test with trichophytin antigen were carried out for every patients. Results: The results of skin test showed 34 patients had immediate hypersensitivity reactions, 10 subjects had no reaction and just 5 cases showed delayed hypersensitivity. Conclusion: It is concluded that allergic reactions in these patients are common and this reaction have intract with cell-mediated immunity, so the exact patients have deficiency to remove dermatophyte infections.

Keywords: Dermatophytosis, Skin test, Trichophytin, Hypersensitivity reactions

538. Comparison of Allergenic Power A. fumigatus, A. Flavus and A. Niger Fungi by Using Patients' Sera with Asthma

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Background: Aspergillus species are common indoor airborn fungi and have been considered as one of the most significant causative agents of human allergic diseases. The goal of this survey was carried out to identify allergenic components of Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger extracts with specific IgE of pooled serum from people suffering from asthma, for comparing allergenic rate of three species of Aspergillus with each others. Materials and Methods: In this study, pooled serum of 32 asthmatic patients attending the Tehran Allergy Clinic were used. After preparing extracts of the mentioned fungi, immunoblotting was used for obtaining allergenic components and anti IgE conjugate with alkaline phosphatase and BCIP and NBT substrates were used to demonstrate reaction results. Results: Immunoblotting results using pooled serum of the patients with asthma showed that A. Fumigatus contains 12 allergenic bands with the maximum band from 18 to 120 kDa, A. niger contains 5 allergenic bands with the minimum band from 50/5 to 178 kDa and A. flavus contains 10 allergenic bands from 28/5 to 91 kDa. Conclusion: It was concluded that abundant allergenic bands of these fungi reacted with sera specific IgE of patients. It was showed that A. fumigatus is more allergenic that the others because of having maximum allergenic band and A. niger has less allergenic power because it has minimum allergenic band.
Keywords: Aspergillus, Allergy, Immunoblotting

539. Evuluation the Number $CD4^+$ $CD25^+$ $FoxP3^+$ *T* reg Cells in Normal Mice Exposed to AFB1 and Treated with Aged Garlic Extract

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Background: Aflatoxin B_1 (AFB_1), a secondary metabolite of the fungus *Aspergillus flavus*, is a hepatocarcinogen in various animal species and human and can suppress immune system. If human receive little dosage of AFB_1daily in long time, it is effect in number of Treg. A wide range of biological activities of garlic in vitro and in vivo have been verified. Our previous studies demonstrated that Aged garlic (keep in dry garlic bulbs in the freezer for six months in -20 ºC) have enriched immunostimulator fractions and reduced immunosuppressor fractions. Recent studies focus on immunosuppressor activity of AFB-1 and immunostimulator activity of Aged Garlic to evaluation of number of Treg in Balb/c mice. Materials and Methods: First of all, Aflatoxin-B1 separated of Aspergillus flavus(PTCC 5004) by HPLC and Aged Garlic extracted by Mantis method and DTH and Hemagglutination test were carried to determinante of Aged Garlic and AFB_1 dosage, which can be effect on immune system. Subsequent experiments were carried out on 20 normal Balb/c mice to estimate the effects of Aged Garlic extract and AFB_1 on number of Treg cell in 4group. Briefly 10µ/kg/day of Aflatoxin-B1 and Aged Garlic extract diluents were administered for 4 consecutive days to group 1: AFB_1, 2: control; 3: garlic extract+ $AFB_1$and 4:garlic extract via intraperitoneal (IP) rout, respectively. Mice were sacrificed and splenocytes harvested and the percentage of splenic Treg cells was measured by Flow cytometry Analysis. Results: According to the findings, Aged Garlic extract could increase DTH reaction and decrease the Treg number rate in spleen ($P<0.05$). AFB_1 could increase the Treg number rate in spleen ($P<0.05$).In group 2(control) and4 (garlic extract) the Treg number rate is decreased (P value>0.05) and in group 1and 3 Treg number rate was increased ($P<0.05$). Conclusion: In general these results introduce some immunostimulator properties of aged garlic extract which modulate the immunosuppressor properties of AF-B1 in vivo.
Keywords: Aflatoxin-B1, Aged Garlic extract, Treg, Immunotherapy

540. IL-22 in Antifungal Immunity

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Deciphering cellular and molecular mechanisms that maintain host immune homeostasis with fungi and the breakdown of this homeostatic tolerance during fungal infections disease is a challenge in medical mycology. In fact, the virulence of fungi may be determined by the interaction between fungi and the host immune status and its classification as a commensal microorganism or a pathogen may shift depending on the balance. In addition to the central role of the IL-12/IFN-γ-dependent Th1 responses in cell-mediated immune protection against fungi, Th17 cells provide protection and inflammation at mucosal surfaces, and Tregs fine-trune immune responses to prevent damage to the host. Recent evidence indicates that IL-22-producing cells, employing primitive antifungal effector mechanisms, contribute to antifungal resistance at mucosal surfaces under conditions of defective adaptive immunity. The fact that IL-22 production is driven by commensals points to the need of an integrated, systems biology approach to improve our understanding of the inherent and intimate mechanisms underlying multilevel host-fungus interactions.
Keywords: allergy; Antifungal immunity, Fungal diseases, IL-22

541. Study of Host Inflammatory Response and Th1 and Th2 Cytokines in Mice with Invasive Aspergillosis

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Background: Invasive aspergillosis (I.A) is a common and devastating complication of immunosuppression which caused by *Aspergillusfumigatus*. Given its poor outcome with current therapy, the precise mechanism of the immune response to *A. fumigatus* is of interest.To further definition of the involvement of innate immunity, inflammatory responses and cytokines in invasive aspergillosis, we investigated the expression of TLR-2, Dectin-1 and the level of cytokine production (IFN-γ, IL-4, TNF-α and IL-10) in a mice model of invasive aspergillosis. Materials and Methods: Invasive aspergillus infection was induced by intravenously injection of *A. fumigatus* conidia in selected groups of mice. Control animals have been injected with normal saline. Fourteen days after infection, mice were scarified and cytokine production of cultured splenocytes was detected by ELISA method. The presences of TLR-2 and Dectin-1 on macrophages were analyzed by flowcytometry. Results: The results show that after infection of mice with *A.fumigatus* conidia, the levels of TLR-2 and Dectin-1 have been increased in compare with control group. Moreover, it showed insignificant decrease in IFN-γ and IL-10 levels and insignificant increase in TNF-a level. The data demonstrated remarkable rise in IL-4 level. Conclusion: Probably I.A causes stimulation in innate immunity and Th2 cells, also some disorganization in cytokine production in CD4+ T helper cells. Other complementary studies could help supporting our hypothesis.
Keywords: invasive aspergillosis, Immune Responses, Cytokines, TLR-2, Dectin-1

IMMUNOLOGY of ORAL DISEASES

Oral Presentation

542. Is Ibuprofen Capable to Change IL-1β, TNF-α and PGE2 Levels in periapical Exudates? A Double Blinded Clinical Trial

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Background: Bone resorption is one of the main features of inflammatory periapical lesions and mediated by cytokines mainly interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and prostaglandin-E2 (PGE2). Recent investigations of these lesions revealed that pharmacological modulation may be possible. The aim of this study was to evaluate the effect of Ibuprofen on IL-1β, TNF-α and PGE2 levels in periapical exudates and compare the results with a group of placebo control. Materials and Methods: Thirty patients with non vital teeth and radiographic lesions were divided into two groups of case and control according to their entrance to the study. Periapical exudates were taken from root canals using absorbent paper points and followed by 400mg Ibuprofen and placebo prescribed one tablet q6h for three days and in the fourth day second samples were taken, then final cleaning, shaping and obturation of the canals were completed. IL-1β, TNF-α and PGE2 levels were determined by enzyme-linked immunosorbent assays (ELISA). Data were analyzed using paired *t-test* and student's *t-test*.Results: showed that PGE2 levels were decreased significantly in the case group to 86.92±72.42 Pg/ml following Ibuprofen treatment comparing with the pre-treatment (164.96± 12.255 Pg/ml) (p=0.02) and placebo group (154.2±97.13 Pg/ml) (p=0.001). But there were no significant differences in IL-1β and TNF-α levels between the two groups and in each group before and after treatment. Conclusion: the data indicate that ibuprofen as a non-steroidal anti-inflammatory drug (NSAID) can be used to block PGE2 releasing, enhance healing of inflammatory periapical lesions and may be inhibit bone resorption.
Keywords: Ibuprofen, IL-1β, TNF-α, PGE2, periapical Exudates

543. The Role of Prophylactic Ibuprofen and N- acetylcysteine on the Levels of Tumor Necrosis Factor alpha, Interleukin 6 And 17 In Periapical Exudates and the Post-Treatment Pain Level

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Background: Periapical lesions are inflammatory disease induced by bacterial infection of the dental pulp. Although various immunological studies concerning cytokines involved in the formation of periapical lesions have been reported, the role of prophylactic drugs on the level of cytokine in periapical lesions remains unclear. The aim of this study was to evaluate the role of prophylactic ibuprofen and N-acetylcysteine (NAC) on the level of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and IL-17 and post-treatment pain level in chronic periapical lesions. Materials and Methods: eighty patients with chronic apical lesions less than 1 cm were randomly assigned to receive NAC tablets(400 mg), ibuprofen tablets(400 mg), NAC (400 mg)/ibuprofen (200 mg) combination and placebo (starch) 90 minutes prior to sampling. Periapical exudates were collected from root canals using absorbent paper points. TNF-α, IL-6, IL-17 levels were determined by ELISA and post-treatment pain was assessed using a visual analog scale (VAS) at 4, 8, 12, and 24 hour after treatment. Cytokine levels in the periapical exudates were compared between groups by using Mann-Whitney test. VAS pain scores were compared using one way ANOVA and Post hoc Tukey test. Results: there was no significant difference between groups regarding TNF-α level (P>0.05). There was significant difference in IL-6 level between ibuprofen group and placebo (P=0.019). Significant difference in IL-17 level was observed between NAC/ibuprofen combination group and placebo (P=0.043). Four hours after treatment, a significant difference was observed in VAS pain score between ibuprofen group and placebo (P=0.017). Eight hours post- treatment, VAS pain score was statistically difference between NAC group and placebo (P=0.033). After 12 hours VAS pain score showed significant difference between NAC group and placebo (P=0.049). Conclusion: the prophylactic ibuprofen and NAC failed to clearly reflect their effect on cytokines levels in exudates of chronic periapical lesions. On the other hand it seems that NAC can be a substitute for ibuprofen for post endodontic pain.
Keywords: Periapical lesion, N- acetyl cysteine, Ibuprofen, IL-6, IL-17, TNF-α

544. Correlation between Inflammatory Cytokines and Smoking in Periodontitis

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Background: Cigarette smoking is considered as an important environmental risk factor for the initiation and progression of periodontal disease. To objective of the present study was to evaluate the effect of smoking on to clinical parameters and the gingival crevicular fluid (GCF) cytokines including IL-10, IFN- γ, IL-6, IL-1β and TNF-α in patients with generalized moderate periodontitis. Materials and Methods: This case-control study was performed on 60 male patients with generalized moderate periodontitis, in the age range 20-60 years old, consist of 30 smokers (Patients group [PG]) and 30 non-smokers (Control group [CG]). After recording our concerned clinical parameters, GCF sample were collected by means of paper points, from four teeth with ≥ 4 mm probing depth per patient, selected in four quadrants. Then cytokines levels were determined by using ELISA, IL-10 and IFN- γ with Bendermed system Kit; IL-6, IL-1β and TNF-α with Diaclone, setup assay. Results: mean age of PG and CG were 42.83 (±8.32), 39.5 (±11.06) Years respectively. Mean levels of IL-10 and IFN- γ in PG and CG were 1.25 (±0.07), 0.82 (±0.08); 1.22 (±0.08), 0.75 (±0.06), respectively. There were no significant difference between its (P>0.05). Although mean levels of IL-1β, TNF-α and IL-6 were 263.73 (±180.37), 301.78 (±212.09), 101.72 (±225.62), respectively, in PG; 257.74 (±201.78), 442.9 (±342), 82.53 (±202.45), respectively, in CG. No significant difference was observed. Clinical parameters include Bleeding Index, Plaque Index, and Probing Pocket Depth no significant difference between groups (P>0.05). Conclusion: The present study did not reveal any difference between concerned parameters in smokers and non-smokers.
Keywords: IFN- γ, IL-10, IL-6, IL-1β, TNF-α, GCF, Periodontitis, smoking, ELISA

545. Comparison of RANKL mRNA Expression in Healthy and Inflamed Periodontal Tissues

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Background: The aim of this study was to assess the expression of RANKL in gingival tissues of patients with periodontal diseases and to clarify possible correlations with clinical parameters. Materials and Methods: Gingival biopsies were obtained from patients with gingivitis (n=17), moderate to severe chronic periodontitis (n=18), as well as healthy subjects (n=15). RANKL mRNA levels were determined using Real time PCR. The intensity of RT-PCR products was analyzed in proportion to the intensity of GAPDH. Results: RANKL mRNA was expressed at significantly higher levels in the inflamed periodontal tissues compared to healthy controls. RANKL expression levels tended to be higher in gingivitis compared to periodontitis although the difference was not statistically significant (P>0.05).Furthermore, a positive correlation was revealed between RANKL and clinical attachment loss ($P < 0.05$). Conclusion: The net increase in the ratio of RANKL in periodontally diseased tissues and association with the clinical parameter of attachment loss correspond well with the critical role of RANKL in immune responses driving osteoclastogenesis and bone loss in periodontal disease and the possible impact on disease progression.
Keywords: Periodontal disease; gingivitis; periodontitis; RANKL

Poster Discussion Presentation

546. Vascular Endothelial Growth Factor (VEGF) in Unstimulated Saliva of the Patient With Minor Aphtous

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Background: VEGF is a multifunctional cytokine and a hetrodimer glycoprotein that has a role in vascularization and healing of the ulcers. Because of unknown pathogenesis of the aphtus and side effect of current treatments and lack of the investigations with clear result in this subject, so aim of this study is to evaluating level of VEGF in 4 clinical stages of the minor aphtus. Materials and Methods: in this case- control study was done among 18 patients with minor aphtus in 4 clinical stages (prodromal- preulcerous- ulcerous- healing) and 18 subjects as a control group. Their saliva was collected by spitting method in specially prepared tubes. Salivary VEGF Level (pg/ml) in each stags was determined and the results were analyzed by Mann- Whitney and Repeated Measures ANOVA test. Results: A significant difference in 4 stage of clinical aphtus were seen (p=0/002). At least level of VEGF were seen in ulcerous stage compared with control group (p= 0/006). Level of VEGF in prodromal stage was more than healing and ulcerous stages (p=0/06) (p=0/014), but no significant difference in Level of VEGF was seen in following stage: healing with ulcerous and preulcerous, prodromal with preulcerous (p=0/158) (p=0/619) (p=0/66), respectively. Conclusion: The results of this research support that VEGF variability specifically decreases in ulcerous stage have a role in minor aphtus ulcers pathogenesis. Because, no over expression of VEGF in aphtus healing stage. It is probable that using the anti - vascularization drugs with not be useful.
Keywords: Minor Aphtus, VEGF, saliva, ELISA

547. Evaluation of Interleukin-1β, and 8 levels and Neutrophil Count in 3-5 Years Old Children's Saliva

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Background: Dental Caries is a multi-factorial and complicated disease and saliva plays the most important role in dental caries. neutrophils are the first barrier defense in immune system, with respect to the infection nature of dental caries and it's Presence in Saliva in dental caries time, the aim of this study was assessing of neutrophils count and IL- lβ, IL-8 Concentration in children's Saliva with dental caries and caries free. Materials and Methods: This case-control Study has been performed on 3-5 years old Preschool children among the kindergarten of the Babol city. Children were divided to three groups accordance to clinical evaluation [caries free, early childhood caries (ECC) and severe early childhood caries (S-ECC)]. Their saliva was collected by spitting method in specially prepared tubes. Investigating the neutrphil's count was done by Gimsa Procedure and to investigate IL- lβ, IL-8 Concentration in Saliva by ELISA method. Data has been analyzed by Kruskal- wallis test and Post hoc Dunnette T3. Results: Ninety 3-5 years old children were involved in the study. The mean 1L- 1β was 47/68$^{pg/ml}$ ($\pm$ 5/52), and IL-8$^{pg/ml}$ 79/41($\pm$ 10/14). The mean number of neutrophils was 2088/33 ($\pm$ 405/47). Mean of IL-1β, IL-8 levels and neutrophil count in caries free group has been found 59/2$^{pg/ml}$ ($\pm$ 52/15), 86/04$^{pg/ml}$ ($\pm$ 96/12) and 1342/66 (+ 2222/412), respectively. In ECC, and S-ECC has been found 36/78$^{pg/ml}$ ($\pm$ 40/88), 76/12$^{pg/ml}$ ($\pm$ 107/01), 2500($\pm$ 3834/61); 48/75$^{pg/ml}$ ($\pm$ 47), 76/77$^{pg/ml}$ ($\pm$ 70/63), 2353/1($\pm$ 4583/81) respectively. There was no significant difference among IL-1β, IL-8 levels and neutrophils count at three groups. (P=0.467, P=0.862, P=382).Furthermore, in an investigation has been done between groups; no Significant difference was observed in IL-1β, IL-8 levels and neutrophil count (P>0.05). Conclusion: IL-1β, IL-8 levels and neutrophil count among 3-5 year old children with caries free and caries didn't show a significant difference but it is need to more studies to give effective idea.
Keyworks: dental Caries, Children, Saliva, neutrophil, IL-8, IL-1β.

548. Leptin-an Adipocytokine and Periapical Granulomas

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Background: It was shown that leptin regulates bone formation and its expression in adipose tissue and its level in circulation are increased after administration of inflammatory stimuli such as lipopolysaccharide (LPS). There is no data about the role of leptin in the inflammatory periapical lesions. So the aim of this study was to evaluate the presence and concentration of leptin in chronic periapical lesions. Materials and Methods: For this purpose, chronic periapical lesions were collected from twenty patients and were cultured for 72 hrs. ELISA method was used in order to determine the concentration of leptin in supernatant fluids of explants cultures. Statistical analysis was undertaken using non-parametric tests (Mann-Whitney U, Chi Square and Spearman's Correlation Coefficient. Results: Leptin was found in all samples with the average concentration of 405.55±102.98 (pg/ml). There was no significant correlation between the concentration of leptin and BMI or the diameters of lesions. Conclusion: It has been concluded that leptin could be considered as an inflammatory mediator during the early phases of dental periapical lesions.
Keywords: Leptin, Periapical Granulomas

549. Correlation between Salivary sCD14 and Induction of Apoptosis of Neutrophils by Saliva in Patients with Gingivitis

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Background: Soluble CD14 (sCD14) competes with membrane bound CD14 (mCD14) for LPS binding and is able to neutralize LPS-induced responses in vitro and in vivo and mediates the LPS-induced activation of non-CD14-expressing endothelial, epithelial and smooth muscle cells. Inhibits binding of LPS to CD14, so it may have some protective role against periodontopathic bacteria. On the other hand it is established that gingival crevicular fluids of patients with periodontal diseases can induce apoptosis of neutrophils. So the aim of this study was to determine the correlation between sCD14 in GCF and the ability of GCF in inducing the apoptosis of neutrophils in patients with gingivitis. Materials and Methods: GCF samples from 20 healthy and 20 diseased sites of 18 patients with gingivitis obtained and the levels of sCD14 in samples were determined by ELISA. After separating neutrophils from the peripheral blood sample of a healthy individual, they were incubated with GCF samples, and then by staining with Annexin V, the percentage of apoptotic, necrotic and vital cells were determined. Results: Although there was significant difference between case and control groups regarding sCD14 levels and the number of apoptotic cells, there was not any correlation between the level of sCD14 and the induction of apoptosis. Conclusion: Elevated levels of sCD14 in gingival inflammation probably does not have any correlation with the induction of neutrophil death.
Keywords: sCD14, Apoptosis, Saliva, Gingivitis

Poster Presentation

550. Estimation of Immunosuppression Level According to Oral Manifestations in HIV-Positive Patients

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Oral lesions might be considered as the initial manifestations of the disease. Oral manifestations of HIV infection are important in the AIDS epidemic and some of them could be used to assess the status of immunosuppression and determine the prognosis of the disease.Some oral lesions may even alter patients' quality of life.Early diagnosis and appropriate treatment of oral lesions have great influence on patients' general health and can reduce the mortality rate of the disease.Although some lesions such as candidiasis and hairy leukoplakia are considered as prognostic factor, recent data have indicated that concurrent existence of multiple and variable oral lesions is accompanied with poor prognosis of the disease.Reduction of circulating CD4 count is the main criteria for assessing the immunosuppression status in HIV-positive patients. The number of circulating CD4 cells ranges from 600 to 1600 cells/mm, but the initial signs of immunosuppression occur when CD4 count is lower than 500 cells/mm. The onset of opportunistic infections in HIV-positive patients is generally associated with a low CD4 count. Oral manifestations can be the first clinical sign of the infection and also determine the progression of disease. The purpose of this study is discuss about importance and prevalence of oral soft tissue manifestations and their relationship with the degree of immunosuppression observed in HIV-positive patients.
Keywords: Immunosuppression, Oral Manifestations, HIV-Positive Patients

IMMUNOLOGY of ORGANS

Oral Presentation

551. Regulatory T-Cells in Autoimmune Hepatitis: Clues for Novel Immunotherapeutic Intervention

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Compelling data generated in animal models indicate that T-regs prevent or cure T-cell mediated disorders, including autoimmune diseases and allograft rejection, by restoring immune-tolerance to auto-antigens and inducing it to allo-antigens. Amongst regulatory cells, CD4 T-cells constitutively expressing the IL-2 receptor (IL-2R) chain (CD25) are central to the maintenance of immune-tolerance.Data from our group have shown that this immune attack is permitted by a numerical and functional impairment of T-regs. T-reg numerical and functional defect is related to the stage of liver disease, being more marked at presentation than during drug-induced remission. The partial T-reg restoration observed during remission indicates that T-regs have the potential to expand in number and regain function. These cells would therefore be ideal for therapeutic reconstitution of self-tolerance. We also examined to see whether impaired immune-regulation in AIH is due to a primary T-reg defect, or to low responsivity of CD4 effector cells to T-reg control. Is Gal-9 expression pivotal to T-reg suppressor function? Does impaired Tim-3 expression lead pathogenic CD4 T cells to avoid T-reg control in AIH? Our data from our group showed that in AIH, Gal-9 expression is reduced in T-regs and Tim-3 is also downregulated on $CD4^{pos}CD25^{neg}$ effector cells suggesting that Reduced expression of Gal-9 characterises T-regs in AIH and is mirrored by down-regulation of Tim-3 on CD4 effector cells. In agreement with others, we were able to expand T-regs *in vitro* following exposure to a polyclonal stimulus, i.e. anti-CD3/anti-CD28 in the presence of high concentrations of IL-2 both in normal subjects and AIH patients. T-regs expanded for up to 4 weeks maintain phenotypic characteristics of the original CD4+CD25+ purified from peripheral blood, express higher levels of FOXP3 and suppress much more efficiently than freshly isolated T-regs, thus representing a potential immunotherapeutic tool.
Keywords: Regulatory T-Cells in Autoimmune Hepatitis: Clues for Novel Immunotherapeutic Intervention

Poster Discussion Presentation

552. Associations between Antibodies against the Endothelial Cell and Toxoplasma Gondii; Cytomegalovirus in Serum of Children with Cochlear Implant Surgery

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Background: Serum Endothelial cell antibodies (AECA) by inducing vascular damage has a prominent role in Immune-mediated sudden sensorineural hearing loss. Goal of study: To compare theAECA in serum and perilymphatic fluid in children (<15y) with cochlear implant surgery. Materials and Methods: A case control study investigated in the cochlear implant wardin Rasoul hospital, Tehran Iran (2008-2010). AECAs was investigated by Indirect immunofluorescence assay in sera and perilymphatic fluids of 47 Idiopathic (case) and 52 (control) Non Idiopathic type of sensorineural hearing loss and compared between the two groups with Independent T-test. Pvalue <0.05 was considered as significant. Results: Serum AECAs was detected in 10 %; perilymphatic fluid AECAs was detected in 12% of cases. AECAs in Perilymphatic fluids had different results between cases with Idiopathic and Non Idiopathic of SNHL (PV=*0.059)*. But serum AECAs had not significant differences between 2 type of SNHL (PV=0.1). The mean age of cases with positive and negative AECAs in serum and perilymphatic fluid had not significant differences between 2 type of SNHL (PV=*0.2*; PV=*0.*2). Conclusion: Idiopathic type of SNHL diagnosed in 47.5 % of cases. Serum AECAs had not significant differences tween 2 type of SNHL. Due to poor outcome of Idiopathic SNHL in cases with positive AECAs, we recommend to search not only serum AECAs but also perilymphatic fluid AECAs in all cochlear implant cases with Idiopathic type of SNHL.
Keywords: ISNHL, SNHL, Cochlear implant, endothelial cell antibodies, Indirect immunofluorescence assay

553. Tolerance and Lymphoid Organ Structure and Function

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This issue of frontiers in immunologic tolerance explores barriers to tolerance from a variety of views of cells, molecules, and processes of the immune system. A lot of laboratory work has been spent over a decade focused on the migration of the cells of the immune system, and dissecting the signals that determine how and where effector and suppressive regulatory T cells traffic from one site to another in order to reject or protect allografts. These studies have led us to a greater appreciation of the anatomic structure of the immune system, and the realization that the path taken by lymphocytes during the course of the immune response to implanted organs determines the final outcome. In particular, the structures, microanatomic domains, and the cells and molecules that lymphocytes encounter during their transit through blood, tissues, lymphatics, and secondary lymphoid organs are powerful determinants for whether tolerance is achieved. Thus, the understanding of complex cellular and molecular processes of tolerance will not come from "96-well plate immunology," but from an integrated understanding of the temporal and spatialchangesthatoccurduringtheresponsetotheallograft.Thestudyoftheprecisepositioningandmovementofcellsinlymphoidorganshasbeendifficultsinceitishardtovisualizecellswithintheir three-dimensionalsetting;insteadtechniqueshavetendedtobedominatedbytwo-

dimensionalrenderings,althoughadvancedconfocalandtwo-photonsystemsarechangingthisview.Itisdifficulttopreciselymodifykeymoleculesandeventsinlymphoidorgans,sothatexistingknockouts,transgenics,inhibitors,andactivatorshaveglobalandpleiotropiceffects,ratherthanpreciseanatomicallyrestrictedinfluences.Lastly,therearenowell-definedpostalcodesortrackingsystemsforleukocytes,sothatwhilewecanusuallytrackcellsfrompointAtopointB,itisexponentiallymoredifficultoreveni mpossibletotrackthemtopointCandbeyond.Webelievethisrepresentsoneofthefundamentalbarrierstounder-standingtheimmunesystemanddevisingtherapeuticapproachesthattakeintoaccountanatomyandstructureasmajorcontrollingprinciplesoftolerance.
Keywords: tolerance, lymph node, structure

554. Monocyte Expression of Toll-Like Receptor 4 (TLR4) and It's Association with TNF-α in Patients who Undergo Percutaneous Coronary Intervention

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Background: Toll-like receptors (TLRs) are a link between the development of cardiovascular diseases and the immune system. Among them, TLR4 plays prominent role in formation of atherosclerosis plaque and ischemic injuries of the heart. The goal of the present work was to study monocyte expression of TLR4 and its association with TNF- α as a major downstream activity of TLR4 in patients with stable angina who are candidates for precutanoeus coronary intervention (PCI). Materials and Methods:The subjects included 41 patients with stable angina who underwent PCI according to the hospital protocols. The exclusion criteria were as follows: MI, unstable angina, inflammatory diseases and using immunosuppressant drugs. We collected blood samples before PCI procedure. We studied expression of TLR4 on the surface of $CD14^+$ monocytes by flow cytometry. We also measured serum levels of TNF-α. The concentration of TNF-α was evaluated using enzyme-linked immunosorbent assay (ELISA). Results: Data are presented in mean ± SD. Monocyte expression of $CD14^+$/ $TLR4^+$ was 18.4 ± 2.3. TNF-α mean concentration was 15.1 ± 2.3 (pg/ml). Pearson analysis showed significant association between TLR4 expression and TNF-α serum concentration (P=0.01) and (r=0.463). Conclusion: We demonstrate that patients with stable angina have enhanced monocyte expression of TLR4, in comparison with previous studies. We conclude that TLR4 sensitization can lead to production of proinflammatory cytokines like TNF-α. Moreover, TLR4 have significant correlation with TNF-α in such patients.
Keywords: Toll-like receptor, monocytes, heart ischemia

555. Expansion of Circulating Toll-Like Receptor 4-Positive Monocytes in Patients with Stable Angina

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Background: Toll like receptors (TLRs) are proteins that transduce signal to different pathogen-associated molecular patterns (PAMPs). Large body of evidence has accumulated suggesting that TLR4 plays key role in formation of atherosclerotic plaque. The purpose of the present work was to investigate monocyte expression of TLR4 and its association with IL-1β as major downstream activity of TLR4 in patients with stable angina who are candidates for precutanoeus coronary intervention (PCI). Materials and Methods: The subjects included 41 patients with clinical evidence of Canadian Cardiovascular Society class II and III stable angina. The exclusion criteria were as follows: MI, unstable angina, inflammatory diseases and using immunosuppressant drugs. We collected blood samples at admission time. We studied expression of TLR4 on the surface of $CD14^+$ monocytes by flow cytometry. We also measured serum level of IL-1β. The concentration of IL-1β was evaluated using enzyme-linked immunosorbent assay (ELISA). Results: Data are shown in mean ± SD. Mean of Monocyte expression of $CD14^+$/ $TLR4^+$ was 18.4 ± 2.3. IL-1β mean concentration was 8.9±14 (pg/ml). Pearson analysis revealed significant correlation between TLR4 and IL-1β (P=0.05) and (r=0.307). Conclusion: In comparison with previous studies we can say that patients with stable angina have augmented monocyte expression of TLR4. We conclude that TLR4 activity can lead to synthesis of inflammatory cytokines. Furthermore, TLR4 have significant correlation with IL-1β.
Keywords: Toll-like receptor, monocytes, heart ischemia

IMMUNOLOGY of SPORT

Oral Presentation

556. Comparisonof the AcuteEffects ofanIncrementalExhaustiveAerobic ExerciseSessionby Upper-Body and Lower-Bodyon theNK andT Cells Response

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Background: The purpose of the present study was to comparing acuteeffects ofanincrementalexhaustiveaerobic exercisesessionby arm andlegon theresponseof NK (CD16, CD56 and CD16/CD56) and T (CD4, CD8 and CD4/CD8) immune cells of athlete male students. Materials and Methods: Twenty male students with mean age 22.4±1.8 years, maximal oxygen uptake 41.7±71 $ml.kg^{-1}.min^{-1}$, BMI 23.3±1.87 kg/m^2 and BF% 19.83±3.3 selected and randomly assigned into two experimental (n=10) and control (n=10) groups.Experimental group carried out two incremental exhaustive aerobic protocols by foot and hands on ergometer cycles in two different days. The control group didn't participate in any physical activity program.Blood samples of both experimental and control groups taken before, immediately after and two hours after the end of the exercise tests and evaluated by flow cytometry analysis for NK and T cell markers. Results: NO significant differences were seen between the two types of training protocols in response of NK and T cells in both arm and leg. Both protocols caused significant increasing in CD8, CD16/56 cells, significant decreasing in CD4 and CD4/CD8 ratio and no significant change in CD16 and CD56 cells. In addition,twohours aftertheend of theprotocols,these changesbackto their levels at the pre-test values. Conclusion: Thisresultsuggeststhatashorttime and intense exercisecausestemporaryandacutechanges intheimmune system. In addition, no significant differences were seen between carrying out of intense exercise by arm and leg in responseofNK andT cells.
Keywords: Aerobic exercise, Upper-body, Down-body, T cells, NK cells

557. A study of one Session of Activity on the Changes in the Most Sensitive Inflammatory Marker Predicting Cardiovascular Diseases in Athletes and Non-athletes

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Background: This study aims to investigate and compare the influence of one session aerobic activity on the inflammatory markers (hs- CRP) in athletes and non-athletes. Materials and Methods: A groups of athletes (10 persons with average height of 1.76±0.129, weight 69.42±3.50, age 24.42±4.67 and VO_{2max} 44.83±2.52), and a group of non-athletes (10 persons with average height 1.74±0.1312, weight 70.71± 4.54, age 23.75 ± 4.20 and VO_{2max} 44.83±2.52) participated voluntarily. They were, then, asked to carry out an exercise plan for 30 min with 60% of Maximal Oxygen Uptake. Their blood samples were measured before, immediately after and also 24 hours after the exercise for(hs-CRP). Results: Before

the test the level of hs- CRP at rest in athlete group compared to non-athletes was 1.80±0.96 and 0.85± 0.79 mgr/lit. Although the hs- CRP level of the athletes reduced immediately and 24 hours after the activity, it was not significant ($p<0.05$). No significant change was traced in the non-athletes after the exercise either ($p<0.05$). It is likely that the slight reduction in the level of hs-CRP in athletes compared to non-athletes was due to their initial high level of hs-CRP and most probably the initial level of hs-CRP in the non-athletes has been too low to be affected by the exercise. Conclusion: These findings show that low intensity exercises can help improve these markers in people with higher baseline level of these markers (for example due to cardiovascular, infectious and inflammatory diseases).
Key terms: aerobic, hs-CRP, exercise plan, VO_{2max}

558. Alteration in Adenosine Deaminase Activity, Cortisol and TNF-α Levels Following Exhaustive Sessions Exercise in Endurance Runners
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Background: Heavy exercise has acute and chronic influences on immune system responses and decreases the athlete's body function. In particular, cortisol and TNF-α has an important role on immune system. Accompaniment of alternations in immune system responses with changes in adenosine deaminase (ADA) activity has previously been. Previous studies have shown a significant increase in cortisol and TNF-α in athlete; however, the alteration of ADA activity in runners has not been previously reported. Therefore, this study was aimed to investigate the effect of exhaustive exercise on cortisol, TNF-α and serum ADA activity in endurance runners. Materials and Methods: All endurance male athletes in Kurdistan province were enrolled in the study. During this study, the participants were submitted to the same experimental protocol used in the exercise trials, they run on a treadmill until exhausting state. Saliva and venous blood sample were collected in resting and immediately after exercise; serums were separated, and stored at -70°C pending simultaneous analysis. Serum was analyzed for ADA activity and TNF-α. Salivary cortisol measured by ELISA method. Results: Cortisol and TNF-α elevated following intensified training compared to resting state (4.4 ± 0.54, 2.8 ± 0.65 ng/mL for cortisol and 78.2 ± 2.1, 54.8 ± 8.3 pg/mL for TNF-α, respectively). There was a weakly increase in serum ADA activity after training (41.13 ± 21.9, 38.12 ± 15.7 IU/L). Conclusion: Based on the obtained data an acute period of intensified training can induce increase of ADA activity accompanies with increase of cortisol and TNF-α. ADA is involved in immune system development. Our results hypothesized that ADA can be associated with an increase in fatigue which lends to reduced physical activity. We showed that excessive exercise can induce an inflammatory response resulting in elevated levels of cortisol and TNF-α and perhaps increased activity of adenosin deaminase.
Keywords: Adenosine Deaminase Activity, Cortisol, TNF-α, Endurance Runners

559. Gene Expression and Activity of Immune System's Enzymes in Young Men Trained: Influenced by a Session Incremental Intensity Exercise
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Background and object: Incremental exercise leads to activity and expression of immune system's enzymes via oxidative stress and inflammatory condition. Mn SOD is locatedinthis group ofenzymesand caused to safety in mitochondria of cells. Mitochondria and other parts of cells are supported by gene expression and antioxidant enzyme activity (such as total antioxidant status). But, mechanisms of these enzymes and gene expression in responding to exercise have not known. Exercise duration and exercise intensity probably change the enzyme gene expression and enzyme activity. Therefore, the purpose of this research was investigation of the Mn SOD gene expression and enzymes activity following incremental intensity exercise in young men trained. Materials and Methods: A repeated measures design was used for this study. Fourteen young mentrained in theage range 23-26 years from Urmia city were volunteered as subjects.Incremental intensity exercise was included 20 minutes running on treadmill in the grade slope 6% and speed of 11 km/h. venousblood sampleswere taken in three stages, beforeexercise, immediately and3 hoursafter exercise.Real time PCRmethodused for analysis of the mRNA of Mn SODgeneexpression and the epectophotometry method for measurement of TAS activity.Results: The mRNA of Mn SOD immediately and 3 hoursafter exercise increased. But it was not significant (P>0/05). Also, significant increase was observed in the TAS levels only in recover or 3 hoursafter exercise(P<0/009). Conclusion: A session of incremental intensity exercise not affects on the Mn SOD gene expression and increases significantly the TAS levels in young mentrained. However, further studies are needed to investigate the role of incremental exercise on the Mn SOD geneexpressionand TAS activity in young men trained.
Keywords: Immune system, men trained, intensity exercise

560. Correlation between White Blood Cells with Maximal Oxygen Pulse in Response to Intensive Exercise in athlete Women
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Background: Studies have shownthatintensiveexerciseleads to loss or suppress of the immune system. WBC (white blood cells) can be an effectiveindicator of the immune system situation in responding to exercise. On the other hand, maximal O2 pulse as a non-invasiveindicator ofoxygentransfer and cardiovascular index is amount of oxygenextractionperheart rateorstrokevolume and it is equal with the milliliter ofoxygenperbeat(ml / beat). Some studies reported O2 pulse increasedby intensive exercise. But relation betweenoxygenpulseand WBC is not clear, therefore, thepurposeofthis studywas to examine the correlation between white blood cell levels and maximal oxygen pulse in responding to intensive exercise in young athlete women. Materials and methods: Twelve healthy youngathlete women in theage range of 20-22 years were volunteered as subjects. Informed consent was obtained from each subject. Subjects participated in the GXT exercise test (speed: 12 km/h, slope grade: 6%, exercise time: 16 minutes). Maximal O2 pulse was estimated; at baseline, end of exercise and2 hoursafter exercise by equation; O2 pulse= SV * (a-v) O2 diff and measurement of VO2 max. Venousblood sampleswere taken atbeforeexercise, immediately and2 hoursafter exercise for evaluation of WBC. Results: The result showed maximal O2 pulse increased at immediately exercise and also revealed significantly higher levels of WBC at end of exercise (P<0/001), but 2 hours after intensive exercise the amounts of WBC was significantly reduced..However we found significant correlation between maximal O2 pulse and WBC (r = 0/763) (P<0/007).Conclusion: Intensity exercise affects on WBC levels and maximal O2 pulse in athlete women, so these changes indicate that there is a significant correlation between maximal O2 pulse and WBC young athlete women.
Keywords: WBC, O2 pulse, athlete women

561. Thestudy of Combined Effect of 8 Weeks of Fish Oil Supplementation and Intense Aerobic Trainings on Plasma Levels of Systemic and Skeletal Muscle Inflammatory Indexes in Trained Male Mice
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Background: Intensive exercise increased pre and anti-inflammatory cytokines. Interleukin 17(IL-17) is a proinflamatory cytokines secreted from T cells and some illness promotion roles had been proposed for this cytokine. The purpose of this study was to studythe combined effects of 8 weeks fish oil supplementation and intense progressive aerobic trainings on plasma levels of systemic and skeletal muscle inflammatory indexes in trained male mice. Materials and Methods:75 healthy male mice 2 months old with 35 grbody mass were selected. At first 10 mice for determining the pretest values were killed, and the other were divided randomly into four groups, control (Cn=30), supplement (S n=15), training (T n=15) and supplement+ training (ST n=15). Two groups (S, ST) consumed0.2 cc fish oil during 8 weeks. The T and ST group train 5 days a

week on animal treadmill for 2 month. Results:The results of study show that there is a significant differences in plasma levels of variables in all groups, at the end of 8 weeks exercises ($p<0.05$). Conclusion: In overall it could be asserted that fish oil supplementation accompanied with intensive aerobic exercise could decrease CRP and subsequently induced reduces in the plasma levels of IL-17 and CK.
Keywords: Fish oil, Inflammation, Interleukin-17, CRP, CK, Aerobic intense exercise.

562. The Comparison of Serum TNF-α, CK, and LDH levels hanges after CON and ECC emphasized RET in Nonathlete

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Background: Recently it's acclaimed that exercise has prefund effects on immune system. Contracting skeletal muscles have interaction with endocrine system by agents called " exercise factors", that induced exercise related metabolic changes in different organs such as liver and brain. Interestingly exercise progressively increased cytokine expression, but regulatory effects of resistance exercise training (RET) in regards of TNF-α expression hasn't been clearly identified. The purpose of study was to compare TNF-α serum level change after separate bouts of Concentric (CON) and Eccentric (ECC) emphasized RET in nonathlete university student. Materials and Methods: statistical population of the study was 14 nonathlete, that performed two bouts of CON and ECC RET 4 days apart with equal load. Blood sampling (5cc) were taken before and immediately after exercise. Statistical analysis of data was done by SPSS.16. Results: after ECC RET significant reduction in TNF-α ($p<0.05$) and insignificant decrease following CON RET. Both CON and ECC RET increased CK and LDH($p<0.05$), that approved training could induced inflammation. Conclusion: It could be justified from our finding that RET have protecting role against skeletal muscle protein loss and cell death that induced byTNF-α specially ECC emphasized RET.
Keyword: Resistance Exercise Training,TNF-α,CK, LDH, Nonathlete

563. The Effect of Combination of Tamoxifen with Interval and Continue Training on Cellular and Humeral Immune Factors in Mice with Breast Cancer Tumor

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Background: Beast cancer is considered as the most common malignancy among females in the world .in Iran, this cancer is also the most prevalent malignancy among women. The aim of this study is to compare the effects of Tamoxifen and interval and continues training on cellular and humeral immunity in Balb/c mice suffering from breast cancer. Materials and Methods: for this reason 30 female balb/c mice were utilized and after transplant carcinoma tumor to mice randomly divided in 4 groups 6 groups as follow: 1: tumor-control 2: tumor-continue training 3: tumor –continue training-Tamoxifen 4:tumor-interval training 5: tumor-interval training-Tamoxifen 6:tumor-tamoxifen. Continues training protocol was done for 6 weeks at 25% to 75% vo_2max and interval training protocol was done for 6 weeks at 20% to 55%vot_2 max between 1 until 10 interval rep *1 min . The drug was injected every day during research program. Blood samples were collected after protocol. The levels of Heat shock protein 70-kDa (Hsp70), interleukin 4 (IL-4), and interferon gamma (IFN γ) were measured with ELISA method, and the resulting data was analyzed with SPSS 10 statistical software. Results: Data analysis showed that the Hsp70 levels in both groups of interval and interval plus Tamoxifen were decreased (P=0.459). Also in continues group was increased (p>0/05).The IL-4 level in both groups of interval and interval plus Tamoxifen showed no significant differences compared to tumor control (P=0.112). The IFN γ level in both groups of interval and interval plus Tamoxifen showed an increase, but was not significant compared to control group(P=0.784). Conclusion: tumor mass in interval training only, interval training and Tamoxifen treated showed a significant decrease in the tumor growth in comparison with control group. But tumor mass in continues group was increased .in comparison control group.
Keywords: Interval Training, Continue Training, Tamoxifen, Tumor Mass, Immune Response

564. Comparison of Execution Concurrent Exercise inthe Morningand EveningonCardiovascular Inflammatory Markersof ActiveMen

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Purpose:The purpose of this study was comparing the response of high sensitivity reactive protein (hs-CRP) and fibrinogen to endurance and strength concurrent exercise in the morning and afternoontime. Materials and Methods: Participants were 10 active male university students (age 25/1 ±1/287 yrs, weight 72/1 ± 6/540 kg, height 177/2 ± 7/775 cm) which voluntarily participated in this study. In a periodized design subjects completed 2 experimental trials during 5 days. Trial one, performing concurrent endurance (running on treadmill for 35 minutes as interval exercise for 3 sets in 7 minutes, 7 minutes rest intervals between each set, at 85 percent of maximum heart rate) and resistance (5 exercise in 3 sets and 8 repetition with 80% 1RM for 45 min) exercise for 80 minutes in the morning. At trial two, subjects performed the same protocol in the afternoon. While the subjects were overnight fast (at least 8h), the first blood draw performed and other Blood samples were taken at immediately and 3 hours after exercise protocol. Blood samples used for measuring acute phase response variables (hs- CRP and fibrinogen). Data were analyzed by using paired t test and analysis of variance with repeated measures. The differences were considered significant at P£0/05. Results: Our findings showed the exercise protocol had no significant effect on serum response of hs-CRP in subjects at different intervals time in the two experimental trials (P =0/432) and there was no significant difference between response of hs-CRP in subjects at different intervals time to selected exercise between the two trials (P =0/331). On the other hand, the findings showed that plasma fibrinogen levels has had significant differences between the first and second trial and after selected exercise protocol at different intervals time(P =0/000). So that plasma fibrinogen levels following the completion of selected exercise in the morning showed significantly increase (12 percent), but in the afternoon showed a small increase (2 percent) that it was not significant. Conclusion: Overall results showed that there were significant differences between the acute phase response of fibrinogen after performing concurrent endurance and strength exercise in both morning and afternoon time.So with regards to the results of this study, from the standpoint of cardiovascular health, is recommended completion of exercises is more secure in the afternoon than the morning and coaches can prescribe this kind of exercise for athleteswith observing the running time of exercise.
Keywords: acute phase response, C- reactive protein, fibrinogen, concurrent exercise

565. Effects of Acute Coenzyme Q10 Supplementation on Serum TNF-α Concentration during Maximal Exercise

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Background: The depression of the immune system function that is typically observed after strenuous exercise is believed to be possibly mediated by stress hormones, cytokines and oxidative stress. The aim of this study was to investigate acute consumption of Coenzyme Q10 supplementation on serum Tumor necrosis factor-alpha (TNF-α) concentration during the maximal activity. Materials and Methods: Twelve healthy active males (age 21/75 ± 0/64 yr, BMI 23/7±0/94 kg/m2) performed 30-min exercise at 80% to 85% HRmax. Subjects 120 minutes pre-exercise received either of the following regimens: Coenzyme Q10 (2 mg per kg body weight) or placebo (food color). Blood samples were obtained prior to supplement consumption and immediately after exercise then groups were reversed after 4 days. The ELISA kit was used to measurement of TNF- α serum level. The data were analyzed using paired and independent t-test. Results: The statistical significance was set at $p<0.05$. Serum levels of TNF-α increase in both supplementation and placebo group (4.2% and 5.12% respectively). Conclusion: The results of

this study showed that although increased serum levels of TNF-α was slower in the supplement group compared with the placebo group after maximal activity but Q10 consumption did not caused a significant decrease between two group (P=0.8).
Keywords: Coenzyme Q10, Serum TNF-α, Maximal Exercise

Poster Discussion Presentation

566. Evaluation of Immune System in Military Divers

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Background: Diving especially in a military situation encounters immune system of divers to the many long term stressors and disasters including psychological stress, changing temperature, extensive physical activity, risk of trauma and occupational damages as well as risk of infections with unknown microorganisms lived in the sea environments. Newly, the effect of pressure overload and hyper-gravity on the lymphocyte subsets was documented in association with hyperbaric oxygen (HBO_2) exposure in divers. This study was design to understand comprehensive effect of military diving on the total branches of immune system in well educated person with long term exposures. Materials and Methods: The cross-sectional study was developed on 25 expert military divers and asses the immunologic parameters (humeral system, cellular system, complement components, innate immune function) comparing to the 25 sex-age matched healthy controls. Results: In the findings of divers comparing to the controls in this study, decreased serum levels of Immunoglobulin (Ig) A and IgM were recorded while the levels of IgG and IgE were elevated significantly. Moreover the Percentage of CD19+ lymphocyte (15.9±6.18 *vs.*23.0±14.3, %, p<0.001) and absolute counts of B cells (379.5±135.6 *vs.* 376.1±210.2, cell /mm^3, p<0.001) was meaningfully lower in the divers group. Despite routine occupational vaccination of military staffs, specific antibody production against Diphteria antigen was defected in divers (p<0.01) but this result was not achieved in verification of Tetaneus antigen. Assessment of divers' cellular arm of immune system showed the lower percentage of CD3+CD4+ lymphocyte (decreased Helper T cells) and reverse ratio of CD4/CD8. However the serum level of CD56+ cells and absolute count of Natural Killer cells (15.9±5.4 % and 376.1±195.8 respectively) was higher in comparison with control group (9.3±1,5 % and 223.2± 92.0, p<0.001). Phagocytosis function of neutrophils was evaluated by NBT test and the result failed to s present any difference between two groups. Furthermore exaggerated activity of classic pathway of complement components was detected in divers (CH50 61.1±10.3 *vs.* 23.8±11.7 p<0.001). Using the logestic regression analysis we also demonstrated the reveres association between duration of diving and total lymphocyte count (r=-0.43, p=0.028), total T helper cell counts (r=-0.44, p=0.025) and cytotoxic T cell counts (r=-0.42, p=0.035) as well as the level of B cells (r=0.419, p=0.037). Previous history of navy executive duty had the relation with CRP levels of divers (r=0.32, p=0.026) and the mean deep of dives had direct effect on lower lymphocyte count, defect in NBT test and secondary specific antibody deficiency. Divers with higher physical activity and severity of services had different inflammatory profiles specially in ESR levels (ANOVA, post Hoc sheffe, f=4.16, p=0.018). Conclusion: Humeral, cellular and complement arms of immune system of divers could be severely involved during long- term diving and should be investigated by further secondary immune test such as AH50 and bone marrow aspiration and biopsy.
Keywords: Hyperbaric oxygen, Diving, Immune system

567. Immunoglobulin and Physical Activity in Chemical Victims: Sardasht-Iran Cohort Study

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Background: Physical activities have useful effects on different organs. The previous studies showed that the effect of exercise on immunoglobulin levels was not completely understood. The goal of this study was to assess the relationship between immunoglobulin classes and physical activity. Materials and Methods: In a historical cohort study, Sardasht-Iran cohort study (SICS), 372 SM exposed participants were studied 20 years after exposure. The global physical activity questionnaire (GPAQ) was used to obtain a self reported measure of physical activity. Serum levels of Immunoglobulins were assessed by Elisa quantitative method. Results: Based on the result of this study, it is found that there is a significant negative relationship between IgE with physical activity (P= 0.05). The mean of IgE in low, moderate and high physical activities was 234.95 (SD=329.97), 196.51 (SD=289.31) and 151.77 (SD=187.31) respectively. There was not a significant relationship between IgM, IgA and IgG with physical activity. Conclusion: Because of poor pulmonary condition of SM exposed people, it seems that physical activity under supervision is a suitable option for them.
Keywords: Immunoglobulin, physical activity, chemical victims

568. Relationship between IL-6 and IL-10 Cytokines with Physical Activity in Chemical Victims: Sardasht-Iran Cohort Study

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Background: Relationship between inflammatory and anti-inflammatory cytokines with physical activity well documented in healthy people. The aim of this study was to determine the relationship between IL-6 and IL-10 cytokines with physical activity in sulfur mustard (SM) exposed people. Materials and Methods: In a historical cohort study, Sardasht-Iran Cohort Study (SICS), 372 SM exposed participants were studied twenty years after exposure. The global physical activity questionnaire (GPAQ) was used to obtain a self reported measure of physical activity. Serum and whole blood culture supernatants samples were used for interleukin 6 and 10 respectively. Cytokines were measured by ELISA method. Results: The mean age of participants were 44 (SD=11) and most of them were married (91%). The result of this study showed that there was a significant relationship between mitogen induced IL-10 and severity of physical activity but there was not relationship between IL-6 and severity of physical activity. Conclusion: It seems that there is a need to provide suitable programs for SM exposed victims by health care system.
Keywords: IL-10, IL-6, physical activity, sulfur mustard

569. The Effects of a Long Prior Aerobic Exercise and High Fat Meal on Inflammatory Markers of Vascular Adhesion Molecules and Lipid Profile inNon-athlete Males

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Background: The purpose of the present study was to examine the effect of an acute bout of postprandial prior exercise with 70 percent VO2max and on markers of inflammation (sVCAM-1) and the lipid profile following a high fat meal in non-athlete men. Plasma concentrations of adhesion molecules and lipid profile are among the most important indicators of the risk of cardiovascular diseases. Materials and Methods: We assigned 20 non-athlete students in a random fashion and based on their body fat to 2 groups of 10 people, one experimental (averaging 21.98±1.30 years, 18.04±2.48 body weight percent) and control (averaging 22.06 ±1.22 years, 18.15±3.54 body weight percentage) groups. The experimental group completed a 90-minute treadmill exercise. A day later both groups received high fat meal. Blood samples in 30 min before and 30 min, 1, 3 and 24 hours following meal were collected. To determine normality of groups, we carried out one sample Kolmogorov Smirnov (PCON=0.996) (PEX=0.999) and to determine the homogeneity of variances we used Leven test and to examine results among and inter- groups independent t-test and statistical test for variance analysis with repeated measures and post-hoc LSD test. Results: The results indicated that one bout of acute prior aerobic exercise reduces sVCAM-1 (P=0.029). Also in 30min and 24 hours following high fat meal there is a reduction of sVCAM-1(P=0.016), (P=0.049). The results also indicated that a bout of prior exercises increased the level of HDL (P=0.000)

but decreased the level of LDL(P=0.012) and vLDL (0.000) and triglycerides (P=0.037). Conclusion: According to the results, high fat meal increases the levels of sVCAM-1, thus leading to inflammation and disease. Prior exercise may contribute to the decrease in sVCAM-1 and lipid profile.which plays a role in decrease of the diseases.
Keywords: Endothelial activation, Postprandial lipemia, Adhesion molecules, Atherosclerosis, Lipid profile.

Poster Presentation

570. The Effect of Exercise Training and Diet on CD3+ T Cells and Blood AST 8 ALT Levels

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Background: The aim of this study was to investigate whether , exercise and diet are effected on the CD3+ T cells and blood AST 8 ALT levels. Materials and Methods: Blood sample of 18 healthy male subjects (divided in to three groups), aged between 22-32 years were performed at rest (Preexercise), on completion of 8 week (Postexercise), and after 48 h of recovery from postexercise of football training. Study subjects recived 30 grams carbohydrate or an equal of placebo perday for 8 weeks.The samples for immune monitoring were first stained with appropriate monoclonal antibodies and then analysed by flowcytometry.Then blood Ast and Alt levels were measured by hematogy Technique. Results: After 8 week prolonged exercise training and carbohydrate intake we found a circulating number of total CD3+ T cells an increased ($P<0.05$) postexercise and 48 h after recovery time . When compared with preexercise trial and control groups. There are insignificant differences ($P>0.05$) between the Ast values after 8 week training period and those before exercise in football players. The Alt values postexercise training period were lower than those before preexercise training. Conclusion: Prolonged Exercise with carbohydrate ingestion influences on CD3+ T cells function. And can damage the body organs .This is an important issue that need to be addressed in future studies.
Keywords: CD3+ T Lymphocytes, AST, ALT, Exercise, Carbohydrate

IMMUNOLOGY of VIRAL DISEASES

Oral Presentation

571. Isolation of scFv Antibody against Glycoprotein B of HSV-1

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Background: HSV-1 is responsible for a wide range of human diseases from the localized infection such as orolabial or corneal lesions to lifethreatening encephalitis, neonatal disease. A critical event in the life cycle resides in the entry of the virus into target cells. Glycoprotein B (gB) has essential function for entry. gB elicits a considerable amount of neutralizing antibodies. Developments in antibody engineering have provided potential to produce of smaller and high affinity recombinant antibody fragments such as single chain antibodies (scFv). In this study a phage antibody display library was used to isolate specific scFvs against gB of HSV-1. Materials and Methods: The phage particles bearing anti-HSV-1 scFv were isolated by panning the phage library against the immunodominant epitope of gB. After rescue with M13KO7 the phages were repanned against peptide a further three times. PCR and *BstN1* DNA fingerprinting were carried out for a number of colonies of the last round of panning. Results: After four rounds of panning against gB epitope, the PCR results randomly selected clones showed the expected 950 base pair bands. The DNA fingerprinting patterns demonstrated 13 scFv types containing one predominant pattern with frequency 45%. This clone was selected for further in vitro studies. Conclusions: until now, there is no cure for latent HSV-1 infection. An effective antiviral therapy in order to block lytic infection, latent reactivation, and transmission are necessary. Although several drugs reduce viral infection, there is an increase in resistance to these agents by the virus. Immunotherapy has recently been introduced as a new therapeutic option for the treatment of several diseases caused by HSV-1. There are some reports of identification of the neutralizing epitopes of gB by monoclonal antibodies. In this study we isolated a specific scFvs by panning process. Further researches are needed to select the neutralizing antibodies for clinical applications.
Keywords: scFv, HSV-1, PCR and *BstN1* DNA fingerprinting

572. Studies on Potential Interfering Effect of HCV Core+1 Protein on Interferon-Based Transcriptional Regulations

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Background: Hepatitis C virus (HCV) genome encodes for three structural (Core, E1/2) and seven non-structural proteins. Recently, anovel HCV proteinknown as ARFP (alternative readingframe protein) or Core+1 protein is discovered which is synthesized via another open reading fameoverlapping the core coding sequence at nucleotide +1 (Core+1 ORF). Emerging data have indicated that HCV has mechanisms to resist the antiviral action of interferon-α. Herein, the effect of Core+1 protein expression on a selected Interferon Stimulating Genes (ISGs) at cellular level was studied. Materials and Methods: Recombinant pcDNA (+) 3.1 vector harboring Core+1 gene was constructed and transiently transfected into Huh-7 cells by electroporation. Expression analysis was assessed via western blotting (WB) and immunoflourescence microscopy (IFM). Transfected Huh-7 cells (with or without IFN-α treatment) were used for RNA extraction and quantitative Real Time PCR (RT-qPCR) using SYBR green was performed to examine the effect of core+1 expression on a selected ISG. Fold induction was then calculated by the Δct method using GAPDH mRNA level to normalize values. Untransfected cells and IFN-α untreated cells were used as controls. Results: Construction of appropriate expression vectors for HCV Core+1 was confirmed by restriction analysis and DNA sequencing. WB and IFM analysis confirmed proper expression of the core proteins inside the transfected hepatic cells. Results of RT-qPCR indicated the potential regulative role of Core+1 protein at the transcriptional level of selected ISGs. Conclusion: These data suggest that HCV Core+1 might have a potential role in establishing persistent infection by subverting an effective immune response.
Keywords: Hepatitis C virus, Core+1, ISGs

573. Defective Expressions of TRIF in Iranian Chronic HBV Infected Patients

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Background: TRIF is of the main intracellular adaptor protein for TLR3 and 4 signaling. Mal-expression of the molecule may lead to an defective immune responses against viral infections including hepatitis B infection. Thus, the aim of this study was to identify the mRNA levels of TRIF in the PBMCs isolated from chronic HBV infected (CHB) infected patients. Material and Methods: This study was undertaken on 60 CHB patients and 60 healthy controls and the mRNA levels of TRIF were examined in parallel with beta-actin (as housekeeping gene) using Real-Time PCR technique. Results: Our results demonstrated that expression of TRIF was significantly decreased in PBMCs isolated from CHB patients in compare to healthy controls. Conclusion: Based on the current results, it seems that CHB patients are unable to express TRIF gene firmly and in

turn properly TLR3 and 4 signaling subsequent to HBV infection. Therefore, our results suggest a probable mechanism which almost partially may define a reasonable fact that why the infection is stable in the CHB patients.
Keywords: Chronic HBV infection, TRIF, Real-Time PCR.

574. Comparing of Chemokine Receptors CXCR1, CXCR2 Gene Expression & HTLV-I Proviral Load in HTLV-I Associated Myelopathy / Tropical Spastic Paraparesis (HAM/TSP)Subgects and HTLV-I Asymptomatic Carriers

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Background: HAM\TSP is a progressive inflammatory disease in which T CD8 and T CD4 cells specific for HTLV-I recruit to CNS and cause bystander demyelisation of spinal cord. In this subject chemokine pattern changes and cause immune cells to recruit to CNS and make damage. Chemokines are known to induce the trafficking of immune cells to the inflammation sites.in HAM\TSP subjects; CXCR1 & CXCR2 chemokine ligands will be increased. For the comparative study of CXCR1 & CXCR2 gene expression, a real-time (RT-PCR) Taqman method was developed in HTLV-I Foundation Lab. Materials and Methods: PBMCs were isolated from peripheral blood of carriers, HAM\TSP subjects and healthy subjects by using Ficoll-hypaque density centrifugation. RNA of each was extracted for cDNA synthesis. A real-time PCR TaqMan method was designed and optimized for evaluation of CXCR1& CXCR2 human gene expression. Protein expression was confirmed by flowcytometery. In this method, CD4 & CD8 cells were evaluated for CXCR1& CXCR2 expression separately.HTLV-I Proviral load kit used to measure the viral load in patients with HAM/TSP and carriers. Results: Using our optimised TaqMan real-time PCR (R>0.95 Efficiency>0.99), the analysed data in this study indicated that there is a significant increase in CXCR2 gene expression of HAM\TSP in comparison with carriers and healthy subjects. The results were confirmed by flowcytometery. The results obtained through the analysed data of CXCR1 were not significant. The results indicated a direct relationship between CXCR2 gene expression and proviral load in HAM/TSP patients. Conclusion: in this study it was shown that CXCR2 gene expression was increased in HAM\TSP patients in comparison with those observed in carriers and healthy subjects.it is highly probable that the increase of CXCR2 would be one of the reasons why the immune cells (especially CD8+ lymphocytes) immigrate to CNS and this may contribute in HAM\TSP pathogenesis.
Keywords: CXCR1, CXCR2, HTLV-I, HAM/TSP

575. Evaluation of Anti-F Protein Ab in Patients with Cirrhotic Hepatitis

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Background: Hepatitis C is an important agent of non-A and non B hepatitis through the word. About 85 % of patients infected with HCV develop chronic hepatitis and from these 20% progress to cirrhosis which put the patients at the risk of progression to hepatocellular carcinoma. As it has been shown in previous studies, the HCV F protein which is derived from the coding region of core protein; may have an important role in multiplication, pathogenesis and carcinogenesis of the virus. In this study the level of anti-F protein antibodies (Abs) were measured in patients with Cirrhotic hepatitis in order to evaluate its association with disease progression to cirrhosis. Materials and Methods: Specific primers were designed for F protein using Generunner software. The F region were amplified and ligated into pET-28a (+) vector and then transfected into Ecoli BL21 strain. The expression of F protein was confirmed using SDS-PAGE assay and western blotting. The recombinant protein was purified by Ni-NTA agarose. Finally the purified protein was coated on 96-well plates in order to measure anti-F protein Abs in patients with hepatitis C cirrhosis and noncirrhotic HCV patients using ELISA assay. Results: In the present study anti-F Ab was detected in all the patients suffering from HCV cirrhosis. Out of 50 cirrhotic subjects, 1 (2%), 13 (26%), 35 (70%) and 1(2%) samples were positive in dilutions of 1/1000, 1/2000, 1/4000 and 1/8000 respectively. It is worth to mention that from 34 chronic non-cirrhotic individuals, 19 samples (55.9%) were positive in terms of anti-F protein Ab that all had a titer of equal or less than 1/2000 dilution. Conclusion: HCV F protein plays an important role in chronic disease progression to cirrhosis. In this study, F protein was detected till dilution of 1/8000 in cirrhotic patients while the highest dilution detected in non-cirrhotic patients was 1/2000. The result highlights the importance of F protein in developing HCV cirrhosis.
Keywords: HCV, Anti-F Protein Ab, pET-28a

Poster Discussion Presentation

576. Increased Plasma Levels of Soluble CD27 among HIV Patients with HCV Coinfectionbut not with GBV-C Coinfection

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Background: Many clinical studies have suggested a beneficial effect of GBV-C virus on the course of HIV infection, but mechanisms involved in such amelioration are not clear. As recent evidence has implicated immune activation in HIV pathogenesis.SCD27 is supposed that the most important bio marker in evaluation of immune activation. So, we investigated the effect of GBV-C viremia on SCD27 concentration in HIV mono infection and also HIV/HCVcoinfected patients. Materials and Methods: Cross-sectionalcomparision of SC27 plasma levels was carried out among 53 HIV mono infected, 14 HIV/CBV-C coinfected, 107 HIV/HCV coinfected and 26 HIV/GBV-C /HCV coinfected patients. CD4+ and CD8+T cellpercentagewere evaluated by flow cytometerythe molecular detection of the GBV-C was performed by reverse transcriptase polymerasechain reaction (RT-PCR). The detection of hepatitis C was carried outusig serologic test(Elisa-Acon). The assessment of SCD27 is measured by serologic test (Elisa- Bender med). Results: HGV-RNA was detected in 20% of the studied population and the frequency of HCV was 65%.among different patient group, patient with HCV coinfection with CD4/CD8<1 had significantly higher SCD27 (p=0.02) as compared to patient with HIV mono infection. Conclusion: The presence of increased plasma level of SCD27 in HIV/HCV coinfected patients could suggest the hypothesis of response biased toward immune activated form in this group in spite of HIV/GBV-C coinfected patients. Still the definitive mechanism of effect of GBV-C used to be identified.
Keywords: CD27, HIV, HCV

577. Peripheral Blood iNKT Cell Functions in HBV Carrier Patients

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Background: Chronic HBV infection may be led to serious liver disease and hepatocellular cancer. NKT cells have important role in HBV pathology. Investigation of NKT cells function in Chronic HBV infection is goal of this study. Materials and Methods: Chronic HBV infection and Healthy Donors were selected. Their Peripheral blood mononuclear cells (PBMCs) isolated and stimulated by α-Galcer and Il-2. IFN-γ and IL-4 production investigated in supernatants' by ELISA method and proliferation of NKT cells analyzed by Brdu colorimetric assay. Results: according to this survey proliferation assay of peripheral blood NKT cells in healthy donors and Chronic HBV infection was similar. IFN-γ and IL-4 also did not show significant differences between two groups. Conclusion: Although this study did not show significant differences between the groups but more investigation in NKT cells in liver tissue strongly recommend.
Keywords: chronic HBV infection, NKT cell, IFN-γ, IL-4

578. Mutational Analysis of HBsAg-Positive Mothers and Their Children Infected Despite Immunoprophylaxis

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Background: Hepatitis B vaccination is safe and effective, although breakthrough infection occasionally occurs in those who receive the vaccine and HBIG prophylaxis. Sequence variation in their antigenic regions is one of the most powerful strategies that are used by viruses to escape recognition by B and T cell-mediated immune responses. Materials and Methods: Six HBsAg-positive mothers and their children who developed HBV infection despite immunoprophylaxis were enrolled. Full HBV genome or surface gene amplification and sequencing were performed. Results: Four children had mutations, at least one of which was involved in functional or immune epitope activity. Of 30 amino acid changes, 11 (36.6%) were located within the different immune epitopes. Three cases had mutations within the "a" determinant region, one of whom inherited the mutation from her mother. Three children harbored wild-type HBsAg, similar to their mothers. With regard to transmission in infected children, immunoprophylaxis had no effect on the isolates, and failure of vaccination was observed in 2 isolates. Conclusion: These findings emphasize the need for an alternative regimen, such as the administration of boosters or a more effective HBV vaccine (a third-generation or HBV DNA vaccine), for high-risk children who are born to HBsAg-positive mothers.
Keywords: HBIG, HBV mutants, HBV vaccine escape mutants, mother-to-infant HBV transmission

579. Low Proviral Load in HTLV-I Infected Subjects in Iranians Compare to Japanese with the Same Signs May Reflect of Roles of Host Associated Factors in the Development of HAM/TSP

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Background: Human T-cell lymphotropic virus type 1 (HTLV-1) associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a neurological disease due to a cell mediated hypersensitivity reaction and observed only in 1–2% of infected individuals. Materials and Methods: Peripheral blood samples of 73 patients (17 male and 56 female) with HAM/TSP and 16 patients (8 male and 8 female) with HTLV- 1 healthy carriers (HCs) from blood donors. A quantitative real-time PCR assay (TaqMan Method) is carried out to measure the proviral load of HTLV-I in PBMCs using specific primers and a fluorogenic probe by a Rotorgen Q 6000. The HTLV-I copy number was referred to the actual amount of cellular DNA by means of the quantitation of the albumin gene as reference gene by the same method. Then HTLV-I DNA concentration and albumin concentration were calculated from two 5 points standard curves. The normalized value of the HTLV-I proviral load is calculated as the ratio of (HTLV-I DNA copy number/ albumin DNA copy number/2) $\times 10^4$and expressed as the number of HTLV-I copies per 10^4 PBMCs. Results: The age of HAM/TSP patients were ranged between 44.85±14.19 years old and 41.25±14.083 in (HCs) group. Variety ranges of complications were observed among HAM/TSP patients but the most common forms were elevation in the liver enzymes then depression along with hypothyroidism respectively. The provirus load was 476.44± 40.61 per 10^4 PBMCs and 144.93±21.16 per 10^4 PBMCs in HAM/TSP and HCs. were measured respectively. HTLV-1 provirus load has been correlated with progression of motor disability and a high amount is associated with an increased risk of progression to disease. Comparison to the previous study about Japanese provirus load in HAM/TSP (800±50) and carrier (120±5) group with our study results. Conclusion: Taken together, even HTLV-I proviral load of Iranian was lower than Japanese, it can be concluded that the proviral load is not the only commitment factor for HAM/TSP progression and host associated factors, particularly immunological activities might be investigated.
Keywords: HTLV-1, HAM/TSP, provirus load, Immunological factors

580. Detection of TT Virus in Patients with Human Immunodeficiency Virus (HIV) Infection

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Background: The TT Virus (TTV) is a small novel DNA virus that was first isolated from a patient with posttransfusion transaminase abnormalities that has been associated with transfusion hepatitis. The aim of this study was to determine the prevalence of this infectious agent in patients with human immunodeficiency virus (HIV) infection and in the relationship to TTV /HIV coinfection. Materials and Methods: This study was carried out on Serum samples of 100 HIV-positive subjects and 150 HIV-negative controls were performed. We investigated TTV in all cases by method of the semi-nested PCR polymerase chain reaction (PCR). Results: Thirty-two percent of HIV-positive patients and 18% of HIV-negative controls were positive for TTV-DNA detected by PCR. The difference in TTV prevalence between the two groups was statistically (χ^2) significant (P= 0.001). The results showed that the level of TTV-DNA titers significantly higher in patients with HIV-positive in comparison with HIV-negative controls (p=0.00) . Conclusion: The aim of this study was to determine the prevalence of TTV in patients with HIV-positive in comparison with HIV-negative controls. Our study has shown a high prevalence of infection with TTV in patients with human immunodeficiency virus (HIV) infection. This study indicates that TTV is commonly present in patients with HIV-positive as well as HIV-negative controls.
Keywords: Transfusion transmitted virus (TTV), Human immunodeficiency virus, confection

581. No Association Between A+2109G IFN-γ Gene Polymorphisms with Response to Antiviral Therapy in Patients Infected with HCV in Fars Province, Southern Iran

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Background: Chronic infection with Hepatitis C virus is a major concern and a huge burden on public health systems. Sustain response will be achieved in approximately 50% of patients. Interferon-γ plays important role in HCV infection. The presence of single nucleotide polymorphisms, G-to-A, in the second intron of IFN-γ gene (A+2109T) has been reported. There is an absolute correlation between the presence of T allele and the ability to produce higher amount of IFN- γ. Accordingly we aim to investigate of the association of IFN-γ +2109 A/G polymorphisms with the outcome of treatment in patients infected with HCV. Materials and Methods: 66 patients were treated with Interferon-α and Ribavirin was included in this study. The presence of HCV infection in patients (before and after therapy) has confirmed by RT-PCR. IFN-γ genotyping was carried out by polymerase chain reaction restricted fragment length polymorphisms (PCR-RFLP) on genomic DNA. Results: No significant difference was observed in allele and genotype frequency in patients achieved sustains response compare to those that fail to response to treatment. Conclusion: According to no significant differences in IFN-γ allele and genotype frequency between patients with sustain response and no response, we suggested that this polymorphisms may has not an important role in response to therapy. Investigation of other polymorphisms in this gene or other cytokine gene may provide valuable information of effect of genetic background on response to therapy.
Keywords: Hepatitis C virus, Interferon gamma, Polymorphisms

582. No Molecular Evidence of HTLV-II Infection among Seroindeterminate Samples in Mashhad, Northeast of Iran

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Background: Although Human T-lymphotrophic Virus Type I (HTLV-I) infection is endemic in different geographical parts of the world including Northeast of Iran, there have been no documents of type-II (HTLV-II) infection in this region. It is reported that one possible reason for seroindeterminate state in HTLV westernblot assays could be the infection with HTLV-II virus. This study is aimed to investigate the presence of HTLV-II infection among blood donors with seroindeterminate westernblot results using molecular techniques. Materials and Methods: Three ml whole blood obtained from 50 blood donors referred to Mashhad Blood Transfusion Organization who had reactive Elisa for HTLV-I and seroindeterminate HTLV westernblot state. DNA was extracted using a commercial DNA extraction kit. A conventional PCR was applied to detect HTLV-I provirus using specific primers while a nested PCR was designed with specific external and internal primers for the detection of HTLV-II. Serologic and molecular data as well as demographic characteristics were registered and analyzed using SPSS V.18.0. Results: The average age of 39 male and 11 female participants was 37.12± 14.36 years. The average OD of the Elisa assay was 1.767± 1.195. The most common indeterminate patterns were Rgp46-II alone (n=12, 27.3%), Rgp46-I alone (n=7, 15.9%), and Rgp46-I with GD21 (n=7, 15.9%). HTLV-I PCR revealed 10 (20%) positive samples while no HTLV-II positive sample were detected by nested PCR. There were no significant age, gender, blood group, Optical Density (OD) of the Elisa assay, and westernblot indeterminate pattern differences between HTLV-I PCR positive and negative samples. Conclusion: No HTLV-II positive sample in this study did not confirm the hypothesis of HTLV-II infection in our region. However, high frequency of HTLV-I PCR positive samples among the seroindeterminate cases implies on the important role of molecular techniques for further confirmation of the infection.
Keywords: HTLV-I, HTLV-II, seroindeterminate state, PCR, Mashhad, Iran

Poster Presentation

583. Immunological response (IgM and IgG) to Crimean-Congo Haemorrhagic Fever (CCHF) in Iranian patients as an emerging disease in recent years

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Background: Crimean-Congo Hemorrhagic Fever virus (CCHFV) is a member of the genus Nairovirus, family Bunyaviridae. Crimean-Congo Hemorrhagic Fever (CCHF) is a viral zoonotic disease that can develop into a severe hemorrhagic fever in human and has a case fatality of 13-50%. After a brief incubation period, the patient has sudden onset of fever, myalgia, nausea and severe headache. Within 3-6 days of the onset of illness, development of a petechial rash and hemorrhagic symptoms such as epistaxi, haematemesis, and melena may be apparent. The most extremely ill patients enter multiorgan failare characterized by shock, hemorrhage and coma. Materials and Methods: From 7June 2000 – 15 November 2011, 2439 serum samples from CCHF probable patients have been collected from different provinces of Iran and transferred according to safety procedures to the Arboviruses and Viral Haemorrhagic Fevers Laboratory (National Ref. Lab) and tested by specific ELISA for IgM and IgG against CCHF. Results: Between 2439 probable patient sera, 863 were confirmed cases (IgM and/or IgG positives) and we had 124 death cases. The Sistan-Baluchistan province, by having the most positive cases, is the most infected province and Khorasan and Isfahan provinces are respectively the second and third most infected provinces in 2011. According to our observation, we saw that the most exposed professions are: slaughterer (17.01%) and farmer (15.62%). Conclusion: CCHF is the most important haemorrhagic fever in Iran. The most infected province is Sistan-Baluchistan, in the southeast of Iran and near the border of Pakistan and Afghanistan where the disease is endemic. After Sistan-Baluchistan, Khorasan and Fars provinces are the second and third infected provinces in 2011, respectively. The majority of confirmed cases in Iran have professions related to infected livestock or their products. So, it seems very important to have some training programs for high risk groups to control disease in endemic area.
Keywords: IgM, IgG, CCHF

584. Comparision of Cytopathic Effect and Hemadsorption Methods of Potency Estimation of Various Mumps Vaccine Strains and Products

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Background: Mumps vaccine potency estimation methods have been subject of research in recent years because of discrepancy of the results obtained in different laboratories using different methods. In attempt to find the optimal method, various methods using real time PCR and plaque assay have been devised and evaluated. We have evaluated the use of Hemadsorption (HAd) to achieve this goal and compared it with CPE method.
Materials and Methods: Four different Mumps vaccine products from 4 different sources i.e., WHO International Standard (IS), Razi Institute of Iran, Serum Institute of India, and Merck of USA containing Urabe, Hoshino, L-Zagreb, and Jeryl-Lynn strains, respectively, were tested in triplicate for virus titer in 96 well cell culture plates on 3 different days and the results based on reading the CPE and hemadsorption positivity were calculated. Changes in titer against time were plotted to see which method reaches sooner to the plateau stage precision was evaluated from results obtained in 3 different runs. Closeness of the result of tests to the stated titer on the IS samples was evaluated as a measure of accuracy. Results: Plateau stage for HAd and CPE methods were reached on days 7-8 and 8-9 respectively. The Intermediate precision of Hemadsorption for all 4 strains (Urabe, Hoshino, L-Zagreb, and Jeryl-Lynn) were 3.6%, 1.45%, 2.87%, 1.34% versus 4.3%, 3.8%, 4.9%, 1.4% for CPE method respectively. Regarding accuracy based on closeness to stated potency (4.5), result of IS for HAd and CPE methods were 4.898 and 4.018 respectively. Positive HAd cases were easy to identify even by the less experienced staff. Conclusion: Hemadsorption method showed superiority to CPE in terms of precision and closeness to the target value of the International Standard and time. The technique is easy to establish and sharp distinction between positive and negative cases makes it easy to read. Various mumps strains do not behave similarly.
Keywords: HAd, CPE, real time PCR

585. Immunological Evaluation Antibody against IgG2b(In- House Antibody) as A Solid Phase in ELISA Assay for Detection Contamination in Active Pharmaceutical Ingredient (API) of Hepatitis B Vaccine

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Background: During Recombinant Hepatitis vaccine purification chromatography column is included IgG2B that used to absorb Hbs ag (hepatitis B surface antigen),so it is possible that a little amount of this antibody separated from Column during washing step and mix with sample .when API(active pharmaceutical ingredient) is prepared , it has to check the amount of IgG2b in API because this protein is known as a foreign antigen by body when vaccine injected .this protein is measured by ELISA method and we try to produce polyclonal antibody against it and use immunoassay exam to evaluated the product . Materials and Methods: In this experience IgG2b in deferent doses with complete freund's adjuvant and incomplete freund's adjuvant was injected to rabbit with special protocol .1 month later the blood of the rabbit was taken and centrifuge. The serum was separated and with ionic chromatography and affinity IgG column antibody was selected .this antibody was compared with commercial kit to approve its activity. Results: in each step SDS PAGE %12 was used to calculated the purity and after purification WHO protocols ICB Q2(R1) and ICH Q2B used to evaluated the antibody and compare with commercial kit. Conclusion: using in house antibody and

kit is a normal method in most biological manufactory and we try to check this method to produce an antibody special for our biological product and we will exam its stability at the future.
Keywords: ICH Q2B, ICB Q2 (R1), ELISA

586. Survey of Polymerase Gene Sequence HBV in HBeAb-positive Patients

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Background: HBV infection ranks as the tenth leading cause of death. Hepatitis B virus replicate via reverse transcriptase by polymerase gene. This gene contains 7 functional domains (A-G) and it lack proofreading activity. Prolonged therapy with nucleotides/nucleosides analogs often cause mutation in polymerase gene. The presence of HBeAg in serum shows virus active replication and HBeAb demonstrates low viral load. The most common method for detection of drug resistance mutation is direct sequencing of the viral pol gen after amplification of a selected fragment, using the polymerase chain reaction (PCR).The aim of this research was to study polymerase gene sequence in HBeAb-positive patients. Materials and methods: A 50 sera of hepatitis B infected patients collected and tested for hepatitis B e antibody (anti-HBe) and hepatitis B e antigen (HBeAg) by ELISA method. HBV-DNA was extracted from these samples and PCR was carried out on extracted HBV-DNA using specific primer. The purified samples were sequenced and analysed by Bechman CEQ 8000 DNA sequencer PCR products software . Results: The results of ELISA showed that of 50 patients, 47 (94%) patients were HBeAb positive. In these samples Only D genotype was detected. 5% of samples had specific substitutions of amino acid L81V, L180M, M204V.Conclusion: This study showed that 5% of patients after Lamivudine treatment had specific mutation that causes drug resistance mutation.
KeyWords: HBeAb-positive, Polymerase gene, Sequencing, Drug resistance mutation

587. The Relationship of the Levels of ALT with Viral Load in Hepatitis B Patients

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Background: The objectives of treatment in chronic hepatitis B patients are suppression of viral replication as well as reduction in hepatic inflammation. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver enzymes that are normally present in the blood. A higher than normal amount of these enzymes in a sample of blood can be a sign of liver damage. Researchers have used elevated alanine aminotransferase (ALT) levels as the key indicator that treatment should be started because elevated ALT levels indicate liver damage is occurring.The purpose of this study was to survey the relationship of the levels of ALT with viral load in hepatitis B infection. Materials and Methods: In this study, 50 sera of hepatitis B infected patients was selected and then serum samples used for determination of the level of liver enzyme (ALT) and viral load. HBV-DNA was extracted from serum samples and HBV viral load detected by Real-Time PCR. To assess the level of ALT, PARS AZMUN kit was used. The results were analyzed by SPSS software. The results considered significant with P<0.05. Results: A Fifty HBV infected patients were studied that 77% of them had viral load less than 100000 IU/ml, and 23% of them had viral load more than 100000 IU/ml. Also, 77% of them had abnormal ALT level and 18% of them had normal ALT level. Conclusion: The result of this study showed that there are significant relationship between liver enzyme and viral load in patients with hepatitisB infection.
Key words: HBV, Viral load, ALT

588. Hepatitis B and C Infections and HCV Genotypes among Haemophilia Patients in Ahvaz, Southwest Iran

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Background: Transfusion-transmitted hepatitis is the most important cause of transmitted infections by the parenteral route in patients with haemophilia. This study was performed to determine the prevalence of HBV, HCV, and different genotypes of HCV among haemophiliapatients in Ahvaz city, southwest Iran. Materials and Methods: A cross-sectional study was conducted on 87 haemophilia patients referred to the Hemoglobinopathy and Thalassemia research centre. Patients' sera were tested for HBsAg and anti-HCV using ELISA and confirmed by PCR (HBV) and RT-PCR (HCV). HCV genotypes were determined with HCV genotype specific primers using HCV genotyping kit. Results: The overall prevalence rate of HBsAg and anti-HCV were 1.1% (95% CI: 0-3.39) and 54% (95% CI: 43.5-64.4), respectively. Forty two of the anti-HCV patients (89.3%) were also HCV RNA positive. The prevalence of anti-HCV seropositivity was significantly higher (p<0.001) among patients who had started to receive transfusions before implementation of blood donor screening. Moreover, the number of transfusion were significantly associated with anti-HCV and HCV RNA positivity (p<0.005 and p<0.03, respectively). The predominant HCV genotype among haemophilia patients in our region was 1a (26/42, 61.9%), although genotypes 1b and 3a were found in 26.1% (11/42) and 11.9% (5/42) of the patients, respectively. Conclusion: It appears stringent donor selection procedures reduced HCV infection in multi-transfused patients, but it is still serious risk for these subjects.
Keywords: Hepatitis B, Hepatitis C, Prevalence, Genotype, Haemophilia

589. Evaluation of Brucellosis Seroprevalence in HIV-Infected Patients in Sanandaj During 2011

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Background: Infection with human immune deficiency virus (HIV) leads to cellular immunity deficiency, and the infected individual is susceptible to infection of various opportunistic pathogen microbes. Therefore, theoretically patients infected with HIV are susceptible to brucellosis. The aim of the current study is determination of brucellosis rate in patients infected with HIV. Materials and Methods: We included 89 HIV patients from Sanandaj Consultation Center for Behavioral Diseases. All patients signed informed written consents and then filled out the questionnaire. Blood sample was taken from each participant. After serum extraction, standard Wright tube, Coombs-Wright, and 2ME-Wright tests were performed on the samples. Moreover, blood samples were obtained from 502 individuals, who were not infected with HIV as the control group, and their results were compared with HIV patients. Results: The mean age of participants in the experiment and control groups were 33.31±7.47 and 34.38±11.29 years, respectively. In the Wright tube test for the HIV group, 71 individuals (79.8%) did not have antibody against *Brucella*, while 18 patients (20.2%) were positive for the antibody. According to the results of Wright tube test for the control group, 63 (12.5%) participants were positive for anti-*Brucella* antibody. The frequency of antibody against *Brucella* in the HIV group was significantly higher than that in the control group (p= 0.042). Conclusion: The results obtained demonstrated that in the HIV group, 20.2% were positive for antibody against *Brucella*, which is higher than that in the non-infected population. Therefore, considering the weak immunity status of HIV positive individuals, in areas endemic for brucellosis, these patients should receive care for brucellosis.
Keywords: *Brucella*, HIV, Sanandaj

590. Prevalence Rate of Herpes Simplex Virus Type 1 and 2 IgG Antibodies in Rasht City

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Background: Herpes simplex virus type 1 and 2 IgG antibodies are common worldwide.The most common manifestations of HSV-1 infections are oral lesions, neonatal disease, genital lesions, encephalitis, ocular infection, asymptomatic infection, non-oral, non-genital skin lesions. Data on prevalence of HSV-*1* and *HSV-2* IgG antibodies are limited in Asia, especially in Iran. The aim of this study was to determine the seroprevalence of Herpes simplex virus type 1 and 2 IgG antibodies based on age , gender , marital status , education , living area , job , Symptoms and history of disease variables . Materials and Methods: We did random blood sampling on 300 cases referred to the Rasht city's clinical laboratories. Demographic data gathered by a well-designed questionnaire and for serological studies, blood samples centrifuged .*HSV-1,2* and *HSV-2* specific ELISA kits used to determine IgG type specific antibodies in sera samples . Results: *HSV-1* and *HSV-2* IgG antibodies were positive in 157 (52.3%) and 16 (5.3%) subjects, respectively. According to our study , there was significant correlation between age , marital status , job , symptoms , history of disease and *HSV* IgG antibodies seroprevalence .Conclusion: This study is the first , to our knowledge , to present the comparative seroepidemiology of *HSV-1* and *HSV-2* IgG antibodyin Rasht city . Our findings were in agreement with prior studies in which *HSV-1* IgG was more prevalent than *HSV-2* IgG antibody and seropositivity increased with age. Prevalence rate of *HSV* IgG antibodies in Rasht is higher than Finland, Netherland and lower than America, most European countries and Africa . The high prevalence of *HSV* infection underlines the need for focusing on preventive efforts and education among the population.
Keywords: *HSV*, ELISA, IgG antibody

591. Improvement of Immune Responses against a Mini Vaccine Candidate from HIV-1 after Co-administration with *Pseudomonas aeruginosa* FLiC Molecule

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Background: HIV infection is one of the global health problems so development of an effective vaccine is necessary. Many candidate vaccines were evaluated but an effective vaccine to be elusive. Immunologic adjuvants such as TLR agonists could increase vaccine efficacy through various mechanisms. Many studies have shown that *Pseudomonas aeruginosa flagellin* as the TLR5 ligands could increase immune responses to vaccines. In this study, HIV-1p24-Nef conjugated to FLiC molecule was injected subcutaneously and intradermally and the immune responses were evaluated. Materials and Methods: BALB/c mice were distributed into different groups and immunized with 20 µg/100µl of HIV-1 p24-Nef conjugated to FLiC, p24-Nef and FLiC which were prepared in Montanide adjuvant and PBS subcutaneously and intradermally under the same conditions. Three weeks after the final boosting injection, lymphocyte proliferation was measured by BrdU method, the response of IL-4 andIFN-γ cytokines, as well as the level of total antibodies and their isotypes were evaluated by using ELISA method. Also IFN-γ ELISPOT was performed. Results:Our data showed that, in compare with control groups, the conjugated HIV-1p24-Nef-FLiC significantly increased lymphocyte proliferation responses, higher levels of cytokines with IFN-γ dominancy compared to IL-4. Also IFN-γ producing lymphocytes significantly increased but the level of total antibody and their isotypes didn't significantly increase. Furthermore the level of immune responses in subcutaneously route was more than intradermal route of immunization. Thus the results showed thatFLiC molecule could increase the level of cellular immune responses compared to candidate vaccine alone and the level of immune responses in the different routes were different. Conclusion: FLiC molecule could be used as adjuvant in combination with vaccines candidate against HIV-1, and modify vaccine formulation does not change the optimal route of vaccine inoculation.

Keywords: FLiC, HIV-1, ELISA

592. Serological Evaluation of Anti-Hbs Antibody in Patients with Suspected Hepatitisb

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Background: Hepatitis B infection is a serious public health problem in worldwide and major cause of hepatitis, cirrhosis and hepatocellular carcinoma.The main objective of this study was to evaluateof anti-HBs antibody level and some of its associated factors in patients with suspected hepatitisB in individuals that refferd to toobaclinic in sari. Materials and Methods: in a cross- sectionall study, Serum from270 individuals (in 3 group ages include1-20 years, 21-40 years and over the 41 years) that were suspected to hepatitis disorder tested for hepatitis B surface antibodies (HBsAb) by enzyme linked immunosorbent assay. Result: 109(40.٢%) subjects of these individuals were male and ١٦١(59.8%) were female.Themean age of all participants was 30.63±20.59 years. 124(45.8%) individuals were HBS Ab positive, (anti-HBs antibody <10 IU/L) and 147 (54.٢%) subjects wereseronegative. there was a significant difference between anti-HBs antibody and ages of groups (Seropositivity was significantly higher at over the 41 years(P≤٠.٠٠٤)).Wefoundno significant relation between serum level of anti-HBs antibody and gender. Conclusions: The results showed that HBsAb titer may decrease over time.Thus, Periodic assessment of anti-HBs antibody level is strongly recommended in all ofage groups especially higher age.
Keywords: Hepatitis B, Anti-HBs antibody, cirrhosis, hepatocellular carcinoma

593. Age-Specific Seroprevalence of Hepatitis a in Sari, Iran

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Background: The declining incidence of hepatitis A virus (HAV) infection in the Islamic Republic of Iran may be reducing the population's natural immunity. This was the first systemized, population-based survey of the seroprevalence of HAV antibodies in urban and rural inhabitants of Sari, Mazandaran province. Materials and Methods: in a descriptive study; Serum from 1034 individuals aged 1–25 years in 2007 were tested for anti-HAV IgG antibody using a commercial enzyme immunoassay kit. Results: The overall seroprevalence was 38.9%. The lowest prevalence (5.2%) was among the younger age group (1–5 years) from urban areas and the highest prevalence (82.0%) in the older age group (15–25 years) from rural areas. Seropositivity was significantly higher at higher age, among females and in rural areas. Conclusion: Sari is no longer classified as an area of high endemicity, and immunization against HAV may be needed in our population in the future.
Keywords: hepatitis, seroprevalence, endemicity

594. Evaluation of CXCL9 and CXCL10 Levels In HAM/TSP Patients and HTLV-1 Carriers and Compare it with Healthy Control Group

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Background: HAM/TSP is a chronic progressive inflammatory neurological disease that associated with HTLV-1 virus. In HAM/TSP, HTLV-1 virus changes the chemokine andchemokine receptor expression in the infected CD4 T cells. These chaotic changes alter the lymphocytes migration pattern. Lymphocyte gene expression appears to be correlated with proviral load. CXCR3 play an important role in transmigration of lymphocytes across the blood brain barrier in a number of CNS inflammatory diseases. CXCL-9 and CXCL-10 also play an *important part* in certain stages of brain inflammation via recruitment of lymphocytes into the inflammatory sites. Therefore increased levels of CXCL-9 and CXCL-10 may reinforce the inflammation in HAM/TSP. Materials and Methods: In this project,three groups including: HAM/TSP patients (22), HTLV-1 carriers (22) and healthy people (30) were selected and blood samples (*10 ml*) *were obtained from each subject*. Chemokines were measured by sandwich ELISA with matched antibody pairs for CXCL9 and CXCL10. All samples were assayed in duplicate and on the same

plate. The detection limits for these assays were 5pg/ml for CXCL9 and CXCL10. ELISA technique was performed on serums of 74 subjects.Results:The serum levels of CXCL9 and CXCL10 were significantly higher in HAM/TSP patients ($P< 0{\cdot}0001$) than in HTLV-I carriers and healthy controls. The median serum levels of CXCL9 for HAM/TSP patients and HTLV-1 carriers were 6502 pg/ml and 2310 pg/ml respectively. The CXCL10 level was significantly associated with HAM/TSP. Conclusion:Increased levels of CXCL9 and CXCL10 chemokines correlated with more CNS lesions because this chemokines play an important role in development of inflammation in the CNS by triggering massive recruitment and transmigration of effector T cells through blood brain barrier.
Keywords: HAM/TSP, CXCL9, CXCL10

595. Construction of HIV-1 *Tat/Env/Pol/Gag* DNA Vaccine Candidate and Its Evaluation in BALB/C Mouse Model

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Background: Despite a huge number of studies towards vaccine development against HIV, no effective candidate has been approved yet, remarking various aspects of research in this area. DNA vaccines offer several potential advantages making them promising against HIV. Herein, a multiepitope DNA vaccine encoding selective epitopic fragments from different HIV-1 antigens (tat, env, pol, gag) is constructed and preliminarily evaluated in vivo. Materials and Methods: HIVtop4 sequence spanning the junction of six amino acid fragments ($Gag_{158-186}$, $Pol_{150-190}$, $ENV_{296-323}$, $ENV_{577-610}$, Tat_{1-20} and Tat_{44-61}) was designed based on computer analysis to reduce the creation of junctional epitopes, improve the cleavage of proteasome and avoid the accumulation of hydrophobic regions. Synthesized nucleotide sequence corresponding to HIVtop4 was cloned into pcDNA3.1+ plasmid. Expression of pcDNA/HIVtop4 plasmid in mammalian cells was confirmed by RT-PCR, dot-blot and western-blotting assays.After then, large scale plasmid purification was carried out using endofree plasmid purification kit.experimentalGroups of BALB/c mice were intramuscularly immunised with either 200 or 100 or 50 μg of endo-free pcHIVtop4 plasmid at a three-injection protocol with 2-week intervals. Immune response analysis was carried out two weeks post-immunisation by proliferation assay (Brdu) and IL-4, IFN-γ cytokine assays (ELISA). Results: According to the obtained data the designed candidate DNA vaccine significantly induced the proliferation of mice lymphocytes in the presence of antigenic peptides, and further induced the secretion of IL-4 and IFN-γ cytokines compared to the control PBS-receiving and mock groups. Conclusion: The results of this preliminary analysis suggested that the constructed multiepitope DNA vaccine was capable of stimulating cellular immune response in mouse, however, more experiments using DNA prime/protein boost strategy should be considered to evaluate the potency of the vaccine.
Keywords: HIV-1, DNA vaccine, ELISA

596. Cloning, Expression and Purification of a Multi-Epitope HIV-1 Tat/Env/Pol/Gag Vaccine Candidate and Immunogenicity Study in BALB/C Mouse

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Background: Designing potential vaccine candidates against HIV-1 is highly demanded. Multi-epitope vaccines offer several potential advantages that may be promising in case of mutable divergent pathogens such as HIV-1. Herein, a multiepitopic recombinant protein containing various HIV-1 antigens was expressed in E. coli cells and its immunogenicity in combination with different adjuvants was initially evaluated in BALB/c mouse. Materials and Methods: HIVtop4 sequence spanning the junction of six amino acid fragments ($Gag_{158-186}$, $Pol_{150-190}$, $ENV_{296-323}$, $ENV_{577-610}$, Tat_{1-20} and Tat_{44-61}) was designed based on computer analysis to reduce the creation of junctional epitopes, improve the cleavage of proteasome and avoid the accumulation of hydrophobic regions. Synthesized nucleotide sequence corresponding to HIVtop4 was cloned into pET23a plasmid. Expression of pET-HIVtop4 plasmid was induced in BL21 *E. coli* cells by addition of 1 mM IPTG during 3 hrs culture. Due to C-terminal fusion of 6xHis-tag, the protein was purified by IMAC and further confirmed against anti-His antibody in western-blotting. Groups of BALB/c mice (n=6) were immunized with of 20 μg of candidate vaccine adjuvanted in Complete Freund's adjuvant , Montanide ISI-70 and Alum. Experimental mice groups were immunized three times with 2 weeks interval subcutaneously with 100μl of each formulation containing 20 μg of vaccine candidate with suitable control groups. Two weeks after last immunization lymphocyte proliferation was measured with Brdu, IL-4 and IFN-γ cytokine with ELISA, total antibody and IgG1, IgG2a and IgM isotypes with indirect ELISA methods. Results: Immunization of mice with HIV-1tat, env, pol, gag led a significant increase in the proliferative responses of lymphocytes, IFN-γ and IL-4 cytokines and total antibody titer with poly-isotypic form in comparison with the control groups. Conclusion: In this study we concluded tat, env, pol, gag with adjutants (Montanide, Alum and CFA) can be considered as a candidate vaccine against the HIV virus.
Keywords: pET23a, pET-HIVtop4, HIV-1

597. Age-Specific Seroepidemiology of Hepatitis a Infection: among1- 30 Years Savadkooh Area, Mazandaran, 2010-2011

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Background: Major geographical variation in the epidemiology of hepatitis A Virus (HAV) infections were exist, which closely correlate with economic status, hygienic and sanitary conditions. During past two decades , results of seroprevalence studies suggests that many hyperendemic countries are undergoing a transition to a lower endemicity as their economic status improved. Earlier HAV seroprevalence studies reports from Mazandaran, WHO ranked Iran as a hyperendemic country. Findings of some seroprevalence studies from different parts of Iran indicated an epidemiological shifting. This study aim was to evaluate an age-specific anti –HAV IgG prevalence rates in Mazandaran and also compare the collected data with results of other studies performed in Mazandaran and other parts of country to determine a proper epidemiological patherns of HAV in Iran. Materials and Methods : The study groups were 984 subjects 1-30 years old age resident of both urban and rural areas of Savadkuh district , divided in 5 age groups: 1-2.9 year n=316 ; 3-6.9 years n=254; 7-10.9 years n=201 ; 11-17.9 years n=115 and 18-30 years n=98 . HAV antibodies were measured by ELISA method. The collected data were analysed by x test. Results: Overall seroprevalence rate was 19.20% with no significant differences between two studies areas. Prevalence rates were increased significantly (p=0.0001) with increasing age: 5.7%, 9.0%, 9.4%, 34.8% and 68% respectively. Also, studies analysis revealed that HAV prevalence rates in children were decreased in Mazandaran and Iran. Conclusion: It seems that HAV epidemiology is shifting from high to a lower level of infection. This pattern necessitate that preventive strategies against HAV should be revised in Mazandaran and Iran.
Keywords: Hepatitis A virus, HAV antibody, Seroprevalence , Endemicity ,Mazandaran, Iran

598. Prevalence Rate of HTLV-1 Antibodies in Beta- Thalassemia Patients Comparative between 2002 and 2010 in the City of Babol North Iran

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Background: Blood transfusion is one the major route of transmission of human T cell leukemia virus type1 (HTLV-1).The aim of this study was to evaluate the prevalence of HTLV-1antibodies conducted to relationship factors age and sex among beta thelassemia patients in 2010 and comparative with 2002. Materials and Methods: In this cross sectional. Comparative surveys were done on HTLV-1antibodies between 143 voluntary beta thalassemia patients in 2010 and with mach age and gender 137 patients in 2002 . The patients' age range were 2 to 24 years with a mean age of 11.19 years in 2002 and mean age 13.3 years in 2009. Patients divided 3 groups ofages 2-8 years, 8-15 years and 16-24 years.

From total of 143 patients' 84 patients were female and 59 patients male. Enzyme linked Immunosorbant assay (ELISA) was utilized to test HTLV-1antibodies and a western blot methods in positive HTLV-1 samples. Results: From total of 137 subjects in 2002 and 143 subjects in 2010, 8 cases (5.8%) and 14 cases (9.7%) respectively were seropostive HTLV-1 antibody with ELISA and western blot methods. Among group 2-8 years of age there were not seropositive case (0%) in 2002 and 1 cases (8%). In age group 8-15 years, 3 cases (5.2%) of subject were serupositive. In age group 16-24 years 20% of cases were serupositive and sex was recognized to be a significant factor since 7 cases (70%) of girls and 3 cases (30%) of boys subjects were seropositive P= 0.454. Conclusion: prevalence HTLV-1 infections between thallasemic patients in 2010 were increase comparative with 2002 study.
Keywords: HTLV-1, Beta- Thalassemia, ELISA, western blot

599. Serological Study Hepatitis B Virus Infection among in 18-24 Year Oldsin Beta Thlasemia and Normal Individuals in Babol, North Iran

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The objective of this study was to estimate the prevalence of HBV infection in 18-24 year olds in Beta thlasemia and normal individuals in Babol city , north Iran. Materials and Methoods: With cros sectional study was during in 2009 and 2010 this studies was done among in 18-24 Beta thlasemia patinet and normal individuals . A total 1644 (380 girls and 1264 boys) of normal individuals and 36 Beta thlasemia patinet (21 girl and 15 boys) were tested for hepatititis B surface antigen (HBsAg) and (anti-HBc) . afterward , seropostive samples were tested for antibodies IgG and IgM to hepatitis B core antigen (anti-HBc) and antibody to HBsAg (anti-HBs) with using a commercially available enzyme-linked immunosorbent assay and PCR for HBV, DNA . Results: Of 1644 normal individuls, 18 cases (1%) were HBsAg and anti-HBc seropositive (6 girls (1.57%) and 12 boys,(0.94%)) P=0.65. All indivduals seropositive were negative for IgM anti-HBc and positive IgG anti-HBc(Chronic infection). 4(4/24) individuals (16.7%) were IgM and IgG anti-HBc positive (acute infectons). All of wich ١٨ samples were negative for anti-HBs marker. The overall prevalence rate of Chronic infection HBV (HBsAg+ , HbcAb(IgM)- , HbcAb(IgG)+, anti-HBc-) was 1%(18/1644) in this study.in total of 36 beta thalassemia 3 cases(3/8%) were positive for HBsAg and anti-HBc. All of wich 3 cases were negative for IgM anti-HBc and anti-HBs. PCR Testes PCR for DNA viruses for all seropostive caces were positive. Conclusion: Prevalence rate hepatitis B virus chronic infection among in young normal puopolation and beta thalessemia in aria are 1% and 8.3% respectively.
Key words: Hepatitis B virus (HBV) , Hepatititis B surface antigen (HBsAg) , Antibodies hepatitis B core antigen (anti-HBc), Chronic infection , blood donor , Iran

600. The Protective Effecacy Rate of Hepatitis B Virus Vaccine in Medical Student

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Background: The aim of this study was to determine percents of groups non responder, low responder, adequate responder and high responder after vaccination in Babol University medical students. Materials and Methods: In this cross sectional, descriptive and analytical study was performed on 181 medical students (157 female 24 males) Vaccins inoculated at three time of 0, 1 and 6 months . Afterward the anti-HBs Ab titer in blood specimen was evaluated by Enzyme-Linked-Immunoassay (ELISA) interval 1 to tree months of last inoculation. Results: From total of 181 students result responder to HBV vaccine were 1 individual (0.6%) non responder (anti-HBs <10 mIU/mL), 6 individuals (3.3%) low responder (10-100 mIU/mL) , 95 individuals (52.2%) adequate responder (100-1000 mIU/mL) and 79 individuals high responder (> 100 mIU/mL). In total (99.4%) of students had HBsAb titers > 10 mIU/mL. From total of 181 students 15 individual (8.3%) had Under BMI 125 indviduals (69.1%) Normal BMI and 41 individuals (21.6%0) had Over BMI. There was nosignificant relationship between mean anti-HBs titer with different groupe BMI and gender (*P*> 0.05). Conclusions: The results of present study show HBV vaccination in yuongh individuals with observe standard methods are efficacy in 99.4% individuals.
Keyworlds: HBV vacchnation, anti-HBs Ab, BMI, medical students

601. Evaluation of HBV Viremia in HBeAb Positive CHB Patients

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Background: Approximtely 400 million people are chronically infected with HBV all over the world.Morbidity and mortality in chronic hepatitis HBV (CHB) are related to viral replication.Transition of active CHB to an inactive phase after immune response is associated with seroconversion from HbeAg to HbeAb and HBV DNA levels decrease to low or undetectable levels.This inactive carrier state can be reactivated by spontaneously or immune suppresion without reappearance of HbeAg due to mutation in precore/core promoter region. Recent guidlines recommended that HBV-DNA viral loads above 2000Iu/ml is an indication for treatment. The aim of this study was to determine HBV viremia in eAg negative/eAb positive Iranian CHB patients. Matherials and Methods: In this cross-sectional study ,700 CHB patients (average age: 40.72, female: 34.1% and male:65.9%) were included.HbeAg/Ab testing were analysed by ELISA method.we investigated HBV viremia using real time PCR by Artus HBV LC PCR kit based on manufacture ٬s instruction in light Cycler instrument (Roche-version 2) . Results: Twenty-two percent of patients had antibody against e antigen.HBV DNA levels were undetectable (<60 Iu/ml), 60-2000 Iu/ml, 2001-20000Iu/ml and above 20000 IU/ml in 24.5%, 49%, 13.4% and 12.8% of subjects, respectively. Conclusion: Although ,traditionally eAg negative/eAb positive phase is called as inactive carrier state in natural history of CHB ,in this presented study we detected clinically significant HBV DNA viremia (>2000 Iu/ml) in considerable proportion (26.2%) of HBeAb positive patients,who should be consider for treatment.
Keywords: HbeAg/HbeAb, Chronic Hepatitis B, Real time PCR

602. Seroprevalence of Cytomegalovirus Infection in Pregnant Women Refferd to Health Care Center of Khorramabad in 2008

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Background: Cytomegalovirus (CMV) is a kind of herpesviruses. It is one of the most common causes of congenital and prenatal infections. CMV infection of pregnant women, especially in the first trimester may lead to congenital abnormalities in the newborns. The prevalence of CMV infection in developed countries is about 40% and in developing countries may be 100%. Because there is no information related to the epidemiology of this infection in Khorramabad city, This study was done to determine the seroprevalence rate of the infection and its associated risk factors in pregnant women who refferd to the health care centers of this city in 2008. Materials and Methods: This cross-sectional study was done in 240 pregnant women. Demografic data were collected by a questionaire. About 3 ml of blood was taken from each patient. Serum samples were aliquoted and froze at -20°C until analysed. The presence of anti-CMV Specific antibodies was assessed by enzyme immunoassays. Data were analysed by fisher٬s exact test and x^2 test using SPSS software version 11.5. Results: Mean age of cases was 26 years and varied between 15-40 years. CMV IgG was found in 217 cases (90.6%) out of 240 cases. 97 people (86%) of the cases who were pregnant for the first time were positive regarding to CMV-IgG. In women who had 1-3 or more than 3 deliveries, this rate was 94% and 100% respectively. There was a significant relationship between the number of deliveries and the positive result of the test (CMV-IgG). There were no significant relationship between age, abortion history and number, education level and the stage of pregnancy with test result (p>0.05). Conclusion: As in other developing countries, the prevalence rate of CMV infection in pregnant women in Khorramabad is high. Since the clinical importance of CMV diseases and not enough informations about the seroepidemiology of this viruses and frequence of maternal infection, it is recommended to perform the sectional studies at all over the country.

Keywords: Cytomegalovirus, Seroepidemiology Enzyme immunoassays, Khorramabad

603. Does IL-28B Related with Predicting Treatment of HCV?

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Background: Hepatitis C infection is a global health problem because of the high rate prevalence and morbidity and mortality worldwide.different factors including; virus,host genom,environment related to disease outcome. The aim of this study was assess the association polymorphism rs12979860 of IL-28B and chronic HCV infection. Materials and Methods: In this study, a total of 123 HCV-RNA positive patients (85(69.1%) males and 38(30.9%) females) with mean age of 37.11+12.61 years, with age, sex and geographical area matched with the patients were recruited. all originating from mazandaran province. We used tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) for amplify of IL-28B gene with specific primers. Results: The frequencies of the IL-28B genotypes (rs12979860) were as follows: CC, 41.5%; CT, 45.5%; and TT, 9.8%. Conclusion: there is association between genetic factors specially IL-28B with disease outcome.
Keywords: IL-28B, T-ARMS-PCR, HCV

604. The IL-10 Promoter Polymorphism is Correlated with Susceptibility to Occult HBV Infection

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Background: Occult hepatitis B infection (OBI) is characterized as a form of hepatitis in which HBsAg is undetectable, whereas detectable amounts of HBV-DNA are obvious in the peripheral blood of patients. The main aim of this study was to investigate whether if there is a relationship between OBI and single nucleotide polymorphisms (SNP) in the -592 region of the IL-10 gene. Materials and Methods:In this study, the polymorphism within -592 region of the IL-10 promoter of 57 OBI cases and 100 healthy controls was performed using PCR-RFLP techniques. Results:Our results showed that patient and control groups had significant differences regarding genotypes and alleles of the -592 region of the IL-10 polymorphism. Conclusion:Based on our results it can perhaps be concluded that the -592 region of the IL-10 polymorphisms in the promoter region of the gene is associated with OBI.
Keywords: HBV, OBI, SNP, IL-10

605. Efficacy of Influenza Vaccine in Iranian Health Workers and Healthy Persons

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Background: As, the Influenza strain composition changes each year, World Health Organization issues an updated recommendation for the vaccine composition, based on the expected circulating strains, annually.Ideally, clinical vaccine efficacy would be established in experimental field trials, but for an annual re-licensure procedure this approach is unrealistic.The clinical immunogenicity data to support the annual re-licensure procedure is necessary. This study was done to evaluate the efficacy of inactivated,split-virus 2010/2011 Influenza vaccine in Iranian health workers and healthy persons by Hemagglutination inhibition assays. The data were analyzed by seroprotection rate, 4 fold increases in antibody titer. Materials and Methods: Forty health workers and healthy persons between the ages of 9 and 64 years were injected withone dose commercial vaccine(Sanofipasteur) comprising 15 microgram of hemmaglutinin. Blood sample was collected from subjects, at the time of vaccination and2 weekspost-vaccination, to determine the serologic response to the vaccine. Hemagglutination inhibition assays were performed on all serum samples. Results: Ahemagglutination-inhibition titer of 1:40 or more was achieved by day 14 in 97.5% of subjects for influenza New A/H1N1 (California).No severe adverse side effects associated with the vaccine were noted. In this group, injection-site or systemic reactions, most mild in nature, were noted in 35% of subjects. Conclusion: Our results indicate that the 2010 seasonal influenza vaccine is >90 %effective against pandemic (H1N1) 2009 virus.This finding showed that one dose of vaccine was highly immunogenicin Iranian health workers and healthy persons.
Keywords: Influenza Vaccine, H1N1, Hemagglutination

606. Prevalence of Anti-Hbs Antibody among Blood Donors without Vaccination in Sistan-O-Baluchistan

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Background: Infection of Hepatitis B is rightly considered as a severe health problem in world-wide due to its high incidence rate and lack of any definite cure. At the present, vaccination is the sole defense mechanism available against this disease. Since Hepatitis B can be easily transmitted via exposure to blood or blood product, careful screening of donor for Hepatitis B is a well-stablished practice in many countries. The purpose of this study was to investigate the prevalence of anti-HBs antibody among blood donors in Sistan-o-Baluchistan who don't have any vaccination. Materials and Methods: In this study, 1500 serum samples were collected from blood donors in Sistan-o-Baluchistan. All sera were tested for HBsAg and anti-HBc by ELISA method, and then all sera that positive for anti-HBc, tested for anti-HBs. Results: Out of 1500 blood donors, 144 donors were positive for Hepatitis B core antibody. among them, 118 persons haven't any vaccination(81.9%) that among them 40 blood donor's(33.89%) showed anti-HBs levels (1-100 IU/L), and in 40(33.89%) persons, anti-HBs titres was ranged from 100-1000 IU/L and 13 persons(11.01%) had titres over than 1000 IU/L and 25 blood donors(21.186%) were negative for this antibody. All subjects were aged between 40 and 60(mean 36.88) that 42(35.6%) blood donors were aged between 20 and 30 that higher than other age groups. Conclusion: Although most of the anti-HBc positive blood units (63.58%) are associated with anti-HBs without vaccination .The interperation could be either a spontaneous anti-HBs formation or previous infection that now are immune and could transfer immunity to recipients.
Keywords: anti-HBs, Sistan-o-Baluchistan

607. SerologicalEvaluation of Infectious Diseases Including HIV, Hepatitis B and C in South Health Centers of Tehran

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Background: New emerged and recurrent infectious diseases are cause of many people death in world. Objective of this study was survey of prevalence infectious diseases including HIV, hepatitis B and C in south health centers of Tehran. Materials and Methods: In descriptive study, 24456 individuals from March 2010 to November 2011 were selected.3-5 ml peripheral blood samples after obtaining informedconsent, and serum was separated immediately and tested for HIV antibody, HCV antibody and HBs Ag by enzyme linked immunosorbent assay.Results: 65%of these individuals were male and 35% were female. 3 individuals out of 8131 (3.68%) were HIV positive. 37 individuals out of 9520(38.86)% were HBs Ag positive and 6 individuals out of 6805(8.81%) were HCV positive.
Conclusion: The present study reveals that 46 of these subjects were suffering from various infectious diseases. This is essential to develop a comprehensive protocol in review prevention programs to reduce the incidence of these diseases in our society.
Keywords: HIV, HBV, HCV

608. Prevalence of CMV Infectious among Renal Recipients in Mashhad University of Medical Science Related Hospitals; PCR and Q-PCR Techniques

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Background: CMV infection is a major cause of morbidity and mortality in immune-compromised individuals. This infection, which can be acquired from the donor, blood products or due to reactivation of a latent infection in recipient can result in prolonged fever, leukopenia, hepatitis, colitis, allograft injury. Therefore, the prevention of CMV disease is a major goal in the management of kidney transplant patients. Reliable and sensitive laboratory techniques for diagnosis of CMV infection before the onset of symptoms are very helpful. Materials and Methods: In the present study, a total of 60 kidney transplant patients were enrolled in this study and recipients' different variables including age, sex, and their para-clinical findings such as CBC differentiation, clinical outcomes and graft function were evaluated. In addition of CMV, other viral infections like EBV, HIV, HBV, HCV and HTLV1 were controlled. Both qualitative PCR and quantitative CMV PCR have been used to detect CMV DNA in the plasma. Results: Most of donors and recipients were (more than 95%) seropositive but according to the PCR results only 58.4% of recipients were CMV positive while 5.1% of them were strong positive and others (38.3%) were negative. An arbitrary cut-off level of900 copies/ml was selected to determine the positive predictive value and negative predictive value of the qRTPCR for the patients. 1,5% of the recipients showed high peak of viral load. All of these patients have developed related symptoms of CMV, unlike the patients were only serpositive. Discussion: Since the number of CMV copies correlated well with the severity of clinical symptoms, early detection of CMV DNA in the blood is of great importance to identify those patients who are at risk of infection. Therefore laboratory techniques such as EIA cannot be used, for diagnosis of CMV infection a reliable and sensitive method like Real-time PCR is suggested.
Keywords: CMV, qRTPCR, Real-time PCR

609. Lack of Correlation between CCR5 Δ32 Mutations and Chronic HBV Infection in a Sample of Iranian Patients

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Background: CCR5 is an important chemokine receptor involved in recruitment of specific anti-viral immune cells (e.g: NK cells and T cytotoxic cells) to the liver. Previous studies indicated that the Δ 32 mutation in CCR5 gene lead to down-regulation in parallel with CCR5 inactivation. The main purpose of this study was to investigate the Δ 32 mutation in CCR5 gene in chronic HBV infected (CHI) patients. Materials and Methods: The study population was a total of 60 CHI patients and 300 healthy controls. Gap-PCR was applied to study the CCR5 Δ 32 mutation. Results: Results of current study demonstrated that none of CHI patients displayed CCR5 Δ 32 mutation while, 3 (1%) of healthy controls revealed heterozigotic form of this mutation. Conclusions: Based on our results it can possibly be concluded that CCR5 gene is not under the influence of itsΔ 32 mutation in the Iranian CHI patients. Keywords*:* Chronic HBV infection, CCR5, Δ 32 mutation

610. Zinc Supplementation: Is Iteffectiveon CD4 Levelin HIV Positivepatients?

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Background: Sufficient zinc is essential in maintaining immune system function, however HIV infected individuals are particularly susceptible to zinc deficiency. In HIV infected patients, low serum levels of zinc have been associated with a more advanced stage of the disease and also with an increase in mortality. However, the HIV virus also requires zinc itself, and excessive zinc intake may stimulate the progression of HIV infection. This study was performed to further assess optimal zinc intakes for HIV infected individuals. Materials and Methods: This clinical trial study was conducted in Navab – Safavi urban health center, in 2009. The patients that referred to this center were randomly assigned to receive zinc supplementation (zinc sulfate 45 mg per day) for about two months. CD4 level before and after the oral administration was counted and data was analyzed using SPSS 16. The chi-square and T-paired tests were used for data analysis. Results: According to the results, mean CD4 levels before and after the intervention was 486.6±226 and 460.6±203.9, respectively. No statistically significant difference was seen in CD4 levels (P=0.19). Conclusion: Our findings are not in line with the results of the previous studies that suggested a connection between zinc supplement and treatment of HIV. Thus more studies with larger number of patients, different doses of zinc supplementation and longer administration of zinc supplement are suggested.We would also like to acknowledge the national elite foundation that supported us attending the congress.
Keywords: Zinc, HIV, CD4

611. Evaluation of IL-21 Gene Expression in HTLV-I Associated Myelopathy/tropical Spastic Paraparesis (HAM/TSP) Subjects and HTLV-1 Asymptomatic Carriersand its Correlation with HTLV-1 Viral Load

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Background: HAM\TSPis aprogressive inflammatory disease in which TCD8 and T CD4 cells specific for HTLV-I recruit to CNS and cause bystander demyelisation of spinal cord. An efficient cytotoxic T lymphocyteresponse to HTLV-1 limits the proviral load and the risk of associated inflammatory diseases such as HAM/TSP.Interleukin-21 is a recently discovered multifunctional and pleiotropic cytokine, shown to exert significant immune-enhancing functions and promotes proliferation and accumulation of Ag-specific CD8+ effector T cells leading to increase of their survival and cytolytic potential. For the comparative study of IL-21 gene expressionlevel, a real-time Taqman method was developed in HTLV-I Foundation Lab. Materials and Methods: PBMCs were isolated from peripheral blood of carriers and HAM\TSP subjects by using Ficoll-hypaque density centrifugation.PBMCs were then cultured in RPMI complete culture medium. Cells were activated with PMAand Ionomycin. Activated cells were harvested and RNA of each was extracted for cDNA synthesis. A real-time PCR TaqMan method was designed and optimized for evaluation of IL-21human gene expression.HTLV-I Proviralloadkit used to measure theviral loadinHAM\TSP and carriers. Results: Using our optimised TaqMan real-time PCR(R>0.95, Efficiency>0.99) ,the analysed data in this study indicated that there is a significant decline in IL-21 expression of HAM\TSP subjectsin comparison with carriersafter PBMCsactivation with PMA and ionomycin.The results indicated an indirect relationship between IL-21 gene expression and proviral load in HAM/TSP subjects. Conclusion: In the present study the results demonstrated that IL-21expression in HAM\TSP was significantly lower thancarriers, with respect to the role of IL-21 in Promoting the cytotoxic potential of CD8 effector T cells and more over an efficient CTL response to HTLV-1 limits the risk of HAM/TSP, It is highly probable that decreased level of IL-21 may contribute in HAM/TSP pathogenesis.
Keywords: HTLV-I, IL-21, HAM/TSP

612. Anti Hepatitis C Virus Antibodies among Blood Donor in Bushehr In 2011

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Background: Blood transmitted infections have always made problems in the use of blood and blood products. Hepatitis C virus infection is one of most common chronic blood borne infection with an estimated 3.9 million persons infected with the virus and has a high rate of development of liver cirrhosis. Materials and Methods: The total of 16755 blood donors donated blood within 2011 in Bushehr. All samples were tested for presence of anti HBV antibodies with ELISA. Data were compared by the Chi-square statistical test. Results: Out of this number 11(0.06%) were positive that except one, all positive were first time blood donor. P value of hepatitis was $p<0.001$ for regular and first-time donors, that this difference was significant. Conclusion: Blood transfusions carry the risk of transmitting infections. Regular blood donation is one of the important steps in blood safety but the HBV infection is still responsible for certain cases of post-transfusion hepatitis world-wide.
Keywords: HCV, ELISA, HBV

613. Seroepidemiological Investigation of HBs Ag among Blood Donors in Boushehr-Iran

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Background: Blood transfusions carry risks of untoward reactions, including the transmission of infections, such as hepatitis B In this survey the seroprevalence of HBs Ag among regular, sporadic (lapsed) and first-time blood donors were compared in 2011. Materials and Methods: Samples of blood donated within 2011 were screened for Hepatitis B virus with ELISA and the prevalence of hepatitis was compared among regular, sporadic (lapsed) and first-time blood donors of Bushehr city. Data were compared by the Chi-square statistical test. Results: The total of 16755 blood donors donated blood in Bushehr; out of these number, 8985 were regular donors (53.6%), 3699 sporadic donors (22%) and 4071 first-time donors (24.3%).Among these donors, 13 (0.07%) HBs Ag positive were detected. The prevalence of hepatitis was less in regular than first-time blood donors. P value of hepatitis was $p<0.001$ for regular and first-time donors, that this difference was significant. Conclusion: Regular blood donation is one of the important steps in blood safety; hence, retention of regular donors, and awareness-raising and recruitment of sporadic and first-time donors can increase the rate of regular donation leading in turn to higher blood safety.
Keywords: HBs Ag, ELISA, Bushehr

614. Anti HCV Seroprevalence in a Tertiary Hospital at Center of Iran

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Background: Hepatitis C virus (HCV) is a major cause of acute and chronic hepatitis worldwide. The rate HCV infection differs in particular countries. The prevalence in developed countries is amount to 0.2%-2.2%, while in developing countries it reaches 7%. However no information has been available concerning the prevalence of HCV in general hospital populations in this geographical area. Epidemiological information on HCV is essential for strategic prevention of chronic hepatitis, liver cirrhosis and cancer. Therefore the objective of this study was to evaluate the seropositivity of HCV antibodies in patients referred to this central teaching hospital.Materials and Methods: This prospective descriptive study was conducted in Vali-Asr central teaching hospital in centre of Iran from January 2010 to January 2011. A total of One thousand and fifty one subjects from outpatient department and hospital admitted patients were included in this study.Results: The mean age and standard deviation of the patients was 33.56 +7.44 years. Anti HCV was positive in 106(10.1%) cases.Conclusion: There was high frequency of seropositivity of anti-HCV in patients in this hospital, demanding for further investigation to determine the root of transmission and risk factors to improve infection control measure.
Keywords: HCV, Anti HCV Seroprevalence

615. Production of Recombinant S1 Glycoprotein of Infectious Bronchitis Virus in Escherichia Coli

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Background: The objective of this work is to provide S1 protein as a recombinant antigen in order to be used in diagnostic kits. Various strains of infectious bronchitis virus (IBV) were detected by the S1 glycoprotein gene. Materials and Methods: A prevalent strain 4/91 was frequently isolated in material from Iran. In this study we extracted RNA from Vaccine strain 4/91 of IBV and amplified the S1 gene using RT-PCR and then cloned the S1 glycoprotein gene of infectious bronchitis virus into a cloning and expression vector. Results and Conclusion: The result confirmed that S1 glycoprotein gene was expressed and amplified. The results also showed that a protein about 33 KDa was expressed. Additionally, western blot assay confirmed the expression of S1 protein.
Keywords: infectious bronchitis virus, S1 glycoprotein, cloning, E.coli

616. Occult Hepatitis B among Healthy Blood Donors

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Background: HBsAg detection is routine for screening blood donors in Iran. An HBsAg negative donation is generally considered safe. In this study we aimed to determine the presence of anti-hepatitis B core (anti-HBc) and HBV genome among Iranian healthy blood donors which have occult hepatitis B (OBI). Materials and Methods: 300 serum samples negative for both HBsAg andanti-HCV collected from healthy blood donors were tested for the presence of anti HBc antibody. All samples positive for anti-HBc antibody then were investigated for determination of anti-HBc titre by enzyme immunoassay (EIA). Each sample which tested positive for anti-HBc was also examined for the presence of HBV-DNA by PCR. Results: Of the 300 samples tested, 25 (8%) blood samples were found to be positive for anti- HBc. HBV DNA was not detected among all of anti-HBc positive specimens. Conclusion: The prevalence of OBI among HBsAg negative blood donors is variable according to the level of HBV endemicity, and to the assays employed in routine serological or NAT screening. Some investigations have shown OBI among Middle Eastern healthy blood donors. On the other hand, special HBV mutations can render HBsAg undetectable by conventional Eliza technique so further studies with more sensitive techniques such as nested PCR or Real time PCR is suggested to ensure about existence of OBI among healthy blood donors.
Keywords: HBsAg, Real time PCR, nested PCR, OBI

617. Seroprevalence of Hepatitis B in Schools in the Port Of Astara of Iran

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Background: HBV is a member of the hepadnavirus family. The envelope contains a protein called the surface antigen (HBsAg), which is important for laboratory diagnosis and immunization. In addition to HBsAg, there are 2 other important antigens: the core antigen (HBeAg), both of which are located p thant antigens: the, core antigen (HBcAg) and the an Important indicator of transmissibilit. The most important laboratory test for the detection of HBV infection is the immunoassay for HBsAg. In This research paper we did Some investigation for revealing The expansion of HBS , HBe and HBc Antibodies in order to reveal The existance Hepatit type B and to study the seroepidemiologic in students over Astara schools. Materials and Methods: In This project our samples are chosen among 7767 students in elementary schools High schools and pre universities High schools and pre universities. They form our statistical population. 480 persons are chosen for random sample. ELISA was used in this study. Results: The questionnaires are given and serological tests were done and we reached to the following conclusions by gathering the information : 15 out of 480 had positive HBs Ag , 18 had positive HBc and 11 had positive HBe Ag with percentage of 3.1% , 3.75% and 2.3% our sample population were positive. Conclusion: We found that the geographical condition (city / country) has important effect in Spreading The disease. Sex and age (From 12 to 18) don't have any important effect. The amount of information about HBV, The anticedant of Liver illness and HBV in parents has serious effect in spreading of HBV in These students. So it is proposed that the information about the disease must be spread at schools and compulsory Vaccination Project must be done at schools in order to prevent from the disease among students.
Keywords: Seroepidemiology, Hepatitis B, students, HBs Antigene

618. Seroprevalence of Human T-cell Lymphotropic Virus type 1,2 Infection (HTLV1,2) inBloodDonorsofEastGilanProvince of Iran
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Background: Human T-cell lymphotropic virus (HTLV) belongs to the family Retroviride. It is known pathogen of Adult T-cell leukemia/lymphoma (ATLL), HTLV-1 Associated Myelopathy/tropical spastic paraparesis HAM/TSP.According to the fact that khorasan province is one of endomic regions for this virus and on the other hand it's outbreak in other provinces is possible, we tried to study its occurrence rate amongst blood donors in Eastern Guilan region. Materials and Methods: In this descriptive-analytic study, 25348 serum of blood donors including 24355 males and 993 females were investigated .Their serum were Anti HTLV screened by using ELISA. positive cases in primary screening tests were tested again in two fold method (pilot and cord of blood bag) under ELISA test . Positive case were validated by western blot. Results: According to the fact that the number of female donors were greatly lower than males, Anti-HTLV occurrence with 47 positive cases was 0.18 %, in primary test, it was 0.098% in secondary test with 25 positive cases and 0.043 % in validation test with 11 positive cases, where all of them were males. Conclusion: Awareness rate of studied cases about this virus and its transformation method was very low and approximately 0.41 % .In present study, relation between age and infection rate was proved so that HTLV frequency rate will increase with age .On the other hand, relation between contaminated blood and its products injection and infection rate was observed. Among 11 positive cases of validation test, 5 cases had a blood injection history in previous years. Thus, general awareness, donated bloods screening and testing infected people especially infected mothers in endemic regions are among preventive method against propagation of this virus.
Keyword: Retrovirus , HTLV, serology, blood donors , Eastern Guilan

IMMUNOPARASITOLOGY

Oral Presentation

619. The Role of Regulatory T Cells in Symptomatic and Asymptomatic Human Cutaneous Leishmaniasis in an Endemic Area of Iran
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Background: Cutaneous Leishmaniasis (CL), caused by either *Leishmania major*or*Leishmania tropica*infections, is a parasitic ailment and a public health concern in Iran. CL clinical features are manifested as self-healing lesions or asymptomatic infections, detected by Leishmanin Skin Test (LST).Aside from the established roles of Th1 and Th2immune responsesto CL, the roles of Th17 and T regulatory (T_{reg}) cells have recently been emerging. The aim of this study was to investigate the role of T_{reg}cells compared toother CD4+ cellsubsets (Th1, Th2& Th17)and their secreting cytokines,in symptomatic and asymptomatic CL casesin an *L. major*infected area. Materials and Methods: Following LST screening, PBMCs from 3 groups of volunteers (all $n \geq 15$: with healed CL (LST+), asymptomatic(LST+) and healthy(LST-) individuals) residing in endemic villages around Damghanwere prepared. Using flow cytometry, the PMBCs stimulated with soluble Leishmania antigen (SLA) or PMA/ionomycin were used to detect the intercellular cytokines (IL-4, IL-10, IL-17 and IFN-γ). Peripheral blood was also used to determine the percentage of T_{reg}(CD4+CD25+FOXP3+) cells by flow cytometry. Results: Samples from the LST+ groupshadrelatively higher IFN-γproductionwhen stimulated with SLA; howeverthe IL-4 and IL-17 productions were not meaningfully different. The members of the healed group had lower IL-10 productions compared to the control group.Thepercentage of theT_{reg} cells in the CD4+ population ofthe asymptomatic volunteers was the lowest among the tested groups. Conclusion: To our knowledge, this is the first comparative analysis ofT_{reg}cell presence with respect to T_{eff} responses in a human CL infected area. The main difference observed between the LST+ groups was the significantly lower proportion of the T_{reg} cells in the asymptomatic cases which seems to be a key factor in prevention of the lesion development.
Keywords: CL, LST,T_{reg}cells

620. Production of polyclonal antibody against SAG1 (P30) protein of *Toxoplasma gondii*
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Background: *Toxoplasma gondii* is an intracellular protozoan parasite that could infect a wide range of warm-blood animals. Human as an intermediate host is infected by ingesting infectious oocyte, tissue cysts or through placenta in pregnant women. 1/3 of people in the world are infected by this parasite approximately. The aim of this study was produce polyclonal antibody against a synthetic peptide derived from SAG1 (P30) protein of *Toxoplasma gondiiin* in order to use in different applications. Materials and Methods: A synthetic peptide from SAG1 protein was designed and conjugated to Keyhole Limpet Hemocyanin (KLH) and used for immunization of a white New Zealand rabbit. The produced antibody was purified by affinity chromatography prepared by coupling immunogenic peptide to CNBr-activated sepharose 4B and the quality of purified antibody was evaluated by SDS-PAGE. Its specific interaction with immunizing peptide was determined by ELISA. Immunoreactivity of the antibody was also tested by Western blotting using *Toxoplasma gondii* RH strain cell lysate. Results: The results showed that produced antibody has desired purity and excellent reactivity with immunizing peptide in SDS-PAGE and ELISA, respectively. Also could detect a band about of 30 kDa corresponding to SAG1 protein of *Toxoplasma gondii*. Conclusion: This antibody might be used as a tool in diagnostic, therapeutic and infection inhibition studies applications.
Keywords: *Toxoplasma gondii*, SAG1 (P30), Antibody

621. Isolation and Characterization of Recombinant Single Chain Fragment Variable Antibodies against *Toxoplasma Gondii* Surface Antigen
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Background: *Toxoplasma gondii*is an obligate, intracellularparasite, which is widely spread in the world. Theparasite is able to infect all warm-blooded hosts including human and farm animals.The infection in humans often occurs after the ingestion ofraw or undercooked meat containing tissue cysts, althoughthe ingestion of food or water contaminated with oocystsexcreted by infected cats is another major transmissionroute.Several methods have been applied to detect this parasite in contaminated foods. The object of present study was to isolate recombinant monoclonal antibodies against this important parasitethat could be used in future in diagnostic tests. Materials and Methods: The purified *Toxoplasma gondii* surface antigen was coated to immunotubes and used as a target for selection of antibodies from the Tomlinson I and J phage display libraries of single chain (scFv) antibodies. Clones able to recognizeantigen were isolated by three rounds of binding, elution and amplification. scFv antibodies chosen from the resulting panel, their specificity were confirmed by ELISA, dot blotting and western blotting. Results: The study showed that several recombinant antibodies were able to bind specifically to antigen with high affinity. Conclusion: Therefore these isolated soluble single chain antibodies are good candidates to apply as monoclonal recombinant antibodies in diagnostic kits for detection *Toxoplasma gondii* in contaminated samples.
Keywords: *Toxoplasma Gondii*, scFv, ELISA, dot blotting, western blotting

622. IL-1β (-511 T/C) Gene Polymorphism not IL-1β (+3953) and LT-α (+252 A/G) Gene Variants Confers Susceptibility to Visceral Leishmaniasis

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Background: Lymphotoxin-α (LT-α) and interleukin-1beta (IL-1β) are proinflamatory cytokines playing important roles in immunity against *Leishmania* infection and the outcome of the disease. As cytokine productions are under the genetic control, this study tried to find any probable relationship between these cytokine gene polymorphisms and the susceptibility to visceral leishmaniasis (VL) in Iranian pediatric patients. Materials and Methods: Ninety five pediatric patients involved with visceral leishmaniasis and 128 non-relative healthy people, from the same area as the patients, were genotyped for LT-α (+252 A/G) and IL-1β (+3953 T/C and -511T/C) gene polymorphisms using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: There was not found any significant differences in allele and genotype frequencies of LT-α (+252 A/G) and IL-1β (+3953) among the study groups. However, the frequency of IL-1β -511TT genotype was higher in the controls (P=0.0004) while the frequency of IL-1β -511CC genotype and C allele were higher in the patients (P=0.008 and P=0.00006, respectively). Furthermore, IL-1β CC (-511/+3953) haplotype was more frequent in VL patients compared with the controls (P=0.0002) and the distribution of TT haplotype was higher in controls compared with the patients (P=0.003). Conclusion: Based on the results IL-1β -511C allele, CC genotype and CC (-511/+3953) haplotype could be considered as the susceptibility factors for visceral leishmaniasis while IL-1β -511TT genotype, T allele and TT haplotype (-511/+3953) might be counted as the influential factors for resistance to the disease.
Keywords: LT-α, IL-1β, *Leishmania*, PCR-RFLP

Poster Discussion Presentation

623. Serum of Individual Patients with Visceral Leishmaniasis Recognizes the Recombinant Protein of LCR1

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Background: *Leishmania infantum* is the causative agent of visceral leishmaniasis (VL). LCR1 is an immunogenic protein, discovered in *L. infantum chagasi*. There is a few data available concerning the human immune response to LCR1. The reactivity of pooled sera from visceral leishmaniasis patients has been already reported. The aim of this study was to characterize the individual immune response against LCR1 in Iranian VL-patients. Materials and Methods: Sera were obtained from six Iranian VL-patients and two healthy controls without any history of VL. Western blotting was carried out by reacting these sera against recombinant LCR1 protein. Host *E. coli* lacking *lcr1*-containing plasmid, and soluble *leishmania* antigen were used as negative and positive controls, respectively. Results: Serum of each VL patient recognizes recombinant LCR-1 (a single band of ~40 kDa) and soluble *leishmania* antigen (several bands), while serum from healthy control individuals did not recognize any of these bands. These data show specificity of reaction of the sera against the recombinant LCR1. Conclusion: Our results showed presence of anti-LCR1 antibodies in all VL-patients but absence of these antibodies in healthy individuals. These data support the possible use of recombinant LCR1 as a diagnostics molecule or a vaccine candidate for leishmaniasis.
Keywords: *Leishmania infantum*, *Leishmania infantum chagasi*, immunoblotting, recombinant protein

624. *Leishmania infantum* Specific IgG1 and IgG2 Immunodetection Patterns by Western Blotting Method in Asymptomatic and Symptomatic Dogs

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Background:*Leishmania infantum* is causative agent of both human and canine leishmaniasis in Mediterranean countries. The immune responses to *L.infantum* in the dogs are included spectrum from humoral to cellular forms. This study aimed to determine immuno-patterns of *Leishmania* specific IgG-1 and IgG-2 antibodies responses in sera of symptomatic and asymptomatic dogs.Materials and Methods: The study was carried out on blood samples obtained from 9 dogs in an endemic area (Baft district in Kerman province). Four dogs were classified as asymptomatic cases and 5 dogs presented as symptomatic, if only two major clinical signs were noted in the exploration (lymph node enlargement and weight loss). This study carried out by western blotting technique on L-infantum extracted protein that exposure to 1/100 dogs diluted sera and specific bands revealed by chemilumincense method. Results: Western blotting results showed that IgG-1 antibody from asymptomatic dogs reacted with a variety of *L. infantum* promastigotes antigens including 15, 20, 25, 50 and 60 kDa bands and IgG-2 antibody from these dogs reacted with 15-20, 20, 40, 50 and 70 kDa antigens. But in symptomatic dogs, IgG-1 antibody reacted with following bands including 20, 25, 30, 60, 70 and 85 kDa and IgG-2 reacted with 15, 15-20, 20, 60, 70, and 85 kDa bands. Conclusion: In 5 cases, symptomatic and asymptomatic dogs showed a predominant IgG1 and IgG2 specific response against 20, 15-20 and 25 kDa bands.These bands in both groups were strong immunogen which stimulate antibody production.Symptomatic animals are characterized by an clearly recognition of 85 kDa band by both IgG1 and IgG2 antibodies. This band was not detected in the resistant dogs. 85 kDa band may be an candidate for determination of susceptibility or resistance of dogs to disease and 20 kDa antigen suggested for evaluation of rising immune system against infection.
Keywords: *Leishmania infantum*, IgG1, IgG2, Western Blott

625. Survay of Diffrence In Immungencity of Wild and Attenuted (H-Line) Types of *Leishmania Infantum* in Dogs

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Background: *Leishmania infantum* is a protozoan parasite causing severe visceral diseases in human and dog. The latter is the most important natural reservoir and the main target of control programs. An attenuated line of *L.infantum* (H-line) has been established by promastigotes culturing in vitro under gentamicin pressure as a vaccine candidate. In the present study, canine anti- leishmanial IgG2 antibodies responses to wild type (W-Type) and H-line proteins was evaluated in dogs sera. Materials and Methods: The study was carried out on sera obtained from 10 dogs in

an endemic area (Baft, Kerman) including vaccinated dogs which stimulated by H-line (n=4), experimentally (intravenous injection of W-Type) infected dogs (n=3) and 3 other naturally infected dogs. This study carried out by western blotting technique on H and W types extracted proteins that exposure to 1/100 dogs diluted sera. Specific bands revealed by chemiluminescence method. Results: In naturally infected Dogs, H-line reacting bands were including; 15,20, 60,70 kDa and W-line reacting bands including; 50 and 70 kDa antigens. Sera of experimentally infected dogs showed reactions in 20,30,40,50.60,70 kDa bands for H-line and 10,15,20,30,40,50 and 60 for W-type. In vaccinated group, following bands including; 20-25,50,60,70,85,100 kDa antigens were revealed reactions for H-line parasites while W-type reacting bands were 10,15,20,25,70 and 100 kDa.Conclusion: IgG2 reaction with 20 kDa revealed a more tendency for W-type in all groups. In H-line, 25 kDa band didn't show any reaction with IgG2 in all samples, while W-type positive reaction observed in vaccinated group. So more tendency in IgG2 (as representative of cellular immunity) reaction with W-type may be H-line based vaccine disadvantage. IgG2 reacted with 70 kDa band in H-line in three groups, and W-type recognition was seen in vaccinated group. Therefore this band considered as an immunogen can stimulate cellular immunity and candidated for vaccine development.

Keywords: H-Line, *Leishmania Infantum*, IgG2

626. Characterization of Vitellin from *Ripicephalus (Boophilus) Annulatus* Tick Larvae

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Background: *Rhipicephalus (Boophilus) annulatus* is an important one host tick in the Mediterranean climates and Iran . This tick transmits the protozoans *Babesia bigemina* and *B. bovis* to cattle. It also transmits bacterium *Anaplasma marginale* to cattle. Today vaccination by tick proteins is one of the methods for Control and prevention against Tick infestations. Thus, the characterization and identification of different tick proteins is necessary. Vitellogenin is the precursor of Vitellin that is produced in mid gut cells and fat body in ticks, exported to tick hemolymph, and then accumulates in oocysts as vitellin. Vitellin is the main yolk component of tick eggs; this phosphorylated heme-lipoglycoprotein is the main source of amino acids and energy as well as hem for the development of tick embryo and unfed larva. Furthermore, this protein acts as hormones carriers related to emberiogenesis. Here we characterized the vitellin protein profiles of *Rhipicephalus (Boophilus) annulatus* larvae using proteomic approaches and also, its immunogenesity was assayed. Materials and Methods: The ticks were cultured in laboratory conditions and their larvae were obtained for preparing the protein extract. Protein profiles of larvae extract were detected using one and two dimentional electrophoresis. Then immunoblot assay was used for detection of protein interactions with hyper immune serum. Results: In one- dimentional method, 48, 70, 100, 130 and 250 KDa protein bands, positively reacted with hyperimmune serum. In two- dimentional method some proteins appeared; of which 10 proteins were selected based on their abundance and Immunogenisity, for mass spectrometry(MALDI TOF TOF). Conclusion: Six of them were positive in immunblotting Protein analysis by MALDI-TOF mass spectrometry analysis showed that amino acids sequence of four immunogenic proteins with molecullar weight of 45, 85 and 97 KDa, were identical with Rhipicephalus (*Boophilus) annulatus* vitelogenin. Furthermore amino acids sequence of protein with molecular weight of 38 KDa was identical with *Dermacentor variabilis* vitellogenin. It seems that obtaining more knowledge about tick proteins and their characterization can be useful for the development of anti tick vaccines.

Keywords: MALDI TOF TOF, *Rhipicephalus*, Immunogenisity, immunblotting Protein analysis

627. Cytokine Production by Infected Macrophage with the Gentamicin Attenuated *Leishmania mexicana*

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Background: *Leishmania* is an obligatory intracellular proteozan parasite. Here, we focused on activation of macrophage by gemtamicin-attenuated *Leishmania mexicana* (*L. mexicana* H-line). We found that *L. mexicana* H-line was phagocytosed by macrophages, but unable to survive whereas, unselected parasites survived and multiplied within infected macrophages. The levels of IL-10 and GM-CSF in the supernatant of macrophages infected with attenuated line or wild-type (WT) parasites were measured. It has been reported that IL-10 suppressed macrophage activation and preventing parasite killing. Moreover, macrophages have been activated with GM-CSF and increased their presenting antigen and T-cell activation. Materials and Methods: Peritoneal macrophages of BALB/c mice were exposed with the stationary phase of promastigotes of *L. mexicana* H-line or *L. me*xicana WT. The percentage of infected macrophages and the number of amastigotes per 100 infected macrophages after 6, 24, 48 hour were detected. The supernatants were also collected and the levels of IL-10 and GM-CSF were measured. Results: The percentage of infected macrophages with the attenuated line after 6 hour was 50%, but decreased to 12% after 48 hours post infection. The number of amastigotes of *L. mexicana* H-line per 100 infected macrophages was 120 after 6 hours decreased to 14 after 48 hours. The levels of IL-10 and GM-CSF in the supernatant of macrophage infected with attenuated line or WT were measuredusing Enzyme-linked immunosorbent (ELISA) assay. Conclusion: BALB/c macrophages uptake *L.mexicana* H-line but the parasites unable to survive within infected macrophages. The activated macrophages with the attenuated cell line induced high level of GM-CSF and low level IL-10.

Keywords: *Leishmania Mexicana*, GM-CSF, IL-10, BALB/c macrophages

628. Time Course Study of Peritoneal Macrophage Responses from BALB/c and C57BL/6 Mice to Leishmania Major Infection

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Background: According to experimental evidences, BALB/c and C57BL/6 mice create different immune response to many stimulations and infections like leishmaniasis and represent two different prototype of immune responses as Th2 or Th1 respectively. However the outcome of an immune response is dependent to the way of shaping immune responses at first hours of immunostimulation.In this study, we show the difference in immune response of macrophages from BALB/c and C57BL/6 mice to L.major at first hours post infection. Materials and Methods: Peritoneal macrophages from BALB/c and C57BL/6 mice was prepared after thioglycolate stimulation. After separation of non-adhesive cells, macrophages were divided at 10^6 cell/well of a 6 well plates as control and test groups. Test groups were infected with 3-5x10 ^{6} L.major. After 3 hours incubation cells were washed to exclusion of free leishmanias. At 0h, 3h,12h,24h,48h and 72h post infection, supernatant were collected for cytokine measurement (IL-4,IL-17,IL-10,IFN-γ and TGF-β) and IDO activity and nitric oxide assay. Results: Cytokine analysis showed that BALB/c and C57BL/6 macrophages have different baseline of cytokine production and after L.major infection create a different pattern of cytokine production during study times.C57BL/6 mice produced more IL-17 and BALB/c mice produced more TGF-β significantly ($p<0.05$). BALB/c mice produced more IL-10 at first hours of infection but after 72h both strain IL-10 production was in the same range. Kynurenine and Nitric oxide amount in the supernatant of infected macrophages from BALB/c and C57BL/6 mice were different significantly and showed different pattern during time course study.Conclusion: According to obtained results, macrophages from BALB/c and C57BL/6 mice respond differently to L.major infection and show different cytokine pattern in time course study. Understanding the early immunological events in leishmania infection, will be helpful for determine how different immune outcomes happen.

Keywords: C57BL/6, BALB/c, Leishmania Major

629. The Effects of Hydatid Fluid Antigens of Echinococcos Granulosus on the Expression Level of Ovine Toll-Like Receptor 2 And 4 (TLR2 and TLR4) in Peripheral Blood Mononuclear Cells (PBMCs)

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Background: Hydatidosis is one of the most important zoonosis disease and represents a major public health and economic burden in many countries. The disease causes by larval stage of Echinococcos Granolosus worm in human and domesticated animals. Immune responses against hydatidosis comprises of various mechanisms of innate and adaptive immunity. In recent years the importance of innate immune responses and in particular, pattern recognition receptors (PRRs) has been recognized as an essential mechanisms for development of an effective immune response. PRRs are the main sensors of pathogen and danger signals in innate immunity. Toll like receptors (TLRs) are the most studied and best characterized PPRs which are responsible for sensing pathogen associated molecular patterns(PAMPs). The role of TLRs in the molecular mechanisms underlying the pathogenesis and immunity in helminths infection has not been clearly defined. Materials and Methods: In this study we aimed on the expression levels of two important ovine TLRs transcripts, namely TLR2 and TLR4 in a culture of ovine lymphocytes exposed to different concentrations of hydatid fluid antigens in a time point experiments. Blood samples were taken from healthy young lambs. After isolation of peripheral blood mononuclear cells (PBMC), the cells were cultured with different concentrations (50µg/ml & 100µg/ml) of hydatid fluid antigens (antigens were extracted and concentrated according to the standard protocols), in different time points. Then, total RNA was isolated from the cell pellets and cDNA was synthesized using Oligo dT primers. Afterwards, the primer pairs for TLR2, TLR4 as target genes and GAPDH as housekeeping and calibrator gene were optimized in a gradient RT-PCR experiment. Subsequently, qPCR analysis was set up to quantify and compare the relative expression levels of TLR2 and TLR4 transcripts in PBMC of antigen treated versus control(untreated) samples. Results: Gene expression analysis showed down-regulation of TLR2 and TLR4 transcripts in treated as compared to the untreated control group which might indicate the presence of Immunosuppressive components in Hydatid fluid antigens.Conclusion: the results presented in this study, can shed more lights to the insight mechanisms behind the molecular immuopathogenesis of hydatidosis which might eventually pave the way for designing novel and more effective therapeutic as well as preventive strategies.
Keywords: TLR2,TLR4, RT-PCR

630. Hydatid Cysts Versus Ovine Innate Immunity: Can antigens from Fertile Protoscoleces Alter Sensor Molecules in Immune Cells?

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Background: Hydatidosis is one of the most important zoonotic diseases, caused by larval stage of *Echinococcus granulosus*, in humans and many domestic and wild mammals. Hydatid cyst is composed of different parts such as cyst wall, cyst fluid and protoscolex; each part contains specific immunogenic molecules, and some of the molecules are common between hydatid cyst and protoscoleces. These immunogenic molecules are able to stimulate/inhibit the immune system through sensor molecules like surface receptors of immune cells, such as TLRs etc. There is countless information on Hydatidosis, but the information on the effect of antigens from fertile protoscolex antigens on sensor molecules in ruminant immune cells is little.The main goal of this study was to examine the effect of protoscolex antigens on the gene expression of key immunological sensor molecules, TLR2 and TLR4 in peripheral blood mononuclear cells (PBMCs) of healthy ovine neonates; and eventually to get insight into how this dangerously zoonotic parasite evades the immune system and growth in the host. Materials and Methods: During a period of 6 months collection of hydatid cysts from liver and lugs of infested sheep in Mahshad slaughter house, the cysts were transferred to the laboratory and protoscoleces were isolated with sterile procedure. The PBMCs were isolated from blood samples of 20 healthy ovine neonates (age 5 -7 days). The ability of the extracted antigens to stimulate/inhibit the sensor molecules was investigated in cell culture system using primary immune cells, PBMCs. In the cell culture media the isolated PBMC were then exposed to 0µg/ml (control group) and 5µg/ml (treatment group) protoscolex antigens for 2 and 6 hours. The RNA were then extracted from treated and control PBMCs. The extracted RNAs were subjected for cDNA synthesis. The synthesized cDNAs were used for conventional PCR and real-time qPCR using primers specific for TLR2 and TLR4. Results: Analyses of the results from qPCR showed that protoscolices antigens significantly increased ($P<0.05$) both TLR2 and TLR4 transcripts in PBMCs of ovine neonates. Moreover, though not significant, the effect on these TLRs tended to be less pronounced at 6 h post incubation compared to 2 h. Conclusion: Depending on the host and the status of the host, the increased in expressions of the TLR2 and 4 on ovine immune cells strongly supports the idea of hydatid cyst larvae might use this strategy for their growth when exposed to the immune cells. Further experimental works on the mechanism and outcome of the effects of hydatid cyst antigens is on progress to better understand the role of molecular aspects of innate immunity on hydatid cyst-parasite interactions.
Keywords: protoscolex antigens, hydatid cyst, ovine neonate, toll-like receptors, peripheral blood mononuclear cells

Poster Presentation

631. Tracking the Proliferation History of Purified $CD4^+/CD8^+$ T Lymphocytes in Cutaneous Leishmaniasis Using Flow Cytometry

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Background: Determination of the function of T cells including proliferation and cytokine production *in vitro* is critical in the study of effector mechanisms against infections. In leishmaniasis, most of the data is drawn from peripheral blood mononuclear cells (PBMCs) culture without separation of T cell subtypes which makes it difficult to judge the role of Th1/Th2 CD4+ cells and CD8+ T cells. Technique described here uses the intracellular fluorescent label carboxyfluorescein diacetate succinimidyl ester (CFSE) for monitoring the proliferation of purified T lymphocyte subtypes. Materials and Methods: Blood samples were collected from volunteers with history of cutaneous leishmaniasis (CL) and healthy controls and PBMCs were isolated by using Ficoll-Hypaque centrifugation. CD4+ and CD8+ T lymphocytes and CD14+CD16- monocytes were purified using cocktail mAbs and magnetic nanoparticles. Purified T lymphocytes were labeled with CFSE solution and co-cultured with 1:10 autologous monocytes following mitomycin C treatment. Cells were stimulated with PHA, soluble *Leishmania* antigen (SLA), or live promastigotes of *L. major* for 7 days, 37° C with 5% CO2, then harvested and analyzed using flow cytometry and green fluorescence was collected. Results: It was possible to separately monitor five consecutive divisions (daughter cells) for each purified T cells. Stimulation of CD4+/CD8+ T lymphocytes from CL subjects with SLA showed a significant difference in proliferation comparing with unstimulated cells ($P< 0.05$). The significant difference in the percentages of divided CD4+ T cells was revealed at each division for every subject. In CD8+ T cells, significant proliferation was evident later. The mean number of divisions in SLA stimulated was significantly greater than in live *L. major* stimulated CD4+/CD8+ T cells ($P=0.007$ / $P=0.012$, respectively).
Conclusion: The proliferation history, number of divisions and percentage of divided cells might be calculated separately. Unlike other usual stains, cells remained alive following CFSE staining and simultaneous functional analysis is possible.
Keywords: SLA, CFSE, $CD4^+/CD8^+$

632. *In Vitro* Anti-Leishmanial Activity of Extracts of *Artemisia Absinthium, Vitex Agnus-Castus, Calendula Officinalis* and *Phytolocca Americana* on *Leishmania Major* Amostigotes, in the Host Macrophages

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Background: Leishmaniasis is a major public health problem caused by species of leishmania, a unicellular kinetoplastid protozoan flagellate, which is transmitted through the bit of female phlebotomine sandfly Spp.The clinical manifestation range from simple cutaneous lesion (CL) to progressive disseminated visceral (VL) fatal disease. Cutaneous leishmaniasis produces skin lesions on the face, arms and legs, and is often self healing. When leishmania first enters the human body, it is in the promastigote form. Promastigotes are engulfed by macrophages but are resistant to proteolysis and degradation in the phagosome. Once inside the macrophage, the organism is termed an amastigote. By continuing to live inside the macrophage, leishmania effectively avoids the humoral branch of the immune system. During each of the steps described, the protozoa evade and at times manipulate the human immune system and avoid digestion. As there is no vaccine, drug treatment (Pentamidine, Amphotericin B, Miltefosine ,…) is the only way to tackle leishmaniasis.But many of the drugs are toxic and the development of resistant parasites to most drugs has been reported. In this study the Anti-leishmanial activity of methanolic extract of 4 plant species (*Artemisia absinthium*, *Vitex agnus-castus*, *Calendula officinalis* and *Pitolocca Americana*) used in Iranian traditional medicine, against Leishmania major amostigot was evaluated. Macrophage were absorbed in 12-well plates and allowed to adhere for 4 hours at 37°C in 5% CO_2. Plate was incubated overnight in RPMI 1640 media. Adherent macroghages were infected with *L.major* promastigotes at a parasite/macrophage ratio of 7:1, incubated in 37°C for 4 hour and free promastigotes removed by washing. Then Treatment of infected macrophages was done by the plant extracts. 2 wells had no extract as a negative control and miltefosine was used for positive control.After 3 days monolayers were washed with PBS, Fixed in methanol and stained with 10% Gimsa. The number of amostigotes were determined by counting at least 100 macrophages in duplicate cultures, and results expressed as infection rate (IR) and parasite survival (PS). The results indicated that some extractions can inhibit the growth of Amostigotes in the host macrophages and PS values for *Aretemisiaabsinthium*, *Phytolacca Americana*, *Vitex agnus-castus* and *calendula officinalis*, respectively, are 33, 43, 45 and 49 percent.

Keywords: *Leishmania major*; plant extract; macrophage cells; Parasite survival

633. TGF-B, IL-12, and IL-1 mRNA Expression in Neutrophils in Response to *Leishmania major*

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Background: Leishmaniasis, which is caused by infection with protozoan parasites of the genus *Leishmania*, is a severe health problem in many regions of the world including Iran. It has been well established that the infection outcome is determined by the balance of Th1 and Th2 cytokines produced. Initial cytokine response in the microenvironment at the early stage of infection essentially determines to which direction (Th1 or Th2) the subsequent immune response will be developed. Neutrophils are one of the earliest cells to arrive at the site of an infection. Neutrophils release cytokines that may influence the development of the subsequent immune response against the parasite. Little is known about cytokines produced by neutrophils particularly human neutrophils in response to Leishmania. In this study, we investigated TGF-B, IL-12, and IL-1 mRNA expressions from neutrophils stimulated with *Leishmania (L.) major* promastigotes. Materials and Methods: Neutrophils were isolated from Heparinized peripheral blood collected from 30 healthy adult volunteers using Histopaque 1119. The cells were coincubated with *L. major* promastigotes for 1h. Total RNA was extracted and cDNA was synthesised using through a reverse transcription reaction. Quantitative mRNA analyses were performed on the Corbett system. B-actin and TATA binding protein were used in each sample as reference gene for normalization. Unstimulated neutrophils were chosen as the calibrator. Specific gene expression was quantitated using $2^{-\Delta\Delta CT}$ method. Results: There were no significant changes in mRNA expression of *TGFB, IL12p40, IL12 p35* gens between unstimulated and *Leishmania*-stimulated neutrophils. However, neutrophils express significantly higher IL1 after stimulation with *Leishmania* ($p<0.001$). The mean fold increase for IL-1 mRNA was 28. Conclusion: The results showed that high levels of IL-1 mRNA were expressed in neutrophils after exposure to *Leishmania major* while TGF-B and IL-12 remains unchanged.

Keywords: IL-1, *Leishmania major*, TGF-B, IL-12

634. Immunization of C57BL/6 Mice with GRA2 Combined with MPL Conferred Short-term, but not Long-term, Immune Protection against *Toxoplasma gondii*

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Background: We previously reported that CBA/J mice immunized with the recombinant GRA2 antigen of *Toxoplasmagondii* were protected against infection with this ubiquitous protozoa parasite. Materials and Methods: To evaluate the consistency and durability of the protection conferred by the candidate GRA2vaccine, another susceptible strain of mice (C57BL6) were immunized with GRA2 that was formulated in monophosphorryl lipid A (MPL) adjuvant. Results: The immunization induced strong antibody responses of the predominantly IgG1 subclass. In addition, a high amount of IFN-γ was produced in spleen cell cultures of the immunized mice restimulated with Toxoplasma lysate antigen, as expected. Substantial amounts of the IL-2and IL-10 interleukins were also produced in the spleen cell cultures of the immunized mice, though they were below the significant level.Mice immunized with GRA2 had significantly fewer brain cysts than those in the adjuvant group, when the challenge infection was performed three weeks after the immunization.In contrast, the brain cysts reduction was not significant when the immunized mice were infected four months after the immunization. Conclusion: Taken together, we conclude that the brain cysts in GRA2 vaccinated animals were significantly lower than the control groups ($p <0.01$), though the potency and longevity of this antigen as a standalone vaccine may be variable in distinct genetic backgrounds. This observation further emphasizes the utility of GRA2 for incorporation into a multi-antigenic vaccineagainst *T. gondii*.

Keywords: Immunization, GRA2, *Toxoplasma gondii*, MPL, Monophosphorryl lipid A

635. Serological Evaluation of Toxoplasma Gondi in Women of Sari, Northen of Iran

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Background: Toxoplasma gondii is an obligate intracellular protozoan parasite that caused common parasitic infections in humans and other warm-blooded animals. Thisinfection has different clinical manifestations;itcauses asymptomatic chronic infection in adults,fatal illness in immunosuppressive persons and abortion in pregnant women. The aim of this study was determination of IgG and IgM antibodies against Toxoplasma in Sari women in 2011. Materials and Methods: Toxoplasma serum prevalence was determined by ELISA method in women referred to Tooba clinic in sari. In this cross sectional study, 620 women were blood sampling randomly after register of the personal information's. Then the toxoplasma IgG and IgM antibodies were examined by ELISA and the titer 1/30 and upper ratio was considered seropositive for Toxoplasmosis. Results: The mean age of all participants was 27.72±20.59 years. Seropositivity was 350 out of 620 (56.45%) and 6 out of 620 (0.95%) for IgG and IgM respectively. The mean of IgG and IgM antibody titer were 55.97%, %0.39 respectively. We found no significant relation between serum level of toxoplasma antibodies and age. Conclusions:According to high prevalence of toxoplasma and importance of toxoplasmosis in women, the obtained rate of prevalence indicated that about 56% of women at risk for toxoplasmosis due to lack of serum antibodies. In addition, it probably causes spontaneous abortion and fetal abnormally.Therefore it seems necessary to health education, increasing awareness of people and other control measures.

Keywords: Toxoplasma gondii, ELISA, Sari

636. A Study on Serum IgE and Eosinophilia in patients infected with giardiasis

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Background: Many studies have described the existence of allergic symptoms such as eosinophilia and elevated levels of IgE in patients with giardia infection. Due to discrepancy of the results of these studies, lack of recent studies showing the relationship between the Giaridsis and allergy among higher age groups, it seems essential to design and perform the currents study. Materials and Methods: Amongst patients who were infected with giardiasis and their infection was definitely diagnosed using stool exam 30 individuals were randomly selected as the case and 30 healthy individuals were also selected as the control group. Total IgE was measured using ELISA while CBC was count using Sysmax cell counter. Data were analysed using Logestic regression and Mann-whitney U test. Results: The mean age was 30.7years in case and 30.6 years in the control groups. More than 80% of participants in both groups were placed in urban areas. The mean CBC among the case and control groups was 6.69×10^3 and 7.45×10^3 respectively. The mean Hb was 13,37 and 13.74 among the case and the control groups respectively. The difference of mean absolute neutrophil count (ANC) between the case and the control groups was statistically significant (P<0. 01). The mean IgE of all patients in all ages was 134.±12 for case and 50.57± 66 for the control group. The odd ratio for IgE of case and control group was 2.7. Conclusion: The incidence of giardiasis is elevated amongst the higher ages compared to the previous rate. The strong relationship of IgE and giardiasis showed a prediction value for this antibody amongst the giardiasis patients so that with increasing the probability of giardiasis the level of IgE can be increased few times. This matter should be monitored in such patients particularly those with allergy.
Keywords: Giardiasis, IgE, Eosinophilia, allergy

637. Importance of Immunoglobulins (IgA and IgE) in Host Defense against *G.lamblia*

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Background: *Giardia lamblia* is one of the most important intestinal parasites that causes both acute and chronic diarrheal disease in human.Symptomatic giardiasis is also characterized by epigastricpain,nausea,vomiting and weight loss. Infection with *Giardia* spp.are usually self-limited in immunocompetentindividuals.Mucosal defenses against *Giardia* must act in the small intestine. Secretory antibodies against *Giardia* spp. Play a role in anti-giardial defense and clearance of parasite. IgE have been demonstrated in serum and milk,and these may contribute to *Giardia* elimination by means of direct cytotoxicity,complementmediated,lysis and opsonization. Material and Methods: Stool examination by two methods of salin-lugoland formalin-ether concentration was performed to find positive individuals for *Giardia lamblia*. Serum was collected from 50 positive samples of men,women and children.40 healthy men,women and children without any health complaints were enrolled in two study.Serum was obtained from both groups. IgA and IgE levels were measured by SRID and Elisa respectively. Results: Mean value of IgA in SG and CG was 309.26 mg/dl and 216.89 mg/dl respectively. Mean value of IgE in SG and CG was 167.34 IU/ml and 35.49 IU/ml respectively. Conclusion: Secretory antibodies of the IgA and IgMisotypes are attractive candidate for immune defense against *Giardia*,because they are secreted in large quantities into the intestinal lumen and their actions are antigen-spesific. The mechanisms by which IgA exerts its anti-giardial functions not well understood,but are likely to involve immune exclusion (e.g. immobilization or detachment of trophozoites from the intestinal epithelium or the mucoselayer)rather than direct killing.
IgE is monomeric antibody and plays an important role in allergy and is especially associated with type 1 hypersensitivity. IgE has also been implicated in immune system responses to most parasitic worms and important during protozoans infections. In our study we have found a significant difference in IgA and IgE in SG and CG. ($p<0.05$)
Keywords: IgA, IgE, *G.lamblia*

638. Evaluation of Immune Response Induced by DNA Vaccine Expressing LACK Gene against Cutaneous Leishmaniasis in Balb/c Mice

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Background: Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania* which, in the infected host are obligate intracellular parasite. Individuals who have recovered from clinical leishmaniasis develop strong immunity against reinfection. DNA vaccines are new type of vaccines that induce expressing protein mammal cells, DNA vaccines can with use one gene or several genes be stimulated cellular and humoral immune responses. Materials and Methods: We evaluated a DNA vaccine containing plasmid encoding the LACK gene of leishmania major (MHRO/IR/75/ER) immune responseand protective efficacy in comparison with control groups. We immunized BALB/c mice intramuscularly three times. For the detection of cytokines, splenocytes from immunized mice were cultured with different stimuli as described for the lymphocyte proliferation assay. Cell-free supernatants were collectedand assayed for IL-4, IFN-γ activity. The concentrations of cytokines were determined by ELISA kit.
Antigen-specific antibodies were measured by ELISA. In this study evaluated humoral response and IFN-γ values after immunization with pcLACK, and the challenge with *L.major*, which were significantly higher than control groups($P\leq0.05$). following immunization and the challenge infection. Results: IL-4 values were increased in the control groups, which were significantly higher than in the pcLACK following immunization and after the challenge with *Leishmania major*. The survival time of the immunized mice with pcLACK group was higher than the control groups. Conclusion: Then DNA vaccine pcLACK have been able to induce protection against infection with *Leishmania major* in mice.
Keywords: DNA vaccine, pcLACK, cutaneous leishmaniasis, Immune response

639. Immunogenic Property of the *Mesobuthus Eupeus* Venom

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Background: Scorpions and their venom constitute a hazard to human life and health. *Mesobuthus eupeus* is a member of Mesobuthus genus, *Buthidae* family. Scorpion venom is very complex mixture of molecules. Finding the antigenic and immunogenic properties of toxin helps to obtain an efficient antidote. Peptides with improved immunogenicity are the main candidates for producing vaccine in other fields. At this study we investigated the immunogenic property of the *M. oupeus* venom. Materials and Methods: Venom was obtained by milking the *M. eupeus* scorpions, followed by lyophilization. Polyclonal antibodies for *M. eupeus* venom were obtained from rabbits according to immunization schedule. The antisera were precipitated with ammonium sulfate. For obtaining specific antibodies CH-Sepharose 4B column was used. The column was prepared by conjugating 20 mgr of *M. eupeus* venom to 7 ml of activated CH-Sepharose 4B. 4 ml of (NH4)2SO4 precipitation-purified rabbit antisera were loaded to this column. after washing the column with 0.01 M PBS and PH 7.2, the antibodies were eluted with 0.1 M Glycine-Hcl, PH 2.5 and neutralized immediately with 1M Tris PH 9.2. Results: Ten mg of this affinity purified antibody conjugated with a

CH-Sepharose 4B column and 5 mg of *M. eupeus* venom was applied to it. The bound proteins were eluted as before. Crude venom and affinity purified fractions of venom were analyzed by SDS-PAGE. The crude venom has 10 detectable bands at the molecular weights of 140, 70, 50,33,27,22,18,14,10 and 5 kDa. Purified venom by Affinity column presented seven bands. 27kDa Band was the most noticeable and the 70, 14 and 10 kDa bands were absent. Conclusion: These results clearly demonstrate that most of the *M. eupeus* venom components are antigenic and immunogenic.

Keywords: *Mesobuthus eupeus*, venom, immunogenic properties

640. Association of Interferon-gamma (IFNγ) Gene Polymorphism with Visceral leishmaniasis in an Iranian Population

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Background: Visceral leishmaniasis (VL) is a parasitic disease caused by a protozoan of Leishmania genus and in Iran by Leishmania infantum. Cytokines have a major role in determining progression and severity of clinical manifestations in VL. Interferon-gamma (IFNG) is a type II interferon playing diverse roles in innate and adaptive immune systems. In murine model, it is generally accepted that Th1 type response is needed for control and protection against Leishmania infections, and interferon gamma (IFNG) secreted by Th1 cells, is the most potent macrophage (Mf)-activating cytokine leading to host resistance to infection with Leishmania parasites. According to the important role of IFNG in immunity against visceral leishmaniasis, this study was conducted to demonstrate the prevalence of genotypes on -179 G/A in promoter region of IFNG gene. Materials and Methods: This descriptive and cross-sectional study was done on 85 pateints with confirmed VL, 106 healthy seronegative controls and 99 seropositive controls. Salting out method was used to extract DNA and the amplification refractory mutation system (ARMS)-PCR procedure was used for detecting polymorphism at IFNG -179 G/A. Results: The frequency of IFNG GG, GA and AA genotypes among all subjects were 41.6%, 45.8% and 12.6% respectively. According to the results, -179 G/A was the dominant genotype among the groups. Statistical analysis of distribution of genotypes was performed using Chi-Square test and do not reveal a significant difference among groups (P =0.053). Conclusion: Polymorphisms of the IFNG gene do not appear to play a major role in the genetic predisposition to VL in Iranian population.

Keywords: IFNγ, Leishmania, VL

641. Comparison Animals and Human Sera as the Accessory Factor in the Sabin – Feldman Dye Test for Detection of Toxoplasmosis

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Background: *Toxoplasma gondii* is an obligate intracellular protozoan parasite, capable of infecting all mammals, including human. Although toxoplasmosis is asymptomatic in healthy individuals it can cause severe complication in pregnant women and immunocompromised patients. Toxoplasmosis is diagnosed by detection of the specific antibodies against parasite in serum using a number of serological methods, includingthe Sabin-Feldman dye test. This test measures principallyIgG antibodies and is both sensitive and specific and so called "gold standard" test. However it needs a source for accessory factor that is difficult to obtain. In this study we compared the sera of animals and human as the source of accessory factor in this test. Materials and Methods: Animals' serum samples for accessory factor from rabbit, rat and guinea pig and also human serum were obtained in the department of parasitoloy, Pasteur Institute of Iran. Each humans and animals serum was screened for toxoplasma antibody by IFAT and dye test .Those which were negative in two tests chose as accessory factor. Then a total of 18 human serum samples were tested to detect the Toxoplasma antibodies by the Sabin - Feldman dye test using these various accessory factors.Results: In the comparison of four accessory factors, the results of the present study showed the benefits of using the rabbit serum as the accessory factor and also demonstrated it is more suitable than other animals. Conclusion: According totheresultsofthisstudy, it is suggested that thecomponents ofrabbit serumis similarto components of humanserum, thus rabbit serum can be used as accessory factor in the Sabin and Feldman dye test.

Keywords: *Toxoplasma gondii*, IFAT, Sabin and Feldman dye test

642. Immuno-Epidemiology of *Ascaris Lumbricoides*Infections in Endemic Populations with Allergic Symptom

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Background: This studyinvestigatedthe relationshipof Immuno-epidemiology of *Ascaris lumbricoides* and allergic symptoms in children from Golestan province. Materials and Methods: The study was conducted between 800 children during the years from 2010-2011. The specimens were examined by simple smear and formalin-ether concentration methods. Results: From the study population, about 11 children had symptoms related to respiratory problems and allergies. Also four children had infected with Ascaris parasite. All children with parasitic infection of Ascaris had allergic symptoms such as cough, shortness of breath and difficulty breathing. Thus, in this study Ascaris infection causes about third of (36.3%) allergic and asthma-like symptoms. Conclusion:The statistical results show that one of the causes of allergies and asthma in school-age children can be owned by a parasitic infection, particularly infection with *Ascaris.* Hence, students' health awareness can be very important in reducing the incidence of both diseases.

Keywords:Asthma, Allergy, *Ascaris Lumbericuides*, Parasiticinfection

643. Evaluation of the Immune Response Induced By DNA Vaccine Cocktail Expressing Complete SAG1 and ROP2 Genes against Toxoplasmosis

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Background: *Toxoplasma gondii*, the pathogen of toxoplasmosis, can infect most mammals and birds.
The high incidence and severe or lethal damages of toxoplasmosis clearly indicate the need for the development of a more effective vaccine. The ROP2 and SAG1 genes of *Toxoplasma gondii* are proposed as a vaccine candidate against toxoplasmosis. Previous studies have reported that the single-gene vaccine with SAG1 or ROP2 could only produce partial protection against *T. gondii*. It was also reported that DNA vaccine cocktail was more effective than single-antigenic vaccine. Materials and Methods: In this work we constructed a DNA cocktail, pcDNA3-SAG1+pcDNA3-ROP2, containing plasmids encoding the full-length Major Surface Antigen 1 and Rhoptry Protein 2 genes of *Toxoplasma gondii* and evaluated its immune response and protective efficacy in comparison with single-gene vaccines and control groups. We immunized BALB/c mice intramuscularly three times. After immunization, the effectiveness of DNA vaccine cocktail and single-gene vaccines were compared using cytokine and antibody measurements. Results: The result of cytokine determination show that the group immunized with pcSAG1+pcROP2 produced high Th1 immune response compared to other groups immunized with single-gene plasmids, empty plasmid or phosphate-buffered saline. Although pcSAG1+pcRoP2 elicited IgG antibody values were greater than that of the sera of mice immunized with empty plasmid or phosphate- buffered saline ($P<0.05$), there was no statistically significant difference between pcSAG1+pcROP2 and single-gene plasmids ($P>0.05$). Conclusion: Moreover, DNA cocktail immunization prolonged survival time against a lethal challeng with the highly virulent *Toxoplasma gondii* RH strain. Our study indicates that the DNA vaccine cocktail, expressing complete SAG1 and ROP2 genes, is more powerful and efficient than single gene vaccine. Also these results support further investigations to achieve a multigene anti-*T. gondii* DNA vaccine.

Keywords: DNA vaccine cocktail, complete surface antigen 1 and rhoptry protein 2, *Toxoplasma gondii*.

644. Prevention of Cryptosporidiosis by Passive Immunization

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Background: *Cryptosporidium parvum* is a zoonotic protozoan parasite that causes enteric disease and sometimes death in immunodeficient humans and animals. Lack of chemotherapeutic agents with consistent efficacy lead to focus on active and nonactive immunization for prevention and treatment of cryptosporidiosis. The aim of this study was to produce enriched colostrum with goat anti whole antigen IgG of *Cryptosporidium parvum*. Materials and Methods: The samples were collected by rectal examination of suspected neonatal calves. Presence of oocysts was determined by modified zielh-neelsen staining. If there were 15 oocysts in each microscopic field under 40 x magnifications, oocystes were extracted by sucrose flotation method. Antigen was prepared by vortexingoocyst suspension alongside with glass beads. Then oocysts was sonicated with GmbH sonicator and freeze and thaw in liquid nitrogen for 5 times. Suspension was centrifuged at 100,000 x g for 2 h at 4°C. The supernatant was separated and used as a source of soluble antigens. To produce enriched colostrum, fifty micrograms of antigen was emulsified with Freund's complete adjuvant and injected to a goat subcutaneously. Two boosters were performed with 30 µg of antigen that were emulsified with Freund's incomplete adjuvant. Blood sample was collected two weeks after final immunization and IgG was purified and added to cattle colostrum. Results: Western blot analysis of enriched colostrum with IgG anti whole antigen of *cryptosporidium parvum*with lysate from *C. parvum*oocyste had positive results. Conclusion: This colostrum could be useful for prevention or treatment of calves cryptosporidiosis.
Keywords: Cryptosporidiumparvum, colostrum, Passive immunization

645. IL-17, IL-22 and TNF-α in Human Cutaneous Infection due to*Leishmania major*

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Background:Clinical outcomes of *Leishmania* infection is a consequence of interactions between the parasite and host immune response. Th17 cells have recently been described as a distinct group of T helper cells that produce interleukin (IL)-17 and IL-22. IL-17, IL-22 as well as tumor necrosis factor-alpha (TNF-α) have interactions as proinflammatory cytokines in immune response. The role of these cytokines is not clear in cutaneous leishmaniasis (CL). We analyzed the alterations of IL-17, IL-22 and TNF-α in the peripheral blood mononuclear cells (PBMCs) culture supernatant of volunteers with CL caused by *Leishmania major*. Materials and Methods: Sixteen patients with CL were enrolled for a follow-up study that consisted of clinical and immunological evaluations during treatment, from most important endemic regions (Ghomrood and Ghanavat Districts) in Qom Province, central Iran. Thirteen healthy controls (without a history of the disease) were included in the study. PBMCs were isolated from whole *blood* using *Ficoll*, before (Active CL) and after (Healed CL) standard antimonial therapy. PBMCs were cultured in the presence of soluble *Leishmania* antigen (SLA). The levels of IL-17, IL-22 and TNF-α were measured by enzyme-linked immunosorbent assay (ELISA) method. Results: The mean±SD concentrations of cytokines were: 300.81±316 *vs.* 239.93±222 pg/mL for IL-17, 1394.06±1614 *vs.* 1613.75±1486 pg/mL for IL-22, 3591.42±4660 *vs.* 1163.33±128 pg/mL for TNF-α in active CL and healed CL, respectively. The mean level of TNF-α was significantly reduced in healed CL compared to active CL volunteers ($P<0.05$). Mean level of TNF-α in active CL was significantly higher than the control group ($P<0.005$). No statistically significant difference was seen in the culture supernatant levels of IL-17 and IL-22 between different groups. Conclusion: According to this study, TNF, but not IL-17 and IL-22 showed alterations in CL. The healing of CL is negatively correlated with the level of TNF-α production in SLA- stimulated PBMC culture.
Keywords: CL, IL-17, IL-22, TNF-α

646. Comparison of Plasma and Mononuclear Cell Culture Supernatant TNF-α Levels in Patients with Active and History of Cutaneous Leishmaniasis

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Background:Tumor necrosis factor-alpha (TNF-α) is an important proinflammatory cytokine in immune response against pathogens with a broad spectrum of biological activities.Present data about the role of TNF-α in cutaneous leishmaniasis (CL) is conflicting, we compared plasma and mononuclear cell culture supernatant levels of TNF-α in CL. Materials and Methods: Blood samples were collected from 16 patients with active CL (ACL), after standard antimonial therapy from the same volunteers as healed CL (HCL), and 13 healthy controls. Plasma and peripheral blood mononuclear cells (PBMCs) were obtained from blood by F*icoll* separation. PBMCs were stimulated with soluble *Leishmania* antigen (SLA) for 72 hrs at 37℃, 5% CO2. Levels of TNF-α production were titrated by using ELISA method. Results: The mean±SD levels of plasma TNF-α was 453.28±274 pg/mL in ACL and 259.23±189 pg/mL in HCL. The mean±SD levels of TNF-α in PBMC culture supernatant was 3591.42±4660 pg/mL in ACL and 1163.33±128 pg/mL in HCL. The mean level of TNF-α was significantly reduced in plasma and PBMC culture supernatants of HCL compared to ACL ($P<0.005$ for plasma, $P<0.05$ for PBMC). The mean level of TNF-α was significantly higher in plasma and PBMC culture supernatant of ACL than control group ($P<0.01$ for plasma and $P<0.005$ for PBMC). The concentration of TNF-α was higher in PBMC culture supernatant*compared* with plasma in all of groups ($P<0.001$ for ACL, $P<0.001$ for HCL and $P<0.001$ for control). Conclusion: The healing of CL is negatively correlated with the level of TNF-α production in both plasma and PBMC samples. Due to simple procedure of collection and preparation, feasibility and cost-effectiveness, it seems that plasma samples could be used instead of PBMC for monitoring of TNF alterations. However, the sensitivity of PBMC might be more than plasma in titration of low TNF production, and in PBMC culture supernatant cytokine production is Ag specific.
Keywords: CL,TNF-α, ACL, PBMC

647. Seroprevalence of Toxocariasis in Children in East Azerbaijan Province, Iran

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Background: Toxocariasis is a zoonotic disease caused by the ascarid of dogs and cats, the main representative of which is a *Toxocara canis*. Distribution of the disease is worldwide and is more prevalent in children. The present study was carried out in children of East Azerbaijan Province, Iran, to determine the toxocariasis seropositivity. Materials and Methods: For the present seroepidemiological study, blood samples were collected at random from children of all the five districts of the East Azerbaijan Province. The sampling was carried from Sep 2008 to Sep 2009. A total of 336 children, 187 males and 149 females in age group of 0-15 years were selected for the present study. ELISA was used for detection of IgG antibodies against *Toxocara* excretory secretary antigen. A questionnaire interview was conducted to obtain the data concerning their age, sex and habits. The particular points in the questionnaire asked were recorded on the format right on the spot. Results: Gender was found a significant risk factor for the *Toxocara* infection in children population. Male children were found more infected (41.71% as compared to females (24.16%). The total seroprevalence of *T. canis* antibodies in children of East Azerbaijan Province was 29.46 %. The risk factors that were found associated with the infection of toxocariasis in children population of East Azerbaijan Province include family back ground, status of living conditions, awareness, etc. Conclusion: The present study reveals high prevalence of *T. canis* infection in children of East Azerbaijan Province. It

is important to raise the awareness of health professionals, public and educators to the fact that toxocariasis is a public health problem. Health promotion by means of a school based educational approach, diagnosis and continuous programme of treatment are necessary.
Keywords: Seroprevalence, Toxocariasis, Children, East Azerbaijan Province, Iran

648. The Effects of Germinal Layer Antigens of Hydatid Cyst on the Expression Level of Ovine Toll-Like Receptor 2 and 4 (TLR2 and TLR4) in Peripheral Blood Mononuclear Cells (PBMCs)

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Background: Echinococcosis is one of the most important zoonosis diseases throughout the world and represents a major public health and economic burden in many countries. Echinococcos Granolosus causes hydatid cyst in human and domesticated animals. Hydatid cyst develops in many organs, mostly in liver and lungs. Immune responses against hydatidosis comprises of various mechanisms of innate and adaptive immunity. In recent years the importance of innate immune responses and in particular, pattern recognition receptors (PRRs) has been recognized as an essential mechanisms for development of an effective immune response. PRRs are the main sensors of pathogen and danger signals in innate immunity. Toll like receptors (TLRs) are the most studied and best characterized PPRs which are responsible for sensing pathogen associated molecular patterns (PAMPs). The role of TLRs in the molecular mechanisms underlying the pathogenesis and immunity in helminths infection has not been clearly defined. Materials and Methods: In this study we focused on the expression levels of two important ovine TLRs transcripts, namely TLR2 and TLR4 in a culture of ovine lymphocytes exposed to different concentrations of germinal layer antigens of hydatid cysts in a time point experiments. Blood samples were taken from healthy young lambs. After isolation of peripheral blood mononuclear cells (PBMC), the cells were cultured with different concentrations (50µg/ml &100µg/ml) of germinal layer antigens (antigens were extracted and concentrated according to the standard protocols) in different time points. Then, total RNA was isolated from the cell pellets and cDNA was synthesized using Oligo dT primers. Afterwards, the primer pairs for TLR2, TLR4 as target genes and GAPDH as housekeeping and calibrator gene were optimized in a gradient RT-PCR experiment. Subsequently, qPCR analysis was set up to quantify and compare the relative expression levels of TLR2 and TLR4 transcripts in PBMC of antigen treated versus control (untreated) samples.Results: Statistical analysis using one way T-test showed an up-regulation of TLR2 and TLR4 in treated as compared to the untreated control group. Moreover, the increased expression of TLR4 was significant. Conclusion: the results presented in this study, can shed more lights to the insight mechanisms behind the molecular immuopathogenesis of hydatidosis which might eventually pave the way for designing novel and more effective therapeutic as well as preventive strategies.
Keywords: PBMC, TLRs, PAMPs

649. First molecular study of s sarcocystis capracanis infection from slaughtered goats in Jahrom

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Background: *sarcocystis capracanis i*sthe species of Microscopic cyst-forming coccidia belonging to the phylum Apicomplexa that stimulates immune mechanismsis and the most infected species in goat, responsible for many serious diseases of humans and domestic animals. This research aimed to detection of sarcocystis capracanis infection in the muscles of different organs (diaphragm, tongue, heart, oesophagus) of slaughtered goats in Fars (Jahrom) by PCR-RFLP for the first time. Materials and Methods: 400 muscle tissues free of macroscopic cysts from slaughtered goats were collected during five months in slaughterhouse of Jahrom in 1390. Total DNA was extracted and a partial sequence 18S rRNA gene of *Sarcocystis capracanis* was amplified by PCR and the PCR product was treated by HincII enzyme. The samples were considered as *Sarcocystis capracanis* that their PCR products were not lysed by HincII enzyme. Results: The results of PCR-RFLP showed that 336 (84%) samples were positive for sarcocystis capracanis infection. There was not significant relationship between sarcocystis capracanis infection and sex ($p>0.05$).Conclusion: *sarcocystis capracanis* may cause clinical illness. symptoms include fever, anaemia and inappetance and associated reduction in productivity. Fever results from the release of interleukin-1 and prostaglandin E_2 while infection of macrophages cause release of tumour necrosis factor-alpha which causes inappetance, toghether with interleukin-1 causes anaemia, suppresses release of pituitary growth hormone, with release of pituitary with resultant weight loss. This molecular study showed a high frequency of *sarcocystis capracanis* infection in goat slaughtered in the jahrom so the veterinary and health service organizations should be carried out preventing operations.
Keywords: *Sarcocystis capracanis*, immunity, clinical disease, goat, Jahrom

650. An Investigation into the Antigenic Property of *Echinococcus* Protoscolex Protein (EPC1) using Bioinformatics Tools

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Background: *Echinococcusgranulosus* is considered to be one of the most important parasitic diseases in endemic regions such as Iran. The most challenging point in this regard has been to establish and evaluate a new antigen of the parasite, which can improve the specificity of the test as far as possible. *Echinococcus* Protoscolex Protein (EPC1) is a new antigenic candidate which can improve the specificity of the serologic tests. Objective bioinformatics analysis software forecasts of *Echinococcus* Protoscolex Protein (EPC1) structure and function of the amino acid sequence. Materials and Methods: Literature search was performed to find proteins from Echinococcus granulosus protoscolex with Antigenic nature. Uniprot server was used for Homology identification. Homology search was performed using BLASTp against PDB database. A subset of protein similar to EPC1 constituents of our dataset was prepared for homology modeling. The structure of these proteins were extracted from PDB database. Then, the data which existed in PDB and has the ≥40% identity were selected and survey in Discovery Studio software. A tertiary structure predicted model of the EPC*1* was constructed with SWISS-MODEL Work Space based on template *Cyprinus carpio* with 40% sequence identity and Evalue$5.9e^{-13}$. EPC1 3D structure compare with related homologous proteins (comparative structure). Results: Homology modeling outcome showed it is an EF-hand calcium binding protein, involve 3 antigenic determinants in its sequence. Modeled residue range is 9 to 75. EPC1 3D structure implies significant structural similarity. Conclusion: For the prediction of antigenic determinant site of EpC1 protein of *Echinococcus granulosus*, we got three antigenic determinant sites in the sequence.EpC1 sequence is 76 residues long involve 3 antigenic determinants at positions 11-20, 28-44, and 48-57. The highest pick is recorded between Amino Acid 28 to Amino Acid 44. The sequence of Amino Acid in this region is 'GKISCAELKSALQSCSA'.
Keywords: EPC1, prediction of antigenic, Bioinformatics

651. Apoptotic Effects of Antimony Sulfide Nanoparticles Synthesized by Biological Methods on Promastigotes of *Leishmania*

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Background: Leishmaniasis is an important public health problem in all over the world. Leishmaniasis, malaria, schistosomiasis, filariasis, trypanosomiasis and tuberculosis, are considered by World Health Organization (WHO) as the six of the most important tropical diseases. Cutaneous leishmaniasis is an endemic infectious disease, which is one of the major health problems in many countries, including Iran. Since there is no vaccine against the disease, its prevalence is increasing in many areas of the world. Using pentavalent antimony compounds is the first line drugs for treatment. In this study nano sizes of these compounds were synthesized by biological methods in Tehran and Kerman University of medical sciences and then apoptotic effects of these particles were evaluated by flowcytometery methods in Tarbiat Modares University. Our method was briefly: 2×10^6 cells/ml of promastigotes were treated with various concentrations of antimony sulfide NPs in ELISA plates and incubated at 24°C for 72 hours. Finally these plates were stained with Annexin-V FITC solution and PI (Propidium Iodid) solution. The percentages of apoptotic, necrotic and viable cells were determined. Cellular apoptosis in our study was detected by using *Annexin-V FLUOS* staining kit (*Roche, Germany*). The procedure was performed according to manufacturing protocol in the dark place. Induction of apoptosis in promastigotes of *Leishmania* was analyzed by flow cytometery FACSCalibor system after staining with Annexin-V and PI. Then results were analyzed by Cellquest software. From these results can be concluding antimony sulfide NPs were induced apoptosis in promastigote cells.

Keywords: Leishmaniasis, *Annexin-V FLUOS,* Annexin-V and PI

652. Effects of *artemisinin* on IFN-γ and IL-4 Cytokines Response in Infected Mouse with *Leishmania major*

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Bachground: Cutaneous Leishmaniasis is prevalent in many areas of the world. Recently many studies were indicated that the number of cutaneous leishmaniasis has increased. Cutaneous leishmaniasis is cause by Leishmania parasite such as *Leishmania major* and *tropica* while *Leishmaniamajor* is the most causative agent of CL in Iran. The current drugs which are used as treatment to Leishmaniasis cannot be easily handle because of problems such as high toxicity, various side effects and etc. artemisinin derivatives, dihydroartemisinin (DHA), artesunate, artemether and arteether, are currently used for the treatment of malaria in artemisinin combination therapies. Materials and Methods: for evaluation of IFN-γ and IL-4 cytokines in test and control mice treated with artimisinin DuoSet ELISA Development IFN-γ and IL-4 Kits (R&D) was used. This procedure was performed according to manufacturing protocols and analyzed by ELISA system. Results: Cytokines results in treated mice were compared with IFN-γ and IL-4 standard curves and control groups. The ANOVA statistical test was employed for comparison and analysis of IL-4 and IFN-γ cytokines results. Conclusion: These results were showed that artimisinin was increased IFN-γ and IL-4 cytokine levels in treated mice rather than control groups ($p\leq0.05$)

Keywords: *Leishmania major, Cutaneous leishmaniasis, Artimisinin,* IFN-γ *and IL-4 cytokines*

653. Nanoselenium and INF-γ and IL-4 cytokine responses against cutaneous leishmaniasis

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Background: Selenium plays a remarkable role in breeding and health of animals and has been considered very important in veterinary medicine. Results of many investigations revealed interactions between selenium and health of mammalians Antioxidants effect of selenium has been shown that protect phagocytic cells and surrounding tissues from attacking free oxidative radicals produced by the respiratory chain of neutrophils and macrophages during phagocytosis and The role of selenium in immune system has been supported by many studies. Materials and Methods: In this study red nanoselenium was employed against cutaneous leishmaniasis and INF-γ and IL-4 cytokines level in BALB/c mouse were measured. After infection each of mice was treated by 5 and 10 mg/kg of nanoselenium daily for 3 weeks. Results: By in vivo experiments we have found that amastigotes of *Leishmania major* are very susceptible to nanoselenium. Nanoselenium could have decreased the size of ulcer during the treatment and the amastigote rate in spleen and liver of mice. Conclusion: Finally results of our study were revealed that nanoselenium was promoted INF-γ secretion against parasite and enhanced Th1 and macrophage activity in leishmaniasis disease. These findings suggest that nanoselenium can be a potent candidate for leishmaniasis treatment in further studies. Results in our study showed the effectiveness of nanoselenium on proliferation of *Leishmania major*. We suggest use of nanoselenium for future studies against cutaneous leishmaniasis

Keywords: *Nanoselenium, Leishmania major, cutaneous leishmaniasis*

654. The Serological Study of Cystic Echinococcosis in Khorram Abad, Iran

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Background: *Echinococcus granulosus* is a cestode whose larval stage causes cystic echinococcosis in wild animals, livestock, and human. There is a lack of information about the seroepidemiology of *Echinococcus granulosus* infection and surgical cases in the general population of Khorram Abad district, southwest of Iran. Materials and Methods: Anti–*Echinococcus granulosus* antibodies were sought in 617 inhabitants in Khorram Abad with the use of enzyme-linked immunoassays and antigen B. The surgical cases of cystic echinococcosis were investigated in Shohaday-e-Ashayer of Khorram Abad, as referral center, in the years of 2006 to 2010. Results: In total, 95 (15.4%) of 617 participants (mean age 39.6 ± 17.6 yr) had anti–*Echinococcus granulosus* antibodies. Prevalence of infection was more in males (40%) than females (60%) with statistical significance ($P<0.001$). Conclusion: High-titer antibodies were most prevalent highest among aged group 20-29 years. There was significant difference between presence of *Echinococcus* antibodies and the terms of sector of residence, education of volunteers, and occupation ($P<0.05$).

Keywords: *Echinococcus granulosus,* Khorram Abad, enzyme-linked immunoassays

655. The Measurement Amount of Total Antibody Serum Level in Newborn BALB/C Mice Infected by Cryptosporidium and Specify by Dot Blot Technique

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Background: Cryptosporidium Parvum is a unicellular parasite which causes diarrheal in human and animals worldwide. Infection transmission has been reported through oral-fecal by direct or indirect contact with infectious objects through foods and drinks. A nursery school and nursing home for the aged are common places for this infection. The immune system of person has an important role in infection with the parasite. Immuno-competent hosts have ability to limit this infection but in immuno-suppressed hosts such as HIV/AIDS patients may cause potentially fatal complications for example bile duct damage. The immune response pathway is less known in this regard. Materials and Methods: The new born mice aged, 2-3 days were infected with adequate amount of Cryptosporidium oocysts (500,000 per ml), and then total proteins were measured through serum- sampling to verify the existence of serum antibody. To confirm the existence of serum antibody, Dot Blot technique was performed. The oocysts were sonicated and placed on nitrocellulose; then fixed by ultraviolet ray. Following washing with PBS, the nitrocellulose placed in blocking buffer for 1 h. Then the primary antibody (serum) and secondary antibody (antiserum) were added. The stains

on the nitrocellulose were appeared with DAB and hydrogen peroxide and the synthesized antibody was measured by biophotometer. Results: Among the test and the control groups, bolts were appeared in test group which means antibody production, but not any blots were observed in the control groups. The non-characteristic proteins in serum were measured by the biophotometer. Conclusion: In this study, it was planned to determine the condition of antibody serum production against Cryptosporidium oocysts. The detected antibody through Dot Blot technique was our aims which had been conjugated to our characteristic antiserum. The recorded numbers for the controls by biophotometer were related to the non-characteristic proteins in serum.
Keywords: HIV, Cryptosporidium oocysts, biophotometer

656. Study On Influence Of Naloxone On Major Protective Cytokines Genes Of T Cell Responses In Mice Inoculated With Leishmania Major

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Background: Leishmania major is a protozoan parasite and it is shown to be host genotype dependent hence some inbred strains of mouse are susceptible while others are resistant to L.major. The resistance is developed by T-helper (Th1) cells by producing IFN-γ and IL-2 while the susceptibility is conferred by Th-2 cells producing IL-4, IL-5 and IL-10. It is has been shown that IFN-γ activates macrophages to kill the intracellular amastigotes. In contrast, Th2 immune responses limit the action of Th-1 functions via IL-10 and IL-4, which results in deactivation of macrophages and growth of intracellular parasite and subsequent exacerbation of disease progression. Materials and Methods: In this research, WHO confirmed standard strain of L.major, were injected to 42 BALB/c mouse. In the other group WHO confirmed standard strain of L.major along with naloxone were injected to 42 BALB/c mouse. Lymph nodes of mice were separated in different periods of time and total RNA was extracted by using molecular methods and cDNA was made. Then by reverse transcriptase expression of IFN-γ, IL-10, IL-4, IL-2 genes were studied by real time PCR using special designed primers. Results and conclusion: Data obtained by delineating cytokines expression was analyzed and consequently we found that probably the naloxone causes driving immune system toward cellular immunity in BALB/c mouse and in group of mice which parasite a long with naloxane injected, improvement was seen.
Keywords: Leishmania major, Th-1, Th-2, IL-2, IFN-γ, IL-10, IL-4, naloxone

657. Gene Regulation of Pteridine Reductase 1 in *Leishmania* Amastigotes Using an Antisense Plasmid

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Background: *Leishmania* species are intracellular protozoan parasites that cause leishmaniasis with a wide range of clinical symptoms. Pteridine metabolic pathwayisunusual features of *Leishmania*, which is necessary for the growth of parasite. *Leishmania* has evolved a complex and versatile pteridine salvage network which has the ability of scavenging a wide area of the conjugated and unconjugated pteridines especially folate and biopterin. In this study, we focus on the inhibition of PTR1gene expression. Materials and Methods: *L. major* PTR1 gene was cloned into pcDNA3 and digested using KpnI and BamHI. The gene was subcloned so that antisense will transcribe and called pcDNA-rPTR. Mouse pritonean macrophages were transfected using pcDNA-rPTR–tansfected promastigotes. Western blotting was performed on mouse transfected pritonean macrophages using a *Leishmania major PTR1* antibody raised in rabbits. Results: The PTR1 protein was not expressed in mouse macrophage transfected with pcDNA-rPTR– tansfected promastigotes. Conclusion: The present results indicated that this approach might be used to study the pteridine salvage pathway in *Leishmania* or to assess the possibility of using gene expression inhibition in the treatment of leishmaniasis.
Key words: Pteridine reductase 1, antisense, *Leishmania*

658. Survey of Antifungal and Cytotoxic Activity Licorice Extracton Aflatoxinby HPLC Technique

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Background: Aflatoxins are potent carcinogenic and mutagenic metabolites mainly produced by the fungal species *Aspergillus flavus and A. Parasiticus*. These species can contaminate several food commodities including cereals, peanuts and crops. In recent years researches on extracts and medicinal herb such as licorice extract lead to reduce the microbial growth and also have a particular effect on production of aflatoxins as carcinogenic compounds. A positive correlation between aflatoxin contamination of agricultural commodities and primary human hepatocellular carcinoma has been documented. The aim of this study is to explore the antifungal and cytotoxic activity of *Glycyrrhiza glabra* extract by using MIC and HPLC technique. Materials and Methods: In this study after culture fungi (standard strain of *A. Parasiticus* ATCC 15517) in SDA and SDB media, serial dilutions of extracts were produced for fulfilling the MIC technique.in the other hand for determining the rate of aflatoxin produces by the fungi alone and in combination with different dilutions of extract (0.5ml- 2.5ml- 5ml- 10ml) was done by HPLC technique. Results: Results demonstrated that licorice extract can inhibited the mentioned fungus growth at 0.5gr/ml. However, HPLC results showed that the mentioned fungi produced the amount of 28960.00 ppb without licorice extract. In addition, the amount of produced toxin was about 14.60 ppb with 10ml of licorice extract. Regards to this concentration, 5ml of extract shows less inhibitory efficiency than 10ml of it but more efficiency than 2.5 ml. However, the antifungal activity of licorice extract was increased with 10ml of the extract. Conclusion: Since licorice *Glycyrrhiza glabra* extract has an antifungal activity and also it can inhibit the *Aspergillus* growth, therefore, it may be mentioned as an antibiotic staff. Knowledge about activity and determining the effect of licorice extract can help to produce antifungal and anti-aflatoxin drugs.
Keywords: *Aspergillus Parasiticus*, Licorice extract, aflatoxin, MIC, HPLC

IMMUNOPATHOLOGY oF DISEASES

Oral Presentation

659. Footprints of Th17 Cells Presence in Chronic Periodontal Disease

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Background: Periodontitis is a chronic infectious inflammatory disease that ultimately leads to the destruction of periodontal tissues. Although periodontopathic bacteria are the causative agents in periodontitis, subsequent progression and disease severity are determined in large part by the deregulated immune responses. Th1/Th2 paradigm could not show true complexity of the fate decision of helper T cells and failed to completely explain the pathogenesis of autoimmune diseases. The discovery of the Th17 cell lineage marked a new era in the studies of the autoimmune responses. Recently, different independent studies have demonstrated an increase of Th17 cells in many autoimmune and inflammatory disorders. The aim of this study was to identify the specific markers of Th17 cells, and their variations in people suffering from chronic periodontal disease in comparison with normal controls.
Materials and Methods: In 30 patients with periodontitis and 30 normal controls, transcript levels of IL-17A and RORC2 were measured using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). The protein levels of IL-17A and RORC2 proteins were also evaluated by immunohistochemistry method. The levels of these markers were compared between healthy and diseased periodontal tissues by the Mann–Whitney u-test. Results: In periodontal lesions, IL-17 and RORC2 were significantly over-expressed compared with normal tissues. According to our immunohistochemical analysis, the number of IL-17A$^+$ cells and RORC2$^+$ cells was significantly higher in periodontal lesions compared to control sites. Moreover, there was a significant correlation between the presence of IL-17A and RORC2 transcript and protein contents levels in the gingiva of diseased patients. Conclusion: In summary, our study demonstrates an elevated expression of IL-17 and RORC2 (characteristic markers of Th17 cells) in chronic periodontitis in comparison with healthy sites. IL-17$^+$, RORC2$^+$ cells may therefore play an essential role in the immunopathogenesis of periodontal diseases.
Keywords: chronic periodontitis, Th17,IL-17A, RORC2

660. A Study Of CD4$^+$Foxp3$^+$ Treg And It's ICOS$^+$ Subsets In Patients Suffering From Atherosclerosis

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Background: Atherosclerosis is a multifactorial disorder with chronic inflammatory condition which immune cells play significant role in its pathogenic process. Regulatory T cells (Treg) as a part of immune system are involved in controlling autoimmune and inflammatory disease. Numerical and functional alteration of Treg has been shown to play an important role in atherosclerosis. Tregs could have an atheroprotective role and may also promote plaque stabilization. Inducible costimulatory molecule (ICOS) express on a Treg subtype with high suppressive potential may effect on atherosclerosis pathogenesis. Material and Methods: Patients with MI or with stable angina who were approved for atherosclerosis by angiography and a group of normal coronary angiography individuals (NCA) were involved in present study. Peripheral blood mononuclear cells (PBMCs) were prepared, and the specific monoclonal antibodies including PerCP anti-humannCD4, phyco-erythrin (PE) conjugated with anti-human ICOS and FITC anti-human Foxp3were used for molecule staining. Flow cytometry was applied for molecules expression assessment. Results: The percentage of CD4$^+$Foxp3$^+$ Treg cells was reduce in MI group compared with NCA and with SA group (1.93±1.07% Vs 4.09±1.16 %., and 2.9±1.18%, respectively P=<0.005). Similar reduction was obtain for CD4 FOXP3 Treg in the groups. A decreased percentage of ICOS$^+$Tregs was seen in MI group compared with SA (0.92±0.62% Vs. 1.58 ±0.62%, p-value=0.001). The ratio of ICOS$^+$/ICOS$^-$ Treg was lower in MI cases compared to individuals NCA (1.06±0.60vs. 1.63±0.65, P=0.002). No significant differences were found in lymphocyte percentage and CD4 T cell between the groups(p>0.05). Conclusion: Data indicate Treg and also it's ICOS$^+$ subset reduction in patients with MI , suggesting a potential role for Treg decrease in plaque destabilization and the onset of ACS including MI and unstable angina.
Keywords: Acute coronary syndrome, Myocardial infarction(MI), Stable angina(SA), Regulatory T cells, ICOS, Inflammation, Atherosclerosis

661. Relationship between HLA-C Surface Expression and Licensing Of KIR+ Natural Killer Cells and Modulation of KIR Repertoire by Cytomegalovirus Infection

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Background: NK cells require interaction of inhibitory surface receptors with HLA ligands during development to acquire functional competence in a process termed 'licensing'. The quantity of HLA required for this process is unknown. A polymorphism affecting HLA-C surface expression (*HLA-C -35*) has recently been identified, and shown to influence progression of HIV infection. Materials and Methods: We typed a cohort of healthy donors for *HLA-C -35*, *KIR2DL1* and *KIR2DL3* and their respective *HLA-C* ligands and analyzed how HLA ligands influenced licensing status of KIR+ NK cells in terms of degranulation and cytokine production in response to HLA negative target cells. The presence of respective HLA class I ligands increased the function of KIR2DL1+ and KIR2DL3+ NK cells in a dose-dependent manner. In contrast, neither the polymorphism at *HLA-C -35* nor the quantity of cell surface HLA-C had any significant effect on NK cell function. Instead, HLA-Cw7 - an HLA-C allele with low surface expression – licensed KIR2DL3+ NK cells more strongly than any other KIR2DL3 ligand. The quantity of cell surface HLA-C does not appear to influence licensing of NK cells, and the *HLA-C -35* polymorphism is unlikely to influence HIV progression through factors related to NK cell education. Patients carrying activating killer cell immunoglobulin-like receptors (KIR) genes are significantly protected from cytomegalovirus (CMV) associated complications after solid organ or hematopoietic stem cell transplantation. Whether previous infection with CMV affects natural killer cell function in healthy donors is unkwnown. We studied KIR repertoire and alterations of KIR expression after in-vitro exposure to CMV in 54 healthy donors. Results: Neither activating nor inhibitory KIR expression was different at baseline between 23 seropositive and 31 seronegative donors. However, after co-culture with CMV infected fibroblast cells, expression of the inhibitory receptors KIR2DL1 and KIR2DL3 and the activating receptor KIR3DS1 significantly increased in CMV-seropositive donors, whereas no changes were observed in seronegative donors. Expansion of inhibitory KIRs occurred exclusively in donors carrying the cognate HLA class I ligands, whereas presence of the putative ligand HLA-Bw4 was not necessary for expansion of KIR3DS1 NK cells. Conclusion: Our data show that previous infection with CMV does not alter resting NK cell receptor repertoire, but appears to modify how NK cells respond re-exposure to CMV in vitro.
Keywords: NK, HLA-C, KIR, CMV

662. Phenotypical Characterization of the Peripheral Blood T Cells in the Patients with Celiac Disease in Isfahan, Iran: Elevation of γδTCR$^+$ T Lymphocytes

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Background: Celiac disease (CD) is a small intestine enteropathy caused by permanent wheat gluten intolerance. One of the earliest signs of mucosal immune activation in CD is an increase in the intestinal intraepithelial lymphocytes (iIELs) count in the small intestinal epithelium. Though most of those iIELs express αβTCR, CD is characterized by an increase in γδTCR$^+$ iIELs. The present study aimed to establish whether these immunological changes seen in the intestinal epithelium of CD patients could also be detected in the peripheral blood lymphocyte populations with special emphasis on the γδTCR$^+$ T cells. Materials and Methods: Peripheral blood T cells were analyzed by two color flow cytometry in 12 untreated patients with CD and 16 healthy controls. Results: There were significant differences between the mean percentages of γδTCR$^+$ T cells in the patients and the controls (7.5 ± 3.7% in the patients versus 4.9 ± 2.5% in the controls, P < 0.05). However, the mean percentages of the αβTCR$^+$ T cells were significantly lowered in the untreated patients (90 ± 3.5% in the patients versus 93.3 ± 2.8% in the

controls, P < 0.05). There were no significant difference between the mean percentages of T cells expressing the CD4 and CD8 molecules in the patients and the controls. Conclusions: The change in the peripheral blood T cells expressing the γδTCR and αβTCR markers in the celiac patients is, therefore, a consequence of an ongoing immunological process.
Keywords: Celiac disease; Peripheral blood T lymphocytes; γδTCR cells; αβTCR cells; Flow cytometry; Isfahan

663. Serum Levels of Rheumatoid Factor, Anti-Nuclear, Anti-Phosphatidylserine and Anti-Cardiolipin Antibodies in Patients with Ischemic Heart Disease

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Background: Some inflammatory diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus have been associated with ischemic heart disease (IHD).The aim of this study was to evaluate the serum levels of some autoantibodies including rheumatoid factor (RF), anti-nuclear antibodies (ANA), anti-Sm, anti-phosphatidylserine (anti-PS) and anti-cardiolipin (anti-CL) antibodies in patients with IHD.Materials and Methods: A total of 120 patients with IHD as having acute myocardialinfarction (AMI; n=60) or unstable angina (UA; n=60) and 60 sex- and age- matched healthy subjects as a control group were enrolled to study. Serum samples of participants tested for the ANA, anti-Sm, anti-PS and anti-CL antibodies by use of ELISA. Serum levels of RF measured by using turbidimetry method. Results: The mean serum levels of RF and anti-PS antibodies in AMI group and UA group was significantly higher than that observed in control group (P<0.0001). Moreover, the mean serum levels of RF and anti-PS antibodies in AMI patients was significantly higher than UA group (P<0.01 and P<0.001, respectively). The mean serum levels of RF in men with AMI or UA diseases was significantly higher as compared to healthy men group (P<0.0001 and P<0.003, respectively). The differences of the serum levels of ANA, anti-Sm and anti-CL antibodies were not significant between AMI, UA and control groups. The differences of the mean serum levels of RF, ANA, anti-Sm, anti-PS or anti-CL antibodies in patients with a traditional risk factor including hypertension, dyslipidemia, diabetes and smoking were not statistically significant as compared to counterpart groups without a certain risk factor. Conclusion: These results showed higher serum levels of RF and anti-PS antibody in patients with IHD. RF and anti-PS antibody may be an independent risk factor for IHD. The levels of antoantibodies did not influenced by traditional risk factors.
Keywords: Acute myocardialinfarction, Unstable angina, Rheumatoid factor, Anti-nuclear antibody, Anti-Sm, Anti-phosphatidylserine, anti-cardiolipin

Poster Discussion Presentation

664. Evaluation of Super Antigens in Polyp Tissue of Patients with Chronic Rhino-Sinusitis Compared with Control Group

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Background: *Staphylococcus Aureus* secretes numerous exotoxins which have super antigenic characteristics. Virulence of some of them has been clarified well, but their biologic effects are not completely understood. These exotoxins may affect immunological and inflammatory status of various organs including sinus mucosa; and their probable involvement in chronic rhino-sinusitis is taken into consideration by some authors. This study is designed to evaluate role of *staphylococcus aureus* super antigens in polyp tissues of patients with chronic rhino-sinusitis in comparison with control group. Materials and Methods: In this observational case- control study on 28 patients with chronic rhino-sinusitis and 19 healthy individuals, polyp tissue samples of patients and mucosal specimens of control group were evaluated for super antigens with RT-PCR and ELISA methods. Data analysis was performed by descriptive measures and Chi-square and Fischer Exact tests using SPSS program (version 11). Results: Evaluation of sample tissues with PCR revealed that 88.2% of patients with polyp had at least one super antigen and in control group, 45.5% had at least one super antigen which was statistically significant (P value = 0.03). Evaluation of super antigens using ELISA method in patients with nasal polyps represent at least one super antigen in nasal samples of patients (100%) and in control group the rate of super antigen existence was 35.3% which was statistically significant (P value< 0.001). Conclusion: this study indicates there is a relationship between staphylococcal super antigens and nasal polyps and also these super antigens may have a role in pathogenesis of nasal polyposis.
Keywords: Polyp Tissue, *staphylococcus aureus*, Chronic Rhino-Sinusitis

665. Evaluation of Urokinase Plasminogen Activator Receptor (uPAR), soluble uPAR, and β1 Integrin in Patients with Hodgkin's Lymphoma

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Background: Several studies indicated the role of urokinase plasminogen activator receptor (uPAR), soluble uPAR (suPAR), and β1 integrin in tumor growth and invasion. Materials and Methods: In this study, the expression levels of β1 integrin and uPAR on lymph node mononuclear cells from 25 Hodgkin's lymphoma (HL) patients were evaluated using two-color flow cytometry. Moreover, the levels of suPAR in the serum samples of HL were measured and compared with 32 healthy controls. Results: Results indicated no significant association of uPAR with tumor size, different stages, or different histological subtypes of HL; however, an increased expression of β1 integrin was detected in advanced stages of HL. Among different subtypes, higher expression of β1 integrin was detected in Nodular sclerosis compared to Lymphocyte Predominant. No significant difference was observed between serum levels of suPAR in patients with different stages of HL and healthy controls. Moreover, the levels of suPAR were significantly higher in Nodular sclerosis in comparison with other subtypes. Conclusion: This study showed that the levels of suPAR and β1 integrin varied between different histological subtypes of HL. Although uPAR may play only a minor role in growth and metastasis of lymphoma, β1 integrin may be important in predicting prognosis and metastasis in HL.
Keywords: uPAR, suPAR, Hodgkin's Lymphoma, β1 integrin, metastasis

666. Neuroprotective Effect of Tretinoin in Ameliorating Animal Model of Multiple Sclerosis

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Background: A fundamental aspect in multiple sclerosis (MS) is the transition from the early relapsing–remitting form to the more neurodegenerative and treatment resistant secondary progressive form. Increasingly, experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice by immunization with MOG_{35-55} peptide produces a chronic progressive disease, as found in secondary progressive stage of MS. This research was conducted to assess neuroprotective effect of Tretinoin in established EAE. Materials and Methods: EAE was induced by MOG_{35-55} peptide and CFA in female C57BL/6 mice. Mice were allocated in two therapeutic groups (n=7 per group). Treatment withTretinoin (25mg/kg body weight-every other day) was started at day 12 post induction when the treatment group developed a neurologic disability score. Signs of disease were recorded daily until the day 33 when mice were sacrificed. For neuropathological analysis, brains and spinal cords were harvested and sections were stained with H&E and toluidine blue. Results: Tretinoin significantly decreased the clinical outcomes (mean clinical score, cumulative disease index, weight gaining) of established EAE. Blinded analyses indicated that degree of parenchymal edema, cellular infiltration, vacuolar degeneration of neurons in brain and spinal cord sections and area of demyelination in spinal cord sections were

significantly reduced in Tretinoin-treated groups, compared to vehicle-treated group EAE group. Conclusion: This pharmacological approach may be as a promising strategy for the therapy of MS.
Keywords: Multiple sclerosis, Experimental autoimmune encephalomyelitis, Neuroprotection,Tretinoin

667. Aqueous Humor and Sera Levels of Soluble MICA and MICB in Cataract and Glaucoma Patients

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Background: Growing evidence obtained from clinical and experimental studies strongly suggest that the immune system is involved in the immunopathogenesis of Cataracts and Glaucoma. We hypothesize that the expression of major histocompatibility complex (MHC) class I-related chain (MIC) in long-term tissue stress in Cataracts and glaucomatous eyes can be plays an important role in activation of an autoimmune immunopathogenesis process in this patient. This study is an effort to investigate the presence and levels of MICA and MICB in the aqueous humor and sera, patients with cataract and glaucoma. Materials and Methods: in a pilot study serum was collected from 41 Cataracts and 3 Glaucoma patients before eye surgery.In addition, aqueous humor in these patients was collected at commencement of surgery. Concentration of soluble MICA and MICB was measured by an enzyme-linked immunosorbent assay. Results: Mean age of the subjects were 65±2.5 years and Mean Concentration of soluble MICA and MICB in serum samples of Cataract and Glaucoma patients were MICA=223.2pg/mL, 188.7 pg/mL; MICB=87.5pg/mL, 215.3 pg/mL, respectively. Also, mean Concentration of soluble MICA and MICB in aqueous samples of Cataract and Glaucoma patients were MICA=20.6pg/mL, 12.7 pg/mL; MICB=17.9pg/mL, 39 pg/mL, respectively. Conclusion: This preliminary study suggests that MIC proteins can be induced during cellular stress in eye tissue that is a neglected point underlying immunopathogenesis of Cataracts and Glaucomadiseases. In addition to, this concept offers a new perspective of disease pathogenesis and therapeutic approaches to prevent irreversible autoimmune process in eye diseases.
Keywords: Cataract, Glaucoma,MIC, Immunopathogenesis

668. Cellular Death Pattern in Recurrent Spontaneous Abortion (RSA)

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Background: Apoptosis or programmed cell death is normal process in which cells perish in an orderly, highly controlled manner so as to sculpt and control an organism's development. Abortion is the termination of a pregnancy associated with the death and expulsion of the embryo or fetus from the womb. In medicine, all terminations of pregnancy not resulting in live birth are defined as abortions. In common parlance, the terms miscarriage or stillbirth are applied to spontaneous (non-induced) abortions. The aim of this study was to investigate cell apoptosis in women with recurrent spontaneous abortion (RSA) of unknown etiology. Materials and Methods: Twenty four women with a history of recurrent pregnancy losses with unknown etiology were included in this study. Women with anatomical, hormonal, infectious and genetic causes of RSA were excluded. We compared the percentage of mononuclear apoptotic cells by flow cytometry in these patients with controls. The pattern of cellular death (early apoptosis, late apoptosis and/or necrosis) was evaluated using annexinV-FITC and propidium iodide (PI) staining method by flowcytometry. Flowcytometric data were analyzed using FSC Express 3.0 software. Results: The percentages of early apoptosis were 13% and 6.1% in RSA patients and controls, respectively. The percentages of late apoptosis were 6.4% and 1% in RSA patients and controls, respectively. Conclusion: The results suggest that the higher percentage of early and late apoptosis in lymphocytes of RSA patients compared to control group may reflect uterine apoptosis cells. The percentage of apoptotic cell in lymphocyte may indicate the risk of recurrent pregnancy loss.
Keywords: Cellular Death Pattern, RSA, flow cytometry

669. Amelioration of Rheumatoid Arthritis through Inducing Regulatory T Cells

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Background: The aim of present study was to modulate T cell response in experimental model of rheumatoid arthritis. Materials and Methods: Collagen-induced arthritis (CIA) was induced by intradermally immunization of Lewis rats with the emulsion of bovine type II collagen in complete Freund's adjuvant at the base of the tail. Administration of prepared immune modulator (PIM) was started on the first day of immunization and continued until final assessment. During this period, clinical examination was taken intermittently. The paws and knees were then removed for histopathology and radiography assays. Moreover, peripheral blood regulatory T cells were examined by flow cytometry. Results: Our data showed that PIM administration to arthritic rats induced a significant reduction in paw edema. This beneficial effect of PIM was associated with a significant increase in the regulatory T cells compared to untreated rats. Histopathological assessment showed a reduced inflammatory cells infiltrate in joints of treated rats, as well as tissue edema and bone erosion in the paws were markedly reduced following PIM therapy. Moreover, our results in radiography paralleled with histological findings. Conclusion: Our findings shed light on the therapeutic efficacy of PIM in experimental model of rheumatoid arthritis and it may be recommended as an immune modulator component in treatment of rheumatoid arthritis.
Keywords: Rheumatoid arthritis, Regulatory T cells, Immuno modulation

670. Comparison of the Number and Phenotype of the Duodenal Intraepithelial Lymphocytes in the Celiac Patients and Healthy Individuals in Isfahan, Iran

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Background: Celiac disease (CD) is an immunologically-mediated intolerance to dietary prolamins. CD is the most prevalent chronic gastrointestinal disease in Caucasians (1% prevalence) and one of the most common gastrointestinal diseases worldwide. Intestinal intraepithelial lymphocytes (iIELs) represent one of the most abundant lymphocyte populations in the body and comprise phenotypically heterogeneous lymphoid subsets. The predominant phenotype of iIELs (>70%) are $CD3^+$ T cells, of which most expresses TCR-α/β (80%) and these T cells express mostly the CD8 antigen, while only nearly 10% express CD4. Increased numbers of human duodenal IELs are one of the key histological findings in CD. The current studies suggest a wide range of iIELs-namely, 5-60 iIELs/100 epithelial cells (ECs). This study aimed to determine the normal range and phenotype of distal duodenal IELs cell counts in the celiac patients and healthy individuals in Isfahan. Materials and Methods: Duodenal biopsy specimens were taken from the normal-looking distal duodenum of subjects, who were suspicious of CD. The iIELs cells were counted as density/100 ECs using the H&E staining method. The Immunoperoxidase staining method was also performed on the histological frozen and paraffin sections using several monoclonal antibodies. Results: The H&E staining method displayed 34 iIELs/100 ECs. In addition, the immunoperoxidase staining method also showed that the upper normal limit of iIELs counts (mean+2SD of iIELs counts in the controls), were 20 $CD3^+$ IEL T cells, and 14 $CD8^+$ IEL T cells/100 ECs. Conclusions: The H&E staining method showed 34 iIELs/100 ECs. Furthermore, the immunoperoxidase staining method showed iIELs counts lower than 20 for the CD3 marker and 14 for the CD8 marker/100 ECs. These values may consider normal among the general population in Isfahan. Geographic variations, food diets, and endemic infections alter the upper normal limit of iIELs counts. Hence, these factors must consider, when interpreting the duodenal biopsy specimens.
Keyword: Celiac disease; Duodenal Biopsy, iIELs, Normal Range, Isfahan

671. Relationship between Anti-β2GP1 Autoantibody and Acute Coronary Syndrome

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Background: Arteriosclerosis is a chronic autoimmune inflammatory disease that continues to be as the most common cause of mortality in the world. Atherosclerosis affects vascular walls, leading to coronary artery disease. Recent studies show that immune responses, including autoantibodies, play an important role in the morbidity of atherosclerosis. This study was conducted to assess the relationship between acute coronary syndrome and the serum level of anti-β2GP1 autoantibody. Materials and Methods: This cross-sectional study included 225 participants, 115 patients with acute coronary syndrome in the case group, and 110 healthy persons in the control group. A questionnaire was completed for all the patients and the control volunteers. After collecting the blood samples, the sera were obtained and kept at -20º C temperature until the time of examination. ELISA was applied to measure anti-β2GP1 autoantibodies in the sera of the studied groups for each of the IgM and IgG classes separately. The collected data was analyzed via t-test and Fisher's exact test, using the SPSS software. Results: The mean age was 53 in the case group, and 55 in the control group, and the participants were in the age range of 24-90. The means of the antibody levels of IgG and IgM classes in the two groups did not show a statistically significant difference (p=0.063). Conclusions: Our findings showed no relationship between anti-β2GP1 autoantibody and acute coronary syndrome. Therefore, anti-β2GP1 autoantibody probably does not have a role in creating vascular lesions leading to acute coronary syndrome. More studies are needed in this regard to investigate the role of this group of autoantibodies along with other risk factors.
Keywords: Autoantibody, Coronary artery diseases, IgG, IgM

672. Evaluation of L-glutamine Effects as a Non Toxic Heat Sshock Proteins Inducer on Fasting Blood Suger Levels and Pathological Lesions of Autoimmune Diabetes in Male C57BL/6 Mice

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Background: Previous studies demonstrated that immunomodulation maybe an helpful approach for treatment of diabetes type 1.L-glutamine,a non toxic inducer of heat shock proteins(HSP), has immunomodulatory effects .the aim of this study was to evaluate the effects of this drug on the level of fasting blood suger and pancreases lesions in male C57BL/6 diabetic mice. Materials and Methods: Diabetes was induced in male C57BL/6 mice. mice randomly allocated in 4 groups(N=5)(positive control, pre induction treatment ,post induction treatment, normal group).7 days after final streptozotocin (STZ) induced dosage, blood sample collected from mice and evaluated for fasting blood suger by automatic glucometer, in addition,14 days after final STZ induced dosage, mice were euthanized and pancreases were collected and stained with H&E .the slides were evaluated for leukocytic infiltration, oedema, congestion, degeneration and ticknessing of blood vessels in pancreatic langerhans'islands. Results:Mice that received L-glutamine showed significantly decreased levels of fasting blood suger and histopathological lesions of pancreases.($p<0.05$). Conclusion*:* L-glutamine has effective role in treatment of diabetes type1 and maybe used in new future for treatment of human diabetes type1.
Keywords: Suger Levels, Pathological Lesions, Autoimmune Diabetes, C57BL/6 Mice

673. Inverse Correlation of Serum Adenosine Deaminase (ADA) Activity and Tumor Necrosis Factor-α (TNFα) Concentration in chronic Heart Failure

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Background: Adenosine Deaminase (ADA) regulates adenosine concentration which is involved in the production of cytokines such as tumor necrosis factor-α (TNFα). In addition, ADA plays an important role in immune diseases and chronic heart failure (CHF) which has recently been considered as an inflammatory disease, however, the correlation of ADA activity and TNFα production has not been well understood. Materials and Methods: Blood samples were collected from 52 CHF patients and 55 healthy controls. The enzymatic activity of total ADA (tADA) as well as ADA1 and ADA2 isoenzymes was determined using Giusti method and an ELISA kit was used to determine TNFα concentration. Results: tADA activity was significantly lower in CHF patients compared to controls (19.29±9.73 and 24.3±6.01 U/L, respectively). ADA2 activity markedly decreased in CHF patients and showed a direct correlation with tADA (R^2=0.49, p<0001). In addition, the lowest levels of tADA and ADA2 activities were observed in patients with the worse stage of disease, according to the New York Heart Association classification (NYHA). CHF patients showed significantly higher TNFα level than controls (12.54±11.69 and 6.01±6.58 pg/ml, respectively) with the highest level being observed in NHYA-IV subgroup (17.23±13.15 pg/ml). Additionally, analysis showed a negative linear correlation between TNFα and adenosine deaminase activity. Conclusion: we showed a lower adenosine deaminase activity accompanied with a higher TNFα concentration in CHF patients compared with controls indicating an inverse correlation of TNFα concentration and adenosine deaminase activity. It can be concluded that adenosine deaminase may play important roles in the pathophysiology of heart failure.
Keywords: Adenosine deaminase, Heart failure, Isoenzymes, Tumor necrosis factor-alpha

Poster Presentation

674. Identification and Expression of The Toll Like Receptors (TLR) Responsible for Innate Bacterial Defense in The Ovine Reproductive Tract

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Background: The female reproductive tract is immunologically unique in its potential for inducing anergy to normal micro flora in lower parts of tract and response to the pathogenic bacteria so the upper parts of the female reproductive tract, including the uterine body, horn and tubes, are virtually free of organisms. Toll-like receptors (TLRs) are evolutionarily conserved innate immune receptors that recognize pathogen specific molecular pattern (PAMPs) in an efficient, non-selfreactive manner and initiate specific immune signaling that culminates in triggering antigen-specific adaptive responses. The aim of this study was to identify the expression of the Toll like receptors (TLRs) 2, 4, 5 and 6 responsible for evoking of innate bacterial defense in epithelial cells of non-pregnant. Materials and Methods: Using PCR techniques, distribution of TLR2, 4, 5, 6 . ewe's genital system including: vagina, cervix, body of uterus, uterine horns and fallopian tube. Results: Results showed that expression of TLR 2, TLR4 and TLR6 were high in body of uterus and in lower amount in uterine horns. They didn't express in vagina, cervix and fallopian tube of healthy animals. TLR5 was not express in these organs. Conclusion: These results can explain the cause of initiating of inflammation after entering of pathogenic bacteria to endometrium and higher incidence of metritis than vaginitis and cervisitis. Uterine tubes rarely exposed to bacteria because of anatomical and immunological characteristics of female genital tract so apparently these TLRs should not express in normal animal.
Keywords: TLR, Ovine Reproductive Tract, PCR

675. Evaluation of hs-CRP, Antioxidant Markers and MDA in Patients of Coronary Artery Disease (CAD) Containing Non-Smokers and Non-Diabetics

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Background: The oxidative stress and inflammation are cooperative events involved in atherosclerosis development. In the present study we assessed the association of MDA, antioxidant markers, high sensitive C - reactive protein (hs-CRP) and lipid status parameters in the patients with coronary artery disease (CAD). Significant risk factors such as cigarette and diabet were excluded from the study. Materials and Methods: Oxidative stress parameters for example Malondialdehyde (MDA), antioxidant markers including: erythrocyte superoxide dismutase(SOD), Glutathion peroxidase (GPX), Total antioxidant capacity(TAC), The inflammation marker and serum lipid status parameters were measured in 120 subjects including 60 CAD patients with angiographically diagnosed CAD and 60 CAD-free subjects as a control group, also diabetics, smoking patients, patients with malignancy, renal and liver disease, and other disease were excluded from the study. Results: The serum MDA and hs-CRP levels were increased significantly as compared to controls. However, erythrocyte SOD, GPX activities and TAC level were reduced significantly in patients ($P<0.05$ in all cases). The levels of total cholesterol, Triglyceride, LDL-C were significantly higher and that of HDL-C was meaningfully lower than those of control ($P<0.05$ in all cases). Conclusions: The association between oxidative stress parameters, antioxidant markers, the inflammation index and lipid status parameters suggest their involvement in atherosclerosis development that may lead to CAD progression.
Keywords: antioxidant, oxidative stress, coronary artery disease, inflammation, nonsmoker, nondiabetic

676. Effects of Mast Cells Stabilizer and Activator Agents on Experimental the Endometriosis in Rats

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Background: Endometriosis is a common gynecological disorder defined by presence of viable endometrial tissue outside the uterine cavity. It is associated with pain and infertility and affects 10–15% of women in reproductive age. Increasing evidence indicates changes in peritoneal environment including; inflammation, angiogenesis, fibrosis and mast cell (MC) accumulation in sites of peritoneal endometriosis. On the other hand MC plays a pivotal role in angiogenesis, fibrosis, wound healing, inflammation, and tissue remodeling. The exact role of MC in endometriosis pathogenesis is not clear yet. Thus the present study is focused at the effects of mast cells stabilizers or activators on endometriosis development in rat. Materials and Methods: 30 adult female rats were randomly divided into 3groups. Rats transplanted with endometrial segments. Tow group of animals received MC stabilizer (sodium cromolin-30 mg/kg) or MC activator (compound 48/80-100 µg/kg) 7 days after implantation intraperitoneally. Saline injected in shame group. 3 weeks after endometriosis induction, implants were evaluated with staining of Hematoxyline and Eosin, CD31 and Er for evaluation of endometriosis proliferation, angiogenesis and estrogen receptors respectively. Total antioxidant capacity (TAC) of serum measured too. Results: TAC properties in sodium cromolyn (SC) treated animals was higher but it was lower in 48/80 treated in comparison with sham group. Histological evaluations revealed that thickness of implanted endometrial tissue, glands and inflammatory cells infiltrations in SC treated group were lower than the sham group. On the other hand these parameters were higher in 48/80 treated animals. Immunohistological examinations are demonstrating that estradiol receptors and angiogenesis extend are significantly lower in SC treated animals but significantly higher in 48/80 treated than non-treated sham group. Conclusion: Based on our knowledge, this study revealed that SC as MC stabilizer shows protective but 48/80, a MC activator agent has promoting effects on experimental endometriosis induction in rat for first time.
Keywords: Mast Cells, Endometriosis, Rats

677. Evaluation of Angio-Geneic/Anti-Angiogeneic CXC Chemokines CXCL1(Gro-α), CXCL10 (IP-10), CXCL9 (Mig) and CXCL12(SDF-1) in Sickle Cell Patients

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Background: The sickle cell disease (SCD) is characterized by a mild chronic low-grade inflammation and endothelial activation as manifested by circulating leukocytosis and monocytosis along with increased soluble vascular cell adhesion molecules. Inflammation and angiogenesis are key phenomenon involved in the pathophysiology of SCD.Therefore, due to the fact that inflammation, angiogenesis/angiostasis phenomenon are of paramount importance in pathogenesis of SCD, we aimed and designed the present study to examine the serum levels of CXC chemokines CXCL1(Gro-α),CXCL10(IP-10),CXCL9(Mig) and CXCL12(SDF-1) involved in SCD. Materials and Methods: This cross-sectional study was conducted at the Kerman Special Disease Center and Rafsanjan Molecular Medicine Research Center during 2008 to 2010. 70 children and adults with sickle cell disease were enrolled in the study. The serum level ofCXCL1(Gro-α),CXCL10(IP-10),CXCL9(Mig) and CXCL12(SDF-1) were measured by ELISA (R&D systems, UK) in patients and healthy controls immediately after blood collection. Results: Our results showed elevated level of pro-angiogenic chemokines (CXCL12 and CXCL1) in sickle cell patiants.We also demonstrated that the serum level of anti-angiogeneic cxc chemokines (CXCL10 and CXCL9) were decreased in sickle cell patiants in compare to control.
Conclusion: The elevated level of these cxc chemokines confirms the involvement of a pro-inflammatory/pro-angiogenesis pathway in SCD. We may also conclude that the balance of angiogenesis/angiostasis is important in pathogenesis of SCD and cxc chemokines are crucial factors in the occurrence of these pathologies in SCD. Keywords: CXCL1(Gro-α), CXCL10(IP-10), CXCL9(Mig), CXCL12(SDF-1), Sickle cell disease

678. Changes of Inflammatory Cytokines in the Embolic Model of Cerebral Ischemia in Rat

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Background: Increased levels of cytokines including tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), Interleukin-6 (IL-6) and interferon gamma (INF-γ) have been recorded after the onset of ischemia and are usually associated with exacerbation of ischemic injury. Embolic stroke model is more relevant to the pathophysiological situation in patients, because the majority of ischemic injuries in humans are induced by old thrombi that originate from the heart and carotid arteries. Changes of inflammatory cytokines have not yet been investigated in this model. Therefore, the aim of the present study was to investigate changes of inflammatory cytokines after embolic stroke. Material and Methods: Rats were subjected to embolic stroke, induced by a natural old clot which was injected in to Middle Cerebral Artery (MCA), or sham stroke. At 48 h after stroke induction, the levels of 5 cytokines (ILs-1α and β, IL-6, INF-γ and TNF-α) were determined in 500 µg of total protein using the Bio-Plex Rat Cytokine Array (BioRad), as per the manufacturer's instructions in ischemic and non-ischemic cortices. Results: while stroke animals showed infarctions and neurological deficits, we did not observe any cerebral infarction and neurological deficits in sham-operated animals. Compared to the sham animals, level of ILs-1α ($P<0.001$) and β ($P<0.005$), Il-6 ($P<0.01$), TNF-α ($P<0.001$) and INF-γ ($P<0.05$) significantly were increased while no significant change in the level of Il-2 was observed. Conclusion: The findings of the present study suggest that part of ischemic injury in the embolic stroke may be mediated through the increased levels of inflammatory cytokines.
Keywords: Inflammatory Cytokines, Cerebral Ischemia, embolic stroke, Rat

679. Construction of a Library Containing Nanobodies against *CA IX* Antigen

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Backgrounds: *CA IX* is a member of the carbonic anhydrase family andplay a critical role in the pH regulation of tumours. Several researches indicate that *CA IX* expression is significantly increase in cervical and renal carcinomas.
In 1986, a monoclonal antibody withvery restricted expression pattern in normal tissues was described against CA IX. The aim of this study is to produce a library, containing camel single-domain antibody fragments(VHHs) against *CA IX* by phage display technique. Materials and Methods: *CA IX* exposed epitopes were bioinformaticaly analyzed and their DNA sequences were sewn together. After codon bias reformation the sequence was synthesized. The target sequence was subcloned and expressed inpET32a. RNA was extracted from lymphocytes of immunized camel. Nested PCR was performed for VHH amplification. PCR products were digested with SfiI restriction endonuclease and ligated to the pComb3x phagmide. *E. coli* TG1 cells were transformed with recombinant phagmids via electroporation.M13K07 helper phage was added to release phage particles when OD_{600} reached 0.6. Results: Phage particles encoding nanobodies with highest affinity toward *CA IX* antigen were isolated with panning.
Keywords: Nanobodies, *CA IX* Antigen

680. The Effect of Immunomodulator Fraction of Garlic (R10) on Producing Pre-Eclampsia in Pregnant Mice

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Background: According to the effect of garlic on immune system and relationship between immune responses and pregnancy outcome, we designed the study of immunomodulator fraction of garlic (R10) on main parameters in pregnancy period. Materials and Methods: One week after mating of 30 female BALB/c mice, they divided to four groups: received R10 fraction for one week, received R10 fraction for two week and their corresponding control groups. Interaperitoneal injection of 0.2 mg/Kg of fraction continues until 18th days of pregnancy. During this time, measurement blood pressure of mice and collection of their urine for protein. Results: Injection of 0.2 mg/Kg of R10 fraction for two weeks significantly increases systolic blood pressure, decline urine protein in non pregnant mice. Conclusion: Garlic isolated R10 fraction which is used in higher dose compare to immunostimulatory dose increase blood pressure. Since obtain result may cause preeclampsia, was suggested precaution use high dose of garlic and R10 fraction in pregnancy period and more study on pregnancy in human and animal models.
Keyword: Immunomodulator fraction of garlic (R10), Blood pressure, protein, preeclampsia, pregnant.

681. Comparison of Mean Sera Level of INF-γ and TNF-α in Rats Exposed to Wood, oil, Cigarette and Exhaust Smoke Compared to Control Group

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Introduction: Smoking exposure induces some changes in the level of INF-γ and TNF-α cytokines. These changes vary according to the type and the nature of the smoke. The current study aimed to compare changes of INF-γ and TNF-α level in rats exposed to wood, oil, exhaust and cigarette smoke as the case and those without any exposure as the control group. Materials and Methods: This cohort study was carried out on 40 male Wister rats placed in 5 groups, 8 rats each in a designed cage in order to expose them to smoke while the fifth group was kept away from any possible smoke. Four weeks later the blood was drawn from their heart of which sera was extracted and the sample sera were kept in -20° until they were used for measuring the INF-γ and TNF-α levels using ELISA. Results: Among the cigarette, exhaust, wood, oil and control group the mean INF-γ level was 22.26±8.74, 4.25±1.53, 3.40±2.92, 2.60±3.60 and 21.06±11.40 while the mean TNF-α level was 43.14±24.56, 40.1±9.1, 28.25±20.78, 13.22±13.13 and 46±24 respectively. The difference of mean INF-γ among different exposure groups was significant (P<0.001). Although such differences were also detected for the TNF-α level among different exposure groups but the difference was not significant. The mean INF-γ level was decreased significantly among rats exposed to wood and exhaust smokes. Among different exposure groups only mean TNF-α level of oil group was statistically significant compared to the control group (P>0.031). Conclusion: The mean INF-γ level in all smoke exposure groups, except for cigarette, has decreased in comparison with the control group as was seen for the mean TNF-α level. As a conclusion it can be said that decreasing in theses two cytokine levels for all groups that were exposed to smoke is an alarming indicator of suppression of immune system.
Keywords: smoke, immune response, exposure, INF-γ, TNF-α

IMMUNOPHARMACOLOGY

Oral Presentation

682. The Role of Toll-Like Receptor 4 in Central Neuroimmune Activation andDevelopment of Neuropathic Pain in a Rat Model of Neuropathy

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Background: Reports suggest that microglia play a key role in chronic constriction injury (CCI)-induced neuropathic pain. TLR4, a well-known receptor for lipopolysaccharide (LPS) in innate immune responses, has a substantial role in the activation of spinal microglia and the development of hyperalgesia and allodynia after nerve injury.In order to investigate the role of TLR4 on nerve-injury induced microglial activation and development of neuropathic pain in a rat model of neuropathy the present study was done. Materials and Methods: 48 male Wistar rats (230-270g) underwent surgery for induction the CCI model of neuropathy. The animals divided into sham-operated, CCI, vehicle and three CCI groups (n=8) that received minocycline (10, 20,40mg/kg;i.p) from post-operative day(POD)6-13, repeatedly for eight days. Thermal hyperalgesia (plantar test) and mechanical allodynia (Von-Frey test) was performed on POD13.Then animals were sacrificed, the lumbar segment of spinal cord collected for western blotting against anti-TLR4 antibody. The data were analyzed by SPSS and one-way ANOVA test for comparing the difference between groups (p<0.05). Results: The results showed that after surgery allodynia and hyperalgesia were developed in ipsilateral paw and minocycline(20,40mg/kg) significantly attenuated mechanical allodynia andthermal hyperalgesia on POD13 compare to CCI and vehicle groups (P<0.001). Our finding indicated that after CCI,the level of TLR4 increase in CCI and vehicle treated groups compare to sham-operated group, moreover, in groups that received minocycline (20,40mg/kg) the level of TLR4 decrease significantly compare to CCI and vehicle treated groups.
Conclusion: It is concluded that TLR4 plays important role in central neuroimmune activation after nerve injury and Inhibition of spinal microglia activation by minocycline decrease the level of TLR4 in dorsal horn and thereby reduced microglial activity and causes toattenuate allodynia and hyperalgesia after development of neuropathic pain.
Keyword: Neuropathic pain, Microglia, Analgesia; TLR4, Minocycline

683. Nitric Oxide and Hydrogen Peroxide Production in Neutrophils and Monocytes in Patients with Urinary Tract Infection caused by *E.Coli* before and after Ciprofloxacin Therapy

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Background: Antibiotics are widely used as bacteriostatic and bactericidal drugs for therapy in bacterial infections. Besides the respective interaction between antibiotics and bacteria and between immune system and bacteria, antibiotics also directly interact with immune system and have various immunomodulatory effects on phagocytosis, chemotaxis, cytokine production and endotoxine release. In this study, the effect of Ciprofloxacin on NO and H2O2 production by monocytes and neutrophils in patients with urinary tract infection caused by *Escherichia coli* (*E. Coli*) was investigated. Materials and Methods: Before and after therapy with 500 mg ciprofloxacin tablets (pretreatment and post treatment respectively) results were compared to an age and sex-matched reference group. Peripheral blood samples from 45 patients with uUTI were collected at diagnosis and the end of treatment. Monocytes and neutrophils were separated from blood samples and cultured in presence of Ciprofloxacin and activators including IFN-γ and LPS or PMA (for in vitro evaluation) or only the activators (for ex vivo evaluation). Supernatant of the cell culture were collected and production of NO and H2O2 was measured. Results: The results showed that pre-treatment and post treatment NO and H2O2 production were significantly increased compared to the control group ($P<0.05$). NO level in the post treatment group was also significantly increased compared to the pre-treatment group ($P<0.05$), but H2O2 level was not significantly altered ($P>0.05$). Conclusion: It was indicate that in vitro results are different from ex vivo ones and significantly alteration was not seen between pre-treatment and post treatment groups ($P>0.05$).
Keywords: Nitric oxide, Hydrogen peroxide, Urinary tract infection, Antibiotic, Ciprofloxacin, Monocyte, Neutrophil

684. Therapeutic Effect of all-*trans* Retinoic Acid on Experimental Autoimmune Encephalomyelitis and its Role T helper Lymphocytes Responses

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Background: Resent studies demonstrated an essential role for IL-17 in pathogenesis of experimental autoimmune encephalomyelitis (EAE).Furthermore; it has been shown that FoxP3$^+$Treg cells play an important role for suppression of autoinflammatory reactions. Although, previous studies have determined the immunomodulatory potential of all-*trans* retinoic acid (ATRA), the immunomodulation was mostly justified to alteration in Th1/Th2 cytokines. The present study was carried out to investigate the therapeutic effect of ATRA on EAE and its effects on T-helper cells responses. Materials and Methods: EAE was induced by MOG_{35-55} peptide and complete Freund's adjuvant in female C57BL/6 mice. Mice were placed in two therapeutic groups (n=7 per group). Treatment with ATRA (500 µg/mouse-every other day) was initiated 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 33 when mice were sacrificed. Splenocytes were tested for proliferation by MTT test, cytokine production by ELISA and FoxP3$^+$Treg cells frequency by flow cytometry. Results: ATRA significantly reduced the clinical signs of established EAE. Aside from decreased proliferation, ATRA significantly inhibited the production of pro-inflammatory IL-17 as well as IFN-γ upon antigen-specific re-stimulation of splenocytes. FoxP3$^+$Treg cells frequency and IL-10 level were not altered significantly. However, IFN-γ to IL-10 and IL-17 to IL-10 ratios decreased significantly. Conclusion: Parallel to reducing autoreactive lymphocyte proliferation and cytokine production in favor of pro-inflammatory cytokines, ATRA ameliorated established EAE.
Keywords: Multiple sclerosis, Experimental autoimmune encephalomyelitis, All-*trans* retinoic acid, lymphocyte response.

685. Suppressive Effect of Cyclosporine A on Vascular Endothelial Growth Factor (VEGF) Isoforms

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Background: VEGF has a key role in transplantation and angiogenesis. Pharmacokinetics of some immunosuppressive drugs and outcomes of transplantations are altered by inheritance of some gene polymorphisms. Our aim in the present study was to determine the suppressive effect of cyclosporine A on all known VEGF isoforms, including VEGF 121, VEGF 145, VEGF 165, VEGF 189 and VEGF 206 in the individuals with VEGF gene polymorphisms -1154 G and -2578 C. Materials And Methods: Expression of VEGF isoforms in individuals with VEGF –1154 G and –2578 C genotype was determined in cyclosporine A-treated peripheral blood mononuclear cells (PBMCs) by TaqMan real-time RT-PCR. Results: The relative amounts of VEGF 165 isoform were significantly suppressed after cyclosporine A treatment comparing to VEGF 121 and VEGF 189. The mRNA expression of VEGF 121, VEGF 165 and VEGF 189 seemed to be associated to VEGF -1154 G rather than -2578 C polymorphisms. Additionally, gene expression of VEGF 145 and VEGF 206 in PBMCs was very low and no statistically difference has been found between treated PBMCs with cyclosporine A and not-treated cells. Conclusion: The AP-1 binding activity to the VEGF promoter is known to be decreased by cyclosporine A. Suppression of VEGF isoforms by cyclosporine A in our research might be related to the above mentioned effects. The influence of VEGF -1154 G on the VEGF isoforms might be related to the effects of this polymorphism on the transcription factor binding sites in the promoter of VEGF gene.
Keywords: Cyclosporine A, VEGF, polymorphisms

686. Effect of Silymarin on Cell Cycle, IL-2 Production and IL-2 Receptor Expression in Activated T Cells from C3H/HeN Mice

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Background: Silymarin, a mixture of bioactive flavonolignans isolated from Silybum marianum, exhibits anti-carcinogenic, anti-inflammatory and cytoprotective effects. In this study, the in vitro immunomodulatory activity of silymarin was investigated using lymphocytes from lymph nodes of C3H/HeN mice. Materials and Methods: Cell culture results revealed that 50 µM silymarin significantly induced cell cycle arrest at G1 phase in αCD3/αCD28 mAb activated T cells; however no inhibition of cell cycle was detected at lower concentrations. The inhibitory effect of silymarin on lymphocyte proliferation was not due to induction of cell apoptosis, since the proportion of apoptotic cells at SubG1 phase did not change significantly. In order to determine whether an IL-2-dependent immune suppression could be induced by silymarin, production of IL-2 at a protein and mRNA levels was determined by ELISA and RT-PCR analyses. Results: Results indicated that 50 µM silymarin significantly inhibited IL-2 production, both at protein and mRNA levels. Interestingly, the inhibitory effect of silymarin on IL-2 production was also associated with a decrease in IL-2 receptor α (CD25) expression on anti-CD3 activated T cells. Since the overexpression of Glucocorticoid-Induced Leucine Zipper (GILZ) is able to inhibit IL-2 production and IL-2 receptor up-regulation induced by T cell activation, GILZ expression was evaluated in lymphocytes after 6 h simultaneously incubation with αCD3/αCD28 mAb and silymarin using RT-PCR. No significant change of GILZ mRNA expression was detected in anti-CD3 activated lymphocytes. Conclusion: Further studies will be needed to evaluate silymarin as an attractive candidate for development of an immunomodulatory drug.
Keywords: Silymarin, Cell Cycle, IL-2, IL-2 Receptor, C3H/HeN Mice

687. Immunomodulation of Peripheral Blood Mononuclear Cells by PHA and Irradiated K562

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Background: Natural killer (NK) cells are an important subset of cytotoxic lymphocytes and are best characterized by their ability to spontaneously kill virally infected and tumor cells but the mechanisms contributing to deficient NK activity in patients with cancer remains unclear. NKp44 and NKG2D are of the main NK activating receptors involved in recognition and killing of tumors. Here we studied the stimulatory effects of K562 on induction of NKp44 and NKG2D expression on human PBMCs. Materials and Methods: The NK activity of PBMCs against DU-145 was determined with 51Cr-release assay. The PBMCs were stimulated with PHA, on 3 occasions (3-PHA-PBMC) and were incubated with irradiated K562 (iK562). The expression of CD56, NKG2D and NKp44 were detected with reverse transcription-PCR and flow cytometry. Results: PHA stimulation increased the proportion of CD56+ cells and up-regulated NKG2D and NKp44 expression. Co-incubation with iK562 didn't change NKG2D but increased NKp44 expression. NK activity of 3-PHA-PBMC after co-incubation with iK562 was significantly increased. Conclusion: Our results demonstrated that the mitogen and iK562 exposure to PBMCs can significantly improve NK activity which is related to the higher expression of NKp44 and NKG2D. These data may help to improve cancer immunotherapy protocols.
Keywords: NKp44, NKG2D, Natural Killer activity, Immunomodulation

688. The Immunomodulatory Effect of Florida Plorutus on Macrophage Response against Candida Albicans

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Background: The evaluation of immune responses is considered as a useful approach for proper diagnosis and appropriate therapeutic protocols in various diseases including infections, cancer, autoimmune, immunodeficiencies etc. An increase or decrease in immune parameters could be an important criterion of disease status. Immunomodulators are compounds capable to modulate different parts of immune responses. Herbal plants and fungi are considered as a significant source of immunomodulators. There are numerous studies indicating that serious and recurrent fungal infections are seen mostly in somehow immunodeficient patients, at the other hand candidal sepsis is followed by vigorous and harmful inflammatory responses. These points along with drug resistance of Candida and other fungal species encourage the attempts to find a useful immunomodulator. In the present study the immunomodulatory effect of Florida plorutus on innate immune response against Candida is considered. Materials and Methods: The extract and various fractions of Florida plorutus (R100, R50, R30, R10, R5 and F5) were prepared. Peritoneal macrophages of Balb/C mice were separated, washed, counted and cultured in 96-well microtiter plates. The extract and fractions were added at various concentrations to the wells, then the Candida albicans were added. Vital activity of macrophages were assayed using MTT. Statistical analysis using ANOVA and $P<0.05$ were performed. Results: A statistically significant increase in macrophage total activity was observed at high concentrations of the extract and its fractions at macrophage to Candida. Conclusion: The results of this study indicates that the effect of the extract and its fractions is dose dependent with positive (increasing) effect at high concentrations and negative (decreasing) effect at low concentrations on total activity of macrophages.
Keywords: Candida albicans, Florida plorutus, macrophage.

Poster Discussion Presentation

689. Effect of Compounds of Vanadium on Necrosis and Apoptosis of k562 Erythroleukemia Cell Line

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Background: Cancer is the most popular agent of mortality in human being, after cardiovascular system disease and accident, so there is great interest on research of cancer in the diagnostic, treatment and prophylactic points of view. In the present study, the apoptotic and necrotic effects of compounds of vanadium on k562 erythroleukemia cell line were studied. Materials and Methods: In the first step the effective dose range was determined by MTT test, and then 150,250 and 350µg/ml of compounds were used to determined necrotic as well as apoptotic death of cells, using commercial available Annexin V/PI kit and flow cytometry. The results of MTT test showed that the effective dose range of these compound is 25-400µg/ml, the highest cell death was happened in 350µg/ml concentration after 48 hours incubation(83-89%),In comparison to doxorubicin(as standard), these compound killed cells for necrosis rather than apoptosis pathway. Results : the results of apoptosis and flowcytometry examination showed that in mentioned densities the best effect is after 12 hour care with vanadium compounds,and the more apoptosis occure in dose of 350 µg/ml is 37.96%,also the results from MTT indicate that 150,250,350 µg/ml of vanadium compounds on cells of k562 cell line cause a worthy and meaningful decrease in the number of existing cells,in away that after 48 hours of decrease,cellular percent in comparison to cells which not affected by vanadium compounds was meaningful at this level $p<0.001$. Conclusion: we found that these compounds of vanadium had variable but significant effect on killing of k562 erythroleukemia cell line and vanadium shift –base compounds have cytotoxic and anticancer effect in k562 cell line in comparison with T cells in normal cell line.
Keyword*:* Compounds of vanadium, k562, Apoptosis, MTT, Flow cytometry

690. Growth Inhibitory Effect of *Foeniculum vulgare* on Human Leukemia Cell Lines

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Background: *Foeniculum vulgare* (Apiaceae) is widely being used for medical purposes in folk medicine. In the present study we investigated the possible inhibitory and apoptotic effects of this medicinal plant on hematopoietic cancer cell lines. Materials and Methods: Two leukemia cell lines, Jurkat T cell leukemia and k562 myeloid leukemia were treated by various concentrations of dicholoromethane, n-hexane, n-butanol, and water fractions of *Foeniculum vulgare*. MTT (3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)colorimetric assay was used in order to determine the growth inhibitory effect of the fractions on the cells. The half maximal inhibitory concentration (*IC50*) *value* was determined. The fraction with maximum inhibitory effect was selected for AnnexinV /PI flow cytometry assay to find the possible induction of apoptosis. Results: Dichloromethane fraction of *Foeniculum vulgare* decreased the growth of K562 cells at concentrations 50, 100 and 200µg/ml. The IC50 values in 24- and 48 hour-cultured K562 cells in the presence of the fraction were 68.3 and 26.48 µg/ml, respectively. The same as K562 cells, Jurkat cells growth was inhibited at concentrations 50, 100 and 200µg/ml of Dichloromethane fraction. The IC50 value in 24 and 48 hours treatment of these cells with the fraction obtained 66.13 and 21.04 µg/ml,respectively. By AnnexinV /PI assay on K562 and Jurkat cells it was found that the dicholoromethane fraction of *Foeniculum vulgare* at concentration of 200µg/ml induced cell death after 48 hours treatment. Conclusion: This study exhibited that *Foeniculum vulgare* can inhibit the leukemia cells growth through induction of apoptosis in a time and dose dependent manner.
Keywords: Growth Inhibitory Effect, *Foeniculum vulgare*, Human Leukemia Cell Lines

691. The Effect of Diazinon on the Reproductive Hormones and Cytokines Production Gamma Interferon, Interleukin- 4 and 10 Levels in the Male Rats

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Background: Diazinon is one of the organophosphate pesticides of wide spectrum insect- killing power. Diazinon extensive application as an effective pesticide was associated with direct or indirect modulation of major and vital sex hormones and immune mechanisms. In the present study investigated the effect of diazinon toxicity on the level of sex hormones including FSH, LH, and Testosterone and on cytokines Gamma Interferon, Interleukin- 4 and 10 levels in the male rats. Materials and Methods: twenty four male rats divided to 4 groups. Intoxicated at doses of 30mg (Group1), 3mg (Group2), and 0.3mg/kg (Group3) per body weight for 30 days (Five injections per week) administrated intra-peritoneal. Control group were kept and fed in the same environment of that of the toxicated rats. Rats of each group were then scarified after the latest injection. Blood samples were collected after centrifuge, serum were separated and stored –70^{o}C for determination of sex hormones FSH, LH, Testosterone and IFN-γ, IL-4, IL-10 cytokines by using ELISA procedure. The data were analyzed with One Way ANOVA and LSD post hoc tests. Results: Mean of LH, FSH, Testosterone, IFN-γ, IL-4, IL-10 and body weight were 0.93(±0.28), 0.13(±0.015), 1.03(±1.02), 0.26(±0.32), 59.92(±119.46), and 28.54(±14.7), respectively. There was no significant difference between LH, FSH, Testosterone, IFN-γ, and IL-10 ($P>0.05$). But a significant difference was found in IL-4 level and body weight ($P<0.05$). Conclusion: In this investigation the positive effect of diazinon on Testosterone, IL-4 levels and body weight. No observable immunotoxicity effect is expected.
Keywords: Diazinon, sex hormones, Immunotoxicity, cytokines

692. Umbelliprenin is Cytotoxic against QU-DB Large Cell Lung Ccancer Cell Line but Antiproliferative against A549 Adenocarcinoma Cells

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Background: Umbelliprenin is a prenylated compound, belonging to the class of sesquiterpene coumarins. Recently, umbelliprenin has attracted attention for its antitumor activities against skin tumors. Its effect on lung cancer is largely unknown. The aim of our study was to investigate the effects of this compound on lung cancer. Materials and Methods: The QU-DB large cell and A549 adenocarcinoma lung cancer cell lines were treated with umbelliprenin. IC50 values were estimated using methyl thiazolely diphenyl-tetrazolium bromide (MTT) assay, in which a decrease in MTT reduction can be as a result of a cell death or a decline in cell proliferation. To quantify the rate of cell death at IC50 values, flow cytometry using Annexin V-FITC (stains apoptotic cells), and propidium iodide (stains necrotic cells) dyes was employed.Results: Data from three independent MTT experiments in triplicate revealed that IC50 values for QU-DB and A549 were 47 ± 5.3 μM and 52 ± 1.97 μM, respectively. Annexin V/PI staining demonstrated that umbelliprenin treatment at IC50 induced 50% cell death in QU-DB cells, but produced no significant death in A549 cells until increasing the umbelliprenin concentration to IC80. The pattern of cell death was predominantly apoptosis in both cell lines. Conclusion: We found that umbelliprenin is cytotoxic for a large cell lung cancer model and antiproliferative against adenocarcinoma lung cancer cells, indicating its possible different anti-tumor mechanisms in different types of lung cancer.
Keywords: Annexin, Methyl thiazolely diphenyl-tetrazolium bromide, Propidium Iodide, Umbelliprenin, Lung cancer

693. Evaluation of the Anti-Inflammatory effect *Migri-Heal*® on the Immune Cells in Central Nervous System (CNS)

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Background: Evidence from other study's implicates the involvement of glia cells in the neurodegenerative process of several degenerative neurological diseases, including Alzheimer's disease and Parkinson's disease. It remains to be determined, however, whether glial activation plays a role in the initiation stage of disease progression or occurs merely as a response to neuronal death. Activated glia secrete a variety of pro-inflammatory and neurotoxic factors for instance nitric oxide (NO) that are believed to induce and/or exacerbate neurodegeneration. *Migri-Heal*® is an herbal medicine therapeutic drug for migraine headaches. This Iranian drug has a formulation that derived from traditional medicine and modern medicine and pharmacy. This Iranian drug has been registered by number 1228143083-IRC at the Ministry of Health and Medical Education. However, the effects of*Migri-Heal*® on the immune functions of glial cells in CNS have not been well characterized. Materials and Methods: For examining the anti-inflammatory effects of the *Migri-Heal*®, the rat brain mix glial cells were inflamed with LPS, and treated with different concentrations of drug. Then inflammation and survival of Glial cells were investigated by Griess Reagent System and MTT tests, respectively. Results: Results of Griess and MTT tests disclosed that particular doses of drugs reduce inflammation. In contrast, at higher doses, cytotoxic effects of drug was clear. Thus *Migri-Heal*®deserves more attention to be studied as an anti-neuro-inflammatory agent.
Keywords: Anti-Inflammatory effect, *Migri-Heal*®, CNS

694. Evaluation of Doxorubicin Cytotoxicity in Combination with Daidzein against Human Breast Cancer Cells (MCF-7)

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Background: Doxorubicin is a broad spectrum antibiotic used as chemotherapeutic drug in the treatment of breast cancers. Daidzein which is one of the main soy isoflavone are shown to be cytotoxic in high concentrations. Co-administration of daidzein and chemotherapeutic agents suppose to be synergic although there are some controversies in the literature. Materials and Methods: In this study, cytotoxic effects of doxorubicin alone and in combination with daidzein on MCF-7 breast cancer cell line (in vitro) were evaluated. Different concentration of doxorubicin and daidzein were added to the cultured cells and incubated for 24 h. Cell lysis was evaluated using cytotoxicity detection kit (LDH kit). Results: The results indicated that doxorubicin 100 μM cytotoxicity effects on MCF-7 cells in combination with diadzein 150, 125 and 100 μM enhanced from 65% cytoxicity to 74%, 80% and 88%, respectively. However, doxorubicin cytotoxicity in 200 μM in combination with diadzein 150, 125 and 100 μM decreased from 100% cytoxicity to 80±2.5%. Moreover, We also demonstrated that MCF-7 treatment by diadzein in concentration 200 μM in combination with doxorubicin 75 and 50 μM reduced diadzein cytotoxicity from 100% to 81% and 62%, respectively. Conclusion: These data suggest that combined treatment with doxorubicin in combination with daidzein against human cancer cells could be a novel and attractive strategy to potentiate the doxorubicin -Induced Antitumor Activity by regulation of both doxorubicin and daidzein concentration. Given the widespread nutritional administration of soy isoflavones to women with breast cancer, considering and understanding soy isoflavones such as daidzein effects and mechanisms is essential for achieving optimal therapeutic responses. Most important, for the first time, the authors demonstrated that the the efficiency of combination treatment of breast cancer by doxorubicin in combination with daidzein is regulated in dose-dependent manner.
Keywords: Doxorubicin, Daidzein, MCF-7 breast cancer cell line, Cytotoxicity

695. In vitro *Immunomodulatory Effect of R10 Fraction of Garlic on CD8+ T Lymphocytes Viability and Production of TNF-α*

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Background:T-cells, especially CD8 + T lymphocytes are the most important cells in anti-tumor response. Previously R10 fraction of garlic extract was reported as an immunomodulator which induced an effective cellular immunity and Th1 responses. In this study the in vitro immunomodulatory effect of R10 on $CD8^+$ T cells viability and production of TNF-α was evaluated.Materials and Methods: CD8 + Tcells were isolated bymagnet bead method fromspleen cells of Balb/Cmice.R10fractionwas preparedusingultrafiltration. MTT assay was used to evaluate cell viability. TNF-α level were measured in the supernatant d by ELISA. Results:The findings indicate that all dilutions of R10 fraction increased cell viability of $CD8^+$ T cells in comparison with negative control group and in the presence of ConA dilution of 1:50 of R10 fraction significantly increased cell viability of $CD8^+$ T Cells. Secretion of TNF-α was significantly increased by all dilutions of R10 fraction. Conclusion: these findings suggest that R10 fraction of garlic can be used as an Immunomodulator drug candidate for induction of cellular Immunity.
Keywords: CD8+ T cells, R10 fraction, garlic, TNF-α, Viability

696. Isolation of a NovelIndigenous MPA Producer*Penicillium*Strain forProduction of a Preventer ofOrgan TransplantationRejection

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Background: Mycophenolic acid (MPA) is an antibiotic and immunosuppressant drug used to prevent rejection in organ transplantation. MPA and its derivatives, have diverse biological properties, and have been extensively studied for their antineoplastic, anti-inflammatory, antiviral, anti-psoriasis, and antifungal activities. MPA is produced by several *Penicillium*spp, among them *P.brevicompactum* and *P.stoloniferum*indicate a relatively high ability of MPA production. Materials and Methods: In this study in order to isolation of a native MPA producer strain,140 *penicillium*sppwere isolated. The isolated fungi were collected from about 50 samples of soil from forest, greenhouse, and soil in agricultural lands and antibiotic production factory,and mouldy food, fruit and dairy products.All of the *Penicillium*isolates were cultivated in liquid medium for 12 days. Then the supernatants were separated and production ofMPA, was analyzed by HPLC. Results and Conclusion: Three strains with highest MPA productionwere selected as the best. Identification of the best MPA producer strain was performed based on macroscopic and microscopic studiesincluding comparison of hyphae and spore surface as well as alignment of 18srDNA sequencebased on NCBI data bank as molecular analysis. Finally, *Penicilliumglabrum* was identified as anovel native MPA producer strain. The isolated *Penicilliumglabrum*will be usedfor production of MPA as a rejection preventer in organ transplantation.
Keywords: MPA,*Penicillium*, Preventer of Organ Transplantation Rejection

697. Daidzein and Non-Isoflavone Soy Protein Isolate Effects on Cytotoxicity of Estrogen-Dependent Breast Cancer Cells (MCF-7) in a Dose-dependent Manner

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Background One of the predominant isoflavone components of soybeans is daidzein. Soy isoflavones are a class of estrogen-like compounds that have become widely used among postmenopausal women as a 'natural' alternative to hormone replacement therapy. Isoflavones have been considered to reduce the risk of cancer and to have potent anticarcinogenic activities, whereas the risks and benefits of diets and supplements containing the estrogenic soy isoflavone daidzein are not well established. On the other hand, it has been reported in some studies that the health effects of soy supplements and soy isoflavone supplements may differ, so other components in soy may suppose to influence human breast cancer differently. Materials and Methods: In this study, cytotoxic effects of daidzein and Non-Isoflavone Soy Protein Isolate (SPI) on MCF-7 breast cancer cell line were evaluated. Different concentration of daidzein and Non-Isoflavone SPI were added to the cultured cells and incubated for 24 h. Cell lysis was evaluated using cytotoxicity detection kit (LDH kit). Results: The results indicated that diadzein increased cytotoxicity of MCF-7 cells in comparison with low negative control samples with high concentration 125, 150, 200 and 300 μM by by 21%, 55% , 100% and 100% cytotoxicity, respectively. However, diadzein treatment decreased cytotoxicity of MCF-7 cells in comparison with low negative control samples with low concentration 100, 50, 20, 2 and 1 μM by 9%, 18%, 14%, 10% and 10%, respectively. Furthermore, Non-Isoflavone SPI treatment reduced cytotoxicity of MCF-7 cells in comparison with negative control samples with concentration 1000, 500, 200, 100 and 50 μM by 10±2.5%, respectively. Conclusion: Here we present that the cytotoxicity effects of daidzein against human cancer cells could be regulated in a dose-dependent manner. Moreover, this is the first report of cytotoxicity decreasing effects of Non-Isoflavone SPI.
Keywords: Daidzein, Non-Isoflavone Soy Protein Isolate (SPI), MCF-7 Breast Cancer Cell Line

Poster Presentation

698. Additive Effect by Combination of Tretinoin and Atorvastatin in Treatment of Experimental Autoimmune Encephalomyelitis

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Background: One suitable approach to enhancing multiple sclerosis (MS) treatment is combination of available medications to provide more desirable outcomes. Immunomodulatory effects of Tretinoin and Atorvastatin were determined in previous studies. Here, we investigated synergistic/additive effects of combination therapy by suboptimal doses of Tretinoin and Atorvastatin in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Materials and Methods: EAE was induced by MOG_{35-55} peptide and CFA in female C57BL/6 mice. Mice were placed in four therapeutic groups (n=7 per group) and treated intraperitoneally with optimal doses of Tretinoin or atorvastatin, suboptimal doses of combination (half doseof eachdrug) or vehicle alone, respectively. Treatmentwas initiated at day 12 post induction when the treatment group developed a neurologic disability score. Signs of disease were recorded daily until the day 33 when mice were sacrificed. Splenocytes were tested for proliferation by MTT test, cytokine production by ELISA and $FoxP3^+$Treg cells frequency by flowcytometry. Moreover, brains and spinal cords were removed for neuropathological analysis.
Results: Combined treatment with suboptimal doses performs better than their individual administrated optimal doses in established EAE as evidenced by decreased clinical score, improved weight gain, reduced neuroinflammation and demyelination. Without further anti-proliferative effect, combination treatment attenuated pathogenic IL-17 and IFN-γ cytokines and conversely, increased anti-inflammatory IL-10 greater than either drug alone upon antigen-specific restimulation. Furthermore, $FoxP3^+$Treg cells were significantly increased, only in combination treatment. Conclusion: Therapeutic treatment with combination of suboptimal doses of Tretinoin or atorvastatin provides additive effects and leads to better outcomes in EAE mice than optimal doses of either drug alone. Therefore, this pharmacological approach may be as a useful strategy to control MS.
Keywords: Multiple sclerosis, Experimental autoimmune encephalomyelitis, Tretinoin, Atorvastatin

699. Atorvastatin Alleviated Clinical Symptoms of Experimental Autoimmune Encephalomyelitis via Downregulation of Pro-inflammatory and Upregulation of Anti-inflammatory T cell responses

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Background: Recent studies demonstrated an important role for Th-17 and FoxP3$^+$Treg lymphocytes in pathogenesis of autoimmune diseases .Although, previous studies have determined the immunomodulatory potential of statins (such as atorvastatin), the immunomodulation was mostly justified to alteration in Th1/Th2 cytokines. The present study was carried out to investigate the therapeutic effect of atorvastatin on experimental autoimmune encephalomyelitis (EAE) and its effects on T-helper cells responses. Materials and Methods: EAE was induced by MOG_{35-55} peptide and complete Freund's adjuvant in female C57BL/6 mice. Mice were placed in two therapeutic groups (n=7 per group). Treatment with atorvastatin (10mg/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 33 when mice were sacrificed. Then, splenocytes were tested for proliferation rate by MTT test, cytokine production by ELISA and FoxP3$^+$Treg cells frequency by flowcytometry.
Results: Atorvastatin significantly decreased the clinical signs of established EAE. In line with reduced proliferation, atorvastatin inhibited the production of pro-inflammatory IL-17 as well as IFN-γ upon antigen-specific re-stimulation. In addition, the level of anti-inflammatory IL-10 was significantly increased. However, the frequency of FoxP3$^+$Treg cells did not alter significantly. Conclusion: Along with decreasing autoreactive lymphocyte proliferation and cytokine production in favor of pro-inflammatory cytokines, atorvastatin ameliorated established EAE.
Keywords: Multiple sclerosis, Experimental autoimmune encephalomyelitis, Atorvastatin , lymphocyte response.

700. Effect of Ginger Extract on Experimental Autoimmune Encephalomyelitis (EAE)

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Background: The progression and development of multiple sclerosis (MS) is associated with inflammation. Ginger has been observed to have anti-inflammatory effect in experimental and clinical models, but there is no data about its effects on multiple sclerosis (MS) or EAE, as the animal model of MS. This study was done to explore the above issue. Materials and Methods: female C57BL/6 mice (5-7 weeks old) were immunized on day 0 with injection of an emulsion of MOG peptide and complete freund's adjuvant containing mycobacterium tuberculosis to induce EAE. The mice were intra peritoneal (I.P) administered with either vehicle (PBS) in control group or ethanol extract of ginger (200mg/kg BW, every other day) from day +3 to +40 in treatment group and day -14 to +10 in prophylactic group. The EAE clinical scores were evaluated till day 40. Results: The ethanol extract of ginger significantly delayed the onset, reduced the peak clinical score and cumulative disease index of EAE and prevented or significantly attenuated relapses in treated groups compared with controls. Pathological study of CNS tissue of the mice in treated groups also showed less cell infiltration compared to controls. Conclusion: Ginger showed a significant prophylactic effect on EAE. Considering the long term use of it in different nations and its proved safety, it is suggested that ginger has anti-inflammatory effect, and its daily use may be beneficial for prophylaxis and dampen the progress of MS.
Keywords: Ginger Extract, EAE, Mice

701. Potential Role of STI-571 in the Treatment of EAE

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Background: Multiplesclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that leads to an inflammatory demyelination, axonal damage and progressive neurologic disability. STI-571 is a selective protein tyrosine kinase inhibitor that abrogates multiple signal transduction pathways implicated in auto immune diseases. The aim of the present research was to test the therapeutic effect of STI-571 in experimental model of MS (EAE). Materials and Methods: The experiment was done on 7- to 9-weeks-old female C57BL/6 mice.
EAE induction was performed by Hooke Kit. The kit consists of antigen (MOG_{35-55}) in CFA emulsion, and pertussis toxin (PTX) in PBS. The mice were injected subcutaneously on upper back and lower back with 0.1 ml of emulsion respectively. Within 2 hours of injection of the emulsion, the first dose of PTX (0.1 ml per mouse) was injected intraperitoneally. 22-26 hours after injection of the emulsion, the intraperitoneally injection of second dose of PTX into the mice (0.1 ml) were done. The mice were administered orally with STI-571 at the specified dose (60 mg/kg) from day 7 after immunization on six consecutive d per wk for 2 wk. The mice were sacrificed on day 35 post-immunization. Brains, cerebellums and lumbar spinal cords were removed, post-fixed in formalin, embedded in paraffin, sectioned and then stained with Luxol fast blue (LFB) and with eosin and hematoxylin. U87-MG and WEHI-164 cell lines were grown in RPMI media and incubated with rising concentrations ofSTI-571and evaluated MMP-2 expression in two proposed cell lines. Results: Our findings showed that treatment with STI-571 caused a significant delay in the time of onset and a significant reduction in severity of the EAE in treated animals compared with normal groups. Conclusion: Our results suggest that FDA-approved drug STI-571 has a potential therapeutic effects on EAE as an autoimmune demyelinating disease.
Keywords: STI-571, Treatment, EAE

702. Immunoglobulins in Opium Users

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Background: There are a few studies with conflicting results on the effects of opium on the functioning of immune system. The aim of this study was to evaluate concentrations of the serum immunoglobulins IgG, IgM, and IgA in opium (*thariac*) users. Materials and Methods: 44 chronically opium-addicted persons, who voluntarily enrolled for detoxification and 44 sex- and age-, matched healthy individuals with no history of drug abuse as the control group was enrolled to this cross-sectional, case-controlled study. Opium addiction was defined as consume more than 2 grams of opium per day for at least 1 year. They absolutely did not abuse other drugs. Serum samples were collected from all participants and tested for the serum immunoglobulins by use of SRID method. Statistical analysis was performed using Student t test. Results: Results showed a significant increase of serum IgG and IgA in the opium addicts (1592.59±218.75 and 374.81±68.91, respectively) compared to those in the controls (1406.63±238.97 and 221.84±95.52, respectively). The serum IgM of the opium addicts was higher compared with the controls, but the differences were not significant. Conclusion: IgG elevation may be due to bacterial or other contamination. The higher IgA in opium users is postulated to reflect local antibody synthesis.
Keywords: Opium addicts, IgG, IgM, IgA

703. Cytokine Profiles in Chronic Opium Addicts

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Aim: The aim of this study was to evaluate the serum level of interleukin (IL)-4, IL-10 and IL-17 in opium addicts. Materials and Methods: Forty-four chronically opium-addicted persons, who voluntarily enrolled for detoxification and 44 sex- and age-, matched healthy individuals with no history of drug abuse as the control group from the same geographical area was enrolled to this cross-sectional, case-controlled study. Opium addiction was defined as consume more than 2 grams of opium per day for at least 1 year. They absolutely did not abuse other drugs. Enzyme-linked immunosorbent assays were used to determine IL-4, IL-10 and IL-17 concentrations in serum. Statistical analysis was performed using Student's unpaired *t*-test. Results: Results showed a significant increase of mean serum levels of IL-17 and IL-10 in the opium addicts

(1.9±0.06 and 95.48±13.05, respectively) compared to those in the controls (1.6±0.01 and 65.28±2.62, respectively). The mean concentration of serum IL-4 in opium addicts did not differ from that in the control group. Conclusion: Studies evaluating the in vivo effects of opium on cytokines are limited and contradictory. The results could suggest that opioids can stimulate the secretion of IL-17. IL-17 is involved in inducing and mediating proinflammatory responses.Opium enhances Th2/Tr1 (IL-10) cytokines. Furthermore, an immunoregulatory response is occurring with the upregulation of IL-10 in an attempt to control the inflammation.
Keywords: Opium addicts, interleukin (IL)-4, IL-10, IL-17

704. Serum Markers of Inflammation in Opium (*thariac*) Users

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Background: Opium (*thariac*) is most common form of substance abuse in Iran. Several studies have reported an association between serum markers of inflammation and inflammatory diseases. This study was done to determine serum C-reactive protein (hs-CRP) and complement factors (C3 and C4) levels in opium addicts and normal healthy controls. Materials and Methods:The study was conducted among 44 male opium addicts and 44 controls of aged 20-40 years. Control group were healthy individuals with no life -time history of substance abuse. All of the opium abusers were selected from those who had a history of use of opium, as a regular habit, at least for one year, with adaily opium dosage of not less than 2 grams. They absolutely did not abuse other drugs. The opium addicts and the controls were matched with regard to age, socioeconomic and smoking status. An enzyme-linked immunosorbent assay (ELISA) was employed to analyse the serum hs-CRP concentrations. Serum C3 and C4 concentration were determined by the use of Single Radial Immunodiffusion (SRID) method. Results: Serum hs-CRP and complement factors (C3 and C4) levels were markedly higher in the opium addicts (8.93±1.93; 138.47±56.8 and 68.79±29.79, respectively) relative to the control group (0.72±0.09; 93.36±37.05 and 33.08±27.69, respectively). Conclusion: Our data demonstrated that the acute phase response is greatly enhanced in opium addicts compared with healthy individuals. Furthermore, exposure to opium may be positively associated with the risk inflammatory diseases such as coronary heart disease.
Keywords: Opium addicts, hs-CRP, C3, C4

705. Diagnosis and Management of Severe Asthma Fungal Sensitization

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Background: Asthma is a complex inflammatory disorder of the airways characterized by airflow limitation and airway hyper responsiveness. A new phenotype of asthma has been described recently, namely severe asthma with fungal sensitization (SAFS). SAFS can be conceptualized as a continuum of fungal sensitization, with asthma at one end and allergicbronchopulmonary aspergillosis at the other. Fungi can be linked to severe asthma in a multitude of ways, including 1) inhalation of fungal spores; 2) fungal sensitization; and 3) allergic bronchopulmonary aspergillosis (ABPA), a severe degree of fungal sensitization culminating in irreversible bronchopulmonary damage. This review summarizes the current understanding of diagnosis, and management of SAFS. Materials and Methods: Diagnosis of fungal sensitization is defined as the presence of immediate Coetaneous hyperactivity to fungal antigen(s), an increase in specific IgE antibodies to a particular fungus and the discovery of recombinant-specific fungal proteins is the only prospect for an accurate diagnosis of fungal sensitization. Results: This is the most crucial and most difficult step in the diagnosis of SAFS. It ispossiblethat many of patients with CB (central bronchiectasis) and SAFS do, in fact, have ABPA, and are mislabeled as having SAFS. IgE plays a central role in the pathogenesis of allergic diseases, including SAFS. The IgE levels quickly decline once glucocorticoids are administered, but not the skin reactivity and specific IgE levels. Many patients with SAFS are already receiving corticosteroids on a daily basis or in intermittent courses. If the IgE levels are checked during these phases, it is possible that they will fall below 1,000 IU/mL, and patients with ABPA-S or ABPA-CB will be (mis)classified as having SAFS. Conclusion: The initial management of SAFS should be akin to that of severe asthma, as both are primarily immune-med inflammatory phenomenaiated. SAFS and ABPA can be hypothesized as different stages of fungal sensitization with differing immune responses, with asthma at one end and ABPA at the other. The benefit of itraconazole in ABPA led to the hypothesis that antifungal azoles also could be useful in the treatment of SAFS. Furthermore, the demonstration of efficacy of antifungal therapy for asthma could also suggest a role for fungal exposure in the pathogenesis of asthma.
Keywords: ASFS, ABPA, diagnosis, treatment, Itraconazole

706. Clarithromycin and Apoptosis of Tumor Cells

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Background: Clarithromycin is a macrolideantibiotic and has similar antimicrobial spectrum as erythromycin but is more effective against certain gram-negative bacteria. The concentration of clarithromycin in the tissues can be over 10 times higher than in plasma. In addition to its antibacterial effects, recently it was shown that clarithromycin can be used in the treatment of asthma as it has an anti-inflammatory effect and due to its potent inhibitory effect on tumor-induced angiogenesis it could be assumed as a possible candidate for therapeutic application. There is also some evidence for its antiproliferative effect, so the aim of this study was to evaluate the effects of several doses of clarithromycin on apoptosis of CT26 tumor cell line. Materials and Methods: For this purpose, tumor cells were treated with three different doses of clarithromycin. Staining with Annexin V flous was used in order to determine the apoptosis in cells. Results: We found significant differences regarding the percent of apoptotic cells between case and control groups and it was also significant differences regarding the percent of apoptotic cells between different doses of clarithromycin. Conclusion: It is concluded that clarithromycin can be effective in the induction of cell death in colon cancer cell line. Of course more studies are needed in order to approve this hypothesis.
Keywords: Clarithromycin, Apoptosis, CT26 tumor cell line

707. Induction ofCytotoxicity in Melanoma Cell Line by CD8 + T cells Treated with Immunomedulator Fraction of Garlic (R10)

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Background: With extensivestudiesduringthetwo past decadesongarlicthe antitumoreffectsof thismedicinalplanthas beenidentifiedandreported. In this research the effect of immunomodulator R10fractionisolated ofthegarlic was evaluated on Cytotoxicity activity of CD8+ T cells against a melanoma cell line. Materials and Methods: Melanoma Cell linewaspurchased from cellbankofPasteur Institute of Iran.R10fractionwas preparedusingultrafiltration.CD8 + Tcells were isolated bymagnet bead method. Cytotoxicity was measuredwith Cytotoxicity Detection Kit (LDH).Apoptosis was measured with Cell Death Detection ELISA^{PLUS}kit. Results:The findingsshowthat theR10 fractionis ableto induced dose-dependent cytotoxicity and apoptosis effects onthe melanoma cell line through CD8 + Tcells and the optimum effect is achieved in the1/50 dilution of R10.Conclusion:The results show that the fraction of R 10 can induce nonspecific cytotoxicity in target cells via CD8+ T cells
Keywords: apoptosis, CD8 + T cells,Cytotoxicity,melanoma, R10fraction, garlic

708. Trends in Adverse Events after IV Administration of Contrast Media

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Background: Data collected from 1985 to 1999 on adverse events after the IV administration of contrast media were evaluated to identify trends. Materials and Methods: Data collected on 391 adverse events after 90,473 administrations of iodinated contrast media and 19 events after 28,340 administrations of gadolinium were evaluated. Reactions were graded as mild, moderate, or severe. Data were also collected regarding contrast extravasation. Results: When only ionic iodinated contrast material was used, the adverse reaction rate was 6-8%. With the selective use of contrast material, the adverse reaction rate was 0.6% and 0.7%, respectively, for ionic and nonionic agents. The rate decreased to 0.2% with the universal use of nonionic agents. More than 90% of adverse reactions were allergic-like. Seven severe reactions (0.05%) and no deaths occurred in the ionic group. During the selective use period, one death occurred in the nonionic group. No severe reactions or deaths occurred during the first 5 years of universal nonionic use. Since then, 10 severe reactions (0.02%) and one death have occurred. Seven reactions occurred in patients after helical CT angiography. The extravasation rate for iodinated contrast material has remained constant at 0.3-0.4% annually. The adverse reaction rate to gadolinium contrast material was 0.06%. Conclusion: Mild and moderate adverse events are more common with ionic contrast material than with nonionic. Most reactions are allergic-like. Severe reactions are seen equally with ionic and nonionic contrast material but differ in type. The reactions were allergic-like in the ionic group but were predominantly attributable to cardiopulmonary decompensation in the nonionic group. Helical CT angiography may play a role in reactions. Keywords: Adverse Events, IV Administration, Contrast Media

709. The Effect of Garlic Fractions on Raji (human Burkitt lymphoma) Cell Line

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Background: *In vitro* and *in vivo* studies have proved that garlic (Allium sativum) components can inhibit cell proliferation in some specific types of cancer. On the other hand, garlic extract possesses immunomodulatory properties and induces lymphocyte proliferation. Thus, it is encouraging to study the effects of garlic on lymphoma which has both cancerous and lymphoid nature. In this study, the effect of garlic extract and it fractions on lymphoma Raji cell line were evaluated. Materials and Methods:Peeled garlic was mixed in ratio of 1 g of garlic to 1 mL of distilled water. Garlic extract was then run through Amicon ultra-filter system. Extract was fractionated to six fractions based on their molecular weights: R100, R50, R30, R10, R5, and F5. MTT [3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] reduction assay was used for cells viability assessment. Results: The results illustrate that garlic extract and its fractions, in proper clinical dosage, does not have noteworthy cytotoxic effects on this cell line at 24h and 48h. At 72h, R100 and R30 fractions decrease cell viability remarkably. The immunomodulator fraction of garlic (R10), has no significant effect on Raji .Our results suggest that cytotoxic effect of garlic fractions is in dose and time dependent manner. Conclusion: The most significant effects were observed, in some dilutions of R100 and R30 at 72h on Raji cell line. Although this finding is valuable, the effects of garlic extract and its fractions on the other malignancies were more remarkable and it may be due to lymphoid nature of this cell line.
Keywords: Garlic, Immunomodulator, cytotoxicity, Raji cell line, lymphoma

710. Comparative Study of Intraperitoneal Injection of Ecstasy, Crystal, Glass and Heroin on the White Blood Cells in Male Rats

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Background: Abusing psychostimulant amphetamine (ecstasy, crystal meth, glass and heroin) has increased dramatically within the past few years in Iran. Alteration of abusing pattern of addictive drugs from classic opioid (heroin) to psychostimulant drugs (ecstasy, crystal meth and glass) threatens young people life. Ecstasy, crystal meth and glass abusing have been increasing among youth, although this drug has not been incoming Iran for a long time.It is known that these substances have toxic effects on the cardiovascular and immune system. In the present study, effects of chronic ecstasy, crystal meth and heroin exposure on the white blood cells were examined. Materials and Methods: Male adult rats were given intraperitoneal injection of ecstasy, crystal meth, glass or heroin (10 mg/kg, once per day) for 15 days. At the end of 15 days, the blood samples were taken from rats and analyzed for white blood cells. Results: Our data showed that ecstasy and glass significantly decreased the total white blood cells ($P<0.05$). The total white blood cells did not change in heroin group. Conclusion: It seems chronic use of amphetamine can alter immune function. Industrial psychostimulant drugs in comparison with classic opioids have more impairing effects on immune system during short time.
Keywords: Intraperitoneal Injection, Ecstasy, Crystal, Glass, Heroin, White Blood Cells

711. Enhancement of TNF-α Production by Macrophages against Candida Albicans by Aloe vera Gel Extract and its Fractions

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Background: herbal medicine was considered as valuable treatments by more people from the past. The litrature shows that Aloe vera causes to adjust safety response. Macrophages play a vital role in defending host against pathogens by production cytokines like; TNF-α. Also, candida is the fourth infectious agent in hospital infection and is one of the most popular fungal infection. In this study the effect of Aloe vera extract was evaluated on TNF-α production by macrophages. Materials and Methods: Candida Albicans was cultured and the aloe vera gel extraction was prepared and its fractions (R5, R10, R30, R50, and R100) were isolated by amicon ultrafiltration method. Then, peritoneal macrophages of Balb/C mice were separated, washed, counted and cultured in 96-well microtiter plates. The extract and fractions were added at various concentrations to the wells, then the Candida albicans were added. The TNF- α production was assessed by using TNF-α R&D quantitative Elisa kit. Statistical analysis using ANOVA were performed and $P<0.05$ was estimated as significant value. Results: the results showed that TNF-α production by macrophages is increased after exposure to aloe vera gel extract and its fractions. Data also show that TNF-α production is increased by aloe vera gel extract and its high molecular weight fractions (R100, R50) at higher concentration (E1/2, E1/5, E1/10).
Conclusion: This study shows that Aloe vera extract and its fraction has effective role in TNF-α production by macrophage. However, it is necessary to biochemical extraction of aloe vera extract and its high molecular weight fraction and its effects in human and animals models.
Keywords: Aloevera, Candida Albicans, Immunomdoulator, Macrophage, TNF- α

INFLAMMATION

Oral Presentation

712. Effect ofInflammation Induced byInjection of LPS in theNervous System on Calmodulin Kinase II an Expression and Memory Formation in Hippocampus of Rat

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Background: Inflammation of the nervous system is an important factor, associated with memory deficits and degenerating neurons in neurodegenerative disease such as Alzhaimer and Parkinson. Lipopolysaccharid (LPS) obtained from the wall of Gram-negative bacteria acts through stimulation of the innate immune system and reacts with receptors that are expressed on the surface of microglia cells during inflammation causes the inflammatory intermediate production such as cytokines and chemokines and thus reduces the expression of genes involved in LTP and memory formation in the hippocampus. Calmodulin kinase 2(*CaMK II α*) is one the most important proteins at memory

formation. Since the relation between neuro- inflammation and deficits in learning and memory is not completely clear we investigated the effect of LPS on *CaMK II α* expressions in hippocampus. Materials and Methods: In this study, male wistar rats (220-250 g) were used, for inducing neuro- inflammation LPS was administrated via intra peritoneal (ip) injection at 250 µg/kg in two days, 3 hours after second injection, animals decapitated and hippocampus from both hemispheres were rapidly dissected and stored at -80centigrade. At next step, presence of inflammatory factors such as TNF-α and IL-1β at hippocampus tissue were investigated by ELISA kits, and finally by using Real time PCR changes in *CaMK II α* gene was evaluated. Results: using the ELISA method we found that in inflammatory conditions the expression of inflammatory factors increased. Moreover, data indicated that *CaMK II α* expression was decreased. Conclusion: Results of this study show that, one reason for deficits effects of LPS on learning and memory is because of changes in Calmodulin kianse 2 expressions in hippocampus and this effect mediated by inflammatory mediators such as TNF-α and IL-1β. It seems these mediators disrupt gene expression or cause death of hippocampus neurons that it needs more investigations.

Keywords: inflammation, LPS, Nervous System, Calmodulin Kinase II

713. Th1, Th2 and Th17 Cytokines Profile Produced by Proteolipid Protein and PHA Activated Peripheral Blood Mononuclear Cells Isolated from Multiple Sclerosis Patients in Comparison to Healthy Control Group

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Background: Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS) in which abnormal immune response to one or several myelin antigens such as myelin basic protein (MBP) and proteolipid protein (PLP) results in focal lesions, which can eventually lead to disability in the young adult population. Both genetic and environmental factors are thought to play roles in the pathogenesis of MS. A number of studies have provided evidence that Th1 and Th17 cells are active or expanded during MS disease and it's animal model, EAE. Materials and Methods: To study the role of Th1, Th2 and Th17 cells in the pathogenesis of multiple sclerosis, we evaluated the synthesis of IL-4, IL-10, IL-17, TNFα and IFNγ cytokines produced by proteolipid protein (PLP) or phytohemaglutinin (PHA) activated peripheral blood mononuclear cells isolated from 20 Multiple Sclerosis patients (14/6 F/M; mean age 33.9±6.5) in active phase in comparison to 19 healthy control group (15/4 F/M; mean age35.2±6.8) using ELISA. Results and Conclusion: In contrast to other studies, our results indicated that just IL-17 production was significantly higher ($p<0.05$) in peripheral blood mononuclear cells of Multiple Sclerosis patients in relapse phase stimulated by PLP or PHA. There were no significant differences in production of IL-4, IL-10, TNFα and IFNγ cytokines between patients and controls.

Keywords: multiple Sclerosis, Th1, Th2, Th17, Cytokines

714. Changing the Sera Levels of Pro-Inflamotory Cytokines (TNF-A, IFN-Γ, IL-2) in Rats Exposed to Car Exhaust and Cigarette Smoking

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Background: An airborne pollutant may enter the respiratory tract as a volatile gas, as liquid droplets (e.g., sulfuric acid, nitrogen dioxide), or as particulate matter (e.g., components of diesel exhaust, aromatic hydrocarbons). These pollutants interact with the immune system and may cause local and systemic responses ranging from overactive immune responses to immunosuppression. Smoking may lead to clinically recognized chronic obstructive pulmonary disease (COPD) in 15–20% of those who smoke. Cigarette smoke was shown to augment the production of numerous pro-inflammatory cytokines and to decrease the levels of anti-inflammatory cytokines. The current study was conducted to evaluate the smoking exposure and resulted cytokine variations in rat. Materials and methods: Pathogen-free male Wistar rats 4 to 5 weeks old were obtained from the Pasteur Institute of Iran. The rats were placed in three groups each of 8 rats; cigarette smokers, car smokers and the control groups. Rats were placed in some designed racks during smoking so that the smoke can reach them similar to what happens in heavy traffic and what a cigarette smoker does daily. After one month of exposure the blood samples were extracted from the rats and cytokine profiles were measured by ELISA using related diagnostic kit. Data were analyzed using Post Hoc analyzing method and LSD test to compare the mean of cytokine levels within the groups. Results: Car smoke suppressed the production of IL-2, IFN-γ and TNF-α by more than 50 %.amongst all the rat in cases groups while the control group had no changing in their cytokine profile. In contrast, cigarette smoke inhibited production of TNF-α and increased production of the IL-2 and IFN-γ. Conclusion: This study indicated that cigarette and exhaust smoke contains potent inhibitors and/or suppressor of cytokine production even in a short term exposure time.

Keywords: cigarette smoking, proinflamatory cytokines, car exhaust, immune response

715. GM-CSF Signaling Boosts Dramatically IL-1 Production

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Background: Dendritic cells are the heterogeneous and versatile regulators of immune system. The goal of this study was to engineer 'tailor made' DCs with highly "immunostimulatory" properties. As a candidate molecule, we chose GM-CSF which is known for its multifunctional properties ranging from cellular differentiation, proliferation, and activation to inhibition of apoptosis and boosting inflammation. However the molecular actions of GM-CSF are still poorlycharacterized. Here we describe a new surprising facet of this ''old'' growth factor as a key regulator involved in IL-1β secretion. IL-1β is a potent pyrogenic cytokine and a key modulator of immune responses against a variety of microbes as well as several acute and chronic inflammatory disorders. Materials and Methods: We used a series of *in vitro* and *in vivo* experiments to further investigate the contribution of GM-CSF in boosting immunostimulatory capacity of DCs, particularly their production of IL-1β. Results: We found that IL-1β release, a pivotal component of the triggered innate system, is heavily dependent on the signaling induced by GM-CSF in such an extent that in its absence IL-1β is only weakly secreted. GM-CSF synergizes with LPS for IL-1β secretion mainly at the level of pro-IL-1β production via strengthening the NF-κB signaling. In addition, we show that expression of Rab39a, a GTPase required for caspase-1 dependent IL-1β secretion is greatly augmented by LPS and GM-CSFco-stimulation suggesting a potential GM-CSF contribution in enhancing IL-1β exocytosis. The role of GM-CSF in regulating IL-1β secretion is extended also *in vivo*, since GM-CSF $R^{-/-}$ mice are more resistant to LPS-mediated septic shock. Conclusion: Collectively, our findings identify GM-CSF as a key regulator of IL-1β production which could be a potential candidate to boost immunostimulatory capacity of DCs *in vitro* and *in vivo*. These modified DCs could potentially be used in different therapeutic approaches to strengthen and modulate the immune responses.

Keywords:GM-CSF, Dendritic cells, IL-1

Poster Discussion Presentation

716. Evaluation of Diagnostic Value of Soluble Urokinase-type Plasminogen Activator Receptor in Sepsis

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Background: Early diagnosis and assessment of the systemic inflammatory response to infection are difficult with usual markers (fever, leukocytosis, C-reactive protein). The urokinase plasminogen activator receptor has been implicated as an important factor in the regulation of leukocyte adhesion and migration. We studied the ability of it to identify patients with sepsis. Materials and methods: Plasma samples were obtained on ICU admission from patients with systemic inflammatory response syndrome for soluble urokinase plasminogen activator receptor measurement. Results: Soluble uPAR, CRP concentrations and ESR were higher in the sepsis group (n=40) than in the non-infectious SIRS group (n=43; p=0.01, 0.00, 0.00 respectively). C-reactive protein concentrations and ESR were higher in the sepsis group than in the non SIRS group (n=24; p=0.00, 0.00 respectively). In a receiver-operating characteristic curve analysis, ESR, CRP and suPAR had an area under the curve larger than 0.65 (p=0.00), in distinguishing between septic and non-infectious SIRS patients. CRP, ESR, suPAR had a sensitivity of 87%, 71% and 66% and a specificity of 59%, 76% and, 74% respectively in diagnosing infection in SIRS. Conclusion: CRP and ESR performed better than sUPAR and WBC count in diagnosing infection.
Keywords: suPAR, SIRS, CRP, Sepsis

717. The Status of Regulated on Activation Normal T Cell Expressed and Secreted (RANTES) and Interleukin (IL)-6 Serum Levels in Nephropathic Type 2 Diabetic Patients

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Background: Nephropathy complication of type 2 diabetes is a complex disorder which is most likely dependant on several environmental and genetic factors and currently the interplay between these factors yet tobe clearly understood. The present study was aimed to examine the serum levels of the regulated on activation normal T cell expressed and secreted (RANTES) and IL-6 (an inflammatory cytokine) in nephropathic and non-nephropathic type 2 diabetic patients. Materials and Methods: In this study, serum samples were obtained from 100 non-nephropathic type 2 diabetic patients, 100 nephropathic type 2 diabetic patients and 100 non diabetic controls. Serum levels of RANTES and IL-6 were detected by ELISA. Results: Our results showed that the serum levels of RANTES were significantly increased in non-nephropathic type 2 diabetic patients, while, it was not differ in nephropathic complication in compare to healthy controls. IL-6 level was also not differ significantly in three evaluated groups (nephropathic and non- nephropathic type-2 diabetic patients and healthy controls). Conclusion: According to these findings, it can be concluded that the serum levels of RANTES, but not IL-6, are associated with type 2 diabetes without nephropathy in Iranian population.
Keywords: RANTES, IL-6, Type 2 diabetes, Nephropathy

718. Influence of Interleukin-17 on Hemoglobin Levels in Patients with Ulcerative Colitis

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Background: Ulcerative colitis is one of autoimmune disease in gastrointestinal tract with unknown etiology. Inflammatory mechanisms in this disease can have a large impact on other organs. Our recent finding indicating significant elevation of IL-17 in serum of ulcerative colitis patients in comparison with controls. Our purpose in this study was determining the relationship between hemoglobin level and IL-17 in serum of patients with ulcerative colitis. Materials and Methods: 85 patients with ulcerative colitis were enrolled in this study. Serum level of interleukin-17 in these patients was measured using ELISA technique. Statistical tests to find a correlation between IL-17 and hemoglobin levels were performed after measuring the hemoglobin level. Results: Mean serum levels of interleukin 17 in patients with ulcerative colitis was 22.8± 57.6 pg/ml which significantly was lower than controls (*p value*: 0.04). The patients average hemoglobin level was 12.24 g/dl with minimum and maximum of 4 g/dl and 18 g/dl respectively. After determining the relationship between IL-17 and hemoglobin level in UC patients, a significant inverse relationship between them was detected so that one unit increase in IL-17 was associated with 0.22 units decline in hemoglobin level (*p value*:0.03,correlation:0.022). Conclusion:In patients with chronic diseases, including inflammatory bowel disease, it is more likely to increase the excretion of blood in stool with greater involvement in the process of inflammation. Additionally, due to inflammatory responses, including increased secretion of inflammatory cytokines, body organs such as the hemapoietic system may be directly or indirectly suppressed through a disruption in iron metabolism and anemia is developed which is correlated with intensity and duration of disease. Employing these, decreasing in hemoglobin level which is associated with increasing of IL-17 in our UC patients can be explained.
Key words: Ulcerative Colitis, hemoglobin, IL-17

719. IL-23 Receptor Genotype Status and IL-23 Serum Levels In Ulcerative Colitis Patients in Iran

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Background: Crohn's disease (CD) and ulcerative colitis (UC) are two major clinical presentation of inflammatory bowel disease. Many novel candidate genes have been found to associate with increased risk for the disease. At the moment IL-23 receptor gene is identified as an IBD associated gene in a genome-wide studies. On the other hand, recent studies have found increased expression of IL23 in inflamated and non-inflamted mucosa of patients with UC. Our aim in the present study was to ascertain whether rs7517847 and rs1004819 located in the IL-23 receptor gene and IL-23 serum level associations with UC are also observed in our population in Iran. Materials and Methods: IL-23 serum levels were measured in 85 UC patients and in 100 healthy controls by ELISA. In all patients and controls genotyping for 2 UC associated IL23R single nucleotide polymorphisms were performed by PCR-RFLP. Results: There was a highly significant increase in IL-23 serum level in UC patients compared to healthy controls and IL-23 serum levels correlated with disease activity. However, there was no association between rs7517847 and rs1004819 located in the IL-23 receptor gene and ulcerative colitis. Conclusions: we have shown increased serum IL23 levels in patients with UC weather these findings are due to increased inflammation or an important role of T helper17 in the pathogenesis of UC, have to be studied in future. Although the results of the interaction between rs7517847 and rs1004819 located in the IL-23 receptor gene have been negative in this particular case, further functional analysis with other known IL23R genotypes and other candidate genes is necessary to confirm any genetic association with UC in our population.
Keywords: IL-23 Receptor, Ulcerative Colitis, IL-23 Serum Levels

720. Cardiotrophin-1 in Patients with Acute Myocardial Infarction

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Background: Myocardial infarction is the combined result of environmental and personal factors. Prothrombotic factors might play an important role in this phenomenon. Inflammation plays a pivotal role in atherosclerosis and coronary heart disease. Cardiotrophin-1 (CT-1), a member of the IL-6 family of cytokines, was identified as a growth factor for cardiac myocytes that induces cardiomyocyte hypertrophy and stimulates cardiac fibroblasts, protects myocytes from cell death. This study was designed to investigate whether plasma concentration of cardiotrophin-1 (CT-1), in patients who had the first acute myocardial infarction, and to analyze their relationship with traditional cardiovascular risk factors. Materials and Methods: This study was carried out on 45 patients with acute myocardial infarction (AMI) in their first 24 hours of admission as

case group and 36 healthy matched individuals were studied as the control. Plasma level of cardiotrophin-1 was determined by enzyme-linked immunosorbent assay and the results were compared. Results: Plasma CT-1 levels in the patients with AMI on admission 615.279±5.109 pmol/L were significantly higher than those in the control group 534.767±6.750 pmol/L (P= 0.001). Plasma CT-1 level was not correlated with diabetes mellitus, hyperlipidemia, sex, age and smoking. Conclusion: Our findings suggest that high plasma CT-1 level in patients with AMI is indicative of hypercoagulable state that is not related to the traditional cardiovascular risk factors.
Keywords: Cardiotrophin-1, Acute Myocardial Infarction, ELISA

721. Serum levels of Interleukin (IL)-27 in Patients with MyocardialInfarction or Unstable Angina
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Background: Cytokines, the key mediators of immune responses, play an important role in the pathogenesis of cardiovascular diseases. The aim of this study was to evaluate the serum levels of IL-27 in patients withischemic heart disease (IHD) and also to clarify its association with traditional risk factors of disease. Materials and Methods: A total of 120 patients with IHD as having acute myocardialinfarction (AMI; n=60) or unstable angina (UA; n=60) and 60 sex- and age- matched healthy subjects as a control group were enrolled to this cross-sectional, case-controlled study. Serum samples were collected from all participants (for AMI patients at 3-5 days after events and for UA at admission time) and tested for the IL-27 by use of ELISA method. Results: The mean serum levels of IL-27 in AMI group (38.00 ± 14.38 Pg/ml) and UA group (35.77 ± 18.93 Pg/ml) was significantly higher than that observed in control group (24.91 ± 14.96 Pg/ml; P<0.0001 and 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 smoking was significantly higher as compared to control group. Conclusions: These results showed that the higher serum levels of IL-27 were associated with IHD. The presence or absence of a certain traditional risk factors of IHD did not influence the serum levels of cytokine.
Keywords: Acute myocardialinfarction, Unstable angina, Interleukin-27

722. Dietary Excess Methionine Alters Pro-Inflammatory Cytokines Production Induced by LPS in Rat
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Background: Methionine is an essential amino acid that is necessary for maintaining proper growth and development in mammals and its supplementation contributes to better production efficacy. Dietary excess methionine, however, causes various toxic changes including suppression of feed intake and growth and oxidative stress.Materials and Methods: For evaluation of toxicity of excess methionine and its effects on pro-inflammatory cytokines production, 96 Male Wistar rats, 7 week old, weighing 180–200 g were selected. The rats were divided into 8 groups and 3 or 4 rat housed in hanging stainless steel wire cages with free access to the standard diet and water in a room at a controlled temperature. Lights were maintained on a 12-h light dark cycle. After adaptation Group 1 & 2 received the basal diet supplemented with 0.15 molal amounts of methionine (22.5 g/kg diet) as low dose; groups 3 & 4 received the basal diet supplemented with 0.3 molal amounts (45 g/kg diet) of methionine as high dose; groups 5 & 6 as the negative control and groups 7 & 8 as the positive control animals, received no supplements of methionine to the basal diet respectively for 1 & 2 months. After 30 days groups 1, 3 & 5 and after 60 days groups 2, 4 & 6 received LPS (4 mg/kg) as IP and groups 7 & 8 received 0.15 mL normal saline as IP and then blood samples were taken after 30 min, 3 and 6 hours and TNFα, IL-6 and IL-1β were determined in serum. Results:The results of this study showed excess methionine in low and high dose in both 30 and 60 days after administration, without LPS induction, significantly increased TNFα, IL-6 and IL-1β concentration in comparison with negative control group, and LPS induction in groups that received excess high and low methionine caused severe production of pro-inflammatory cytokines in contrast to negative and positive control groups at 30 and 60 days.Conclusion:In conclusion excess methionine can stimulate immune system to produce Pro-inflammatory Cytokines and this phenomenon exaggerates inflammation process by increasing of the production inflammatory cytokines.
Keywords:Methionine, Pro-Inflammatory Cytokines, LPS, Rat

723. Evaluation the Anti-Inflammatory Activity of *Artemisia Khorassanica* in Inflammatory Macrophage Model
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Background: Inflammation is characterized by the release of pro-inflammatory cytokines and inflammatory mediators that are synthesized by inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). In macrophages, they are induced by bacterial endotoxin lipopolysaccharide (LPS) and inflammatory cytokines. This inflammatory cytokines and mediators are involved in the causation of many human diseases including rheumatoid arthritis. Nuclear transcription factor-κB (NF-κB) activates pro-inflammatory genes encoding iNOS, COX-2, TNF-α and IL-1β and its aberrant activity is associated with various inflammatory diseases. Members of the *Artemisia* genus (Astraceae) are important medicinal plants throughout the world. Here, we prepared a sesquiterpene lactone fraction from *Artemisia khorassanica* and evaluated its effect on LPS-induced inflammation in macrophages as a model of inflammatory model *in vitro*. Materials and Methods: The effects of sesquiterpene lactone fraction from *Artemisia khorassanica* (SLAK) on lipopolysaccharide (LPS)-induced nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) production was evaluated in J774A.1 macrophages. Moreover, we evaluated SLAK modulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) enzyme expression by western blot analysis. Also, the activity of nuclear factor, NF-κB was determined in cellular nuclear extracts. Results: Our data revealed that SLAK (10-100 μg/mL), in a dose-dependent manner, inhibits NO, PGE_2, TNF-α and IL-1β production induced by LPS in the J774A.1 macrophages. These data were consistent with the modulation of iNOS and COX-2 expressions. It was also showed that SLAK suppresses the iNOS and COX-2 enzyme expression through the inhibition of NF-κB activity. Conclusion: In this study, we demonstrated that SLAK showed anti-inflammatory effect in LPS-stimulated macrophages. This activity occurs by inhibiting iNOS and COX-2 expression via the inactivation of NF-κB pathway. This inhibitory effect has important implications for the development of anti-inflammatory drugs and strategies to limit pathological inflammation such as rheumatoid arthritis.
Keywords: *Artemisia Khorass*, Macrophage, NO, PGE_2,TNF-α, IL-1β

724. Circulating Adipokines in Ulcerative Colitis: As a New Markers of Inflammation
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Background: Adipokines are fat-derived hormones and cytokines with immune-modulating and metabolic properties. They play an important role in metabolism and in inflammation.Because human metabolism dramatically changes in inflammatory bowel disease (IBD) and chronic inflammation is the hallmark of the disease, we studied serum levels of leptin, adiponectin, and resistin, in patients with ulcerative colitis (UC) (active and remission phase) in comparison with healthy controls (HC). Materials and Methods: For all subjects white blood cell (WBC) count test were performed. We studied serum levels of leptin, adiponectin and resistin in 89 UC patients (49 in remission and 40 active patients) and in 30 healthy controls (HC) using commercially available enzymelinked immunosorbent assays. C- reactive protein was measured by turbidimetric immunoassay. Clinical disease activity was assessed by Truelove and Witts' score. Also body mass index of patients were calculated. Results: Patients with ulcerative colitis showed significantly higher resistin levels compared with controls (P < 0.001).In both, UC patients with active and

remission, resistin concentrations were significantly associated with elevated white blood cell count (P = 0.002), C-reactive protein (CRP) (P= 0.001) and disease activity (P= 0.02). Leptin was similar in patients with UC and controls, whereas in active and inactive disease, adiponectin was increased (p= 0.003) compared with controls. In UC patients, resistin was associated with active disease in multivariate regression analysis after control for sex, age, body mass index and white blood cell count (odds ratio 1.015, 95% confidence interval 1.002–1.029, P = 0.02). Conclusion: Serum levels of adiponectin, resistin are increased in patients with ulcerative colitis. Resistin levels are an independent predictor of disease activity in active patients. Further studies are needed to elucidate the role of adipokines in ulcerative colitis.
Keywords: ulcerative colitis, resistin, leptin

725. Added Predictive Ability of New Markers (RDW and MPV) to Inflammatory Parameters in Disease Severity of Ulcerative Colitis in Clinical Remission

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Background: Recently some noninvasive tests have been studied for determining the clinical activity of disease in ulcerative colitis (UC). Mean platelet volume (MPV) and Red cell distribution (RDW) are influenced by inflammation. In several studies, these markers have been correlated with disease activity index. The aim of this study is to determine the added predictive ability of RDW and MPV in regard to inflammatory markers in disease severity of UC patients in clinical remission. Materials and Methods: forty eight patients with ulcerative colitis were enrolled in this study. For all subjects WBC count, platelet count, RDW and MPV tests were performed. Serum high-sensitivity C-reactive protein (hs-CRP) and pro-inflammatory cytokine; interleukin 12 (IL12) levels were also measured. Clinical disease activity was defined using Simple Clinical Colitis Activity Index (SCCAI). Results: According to SCCAI, all patients were in remission. There was significant correlation between hs-CRP levels and disease activity score (P = 0.01), while serum levels of IL12 has showed no correlation with disease activity. Group markers including RDW, MPV, PLT count have added the predictive ability of disease activity about 20.6%. From these markers, RDW consider as an independent predictor of clinical disease activity. Conclusion: Our results suggest that easily available and inexpensive markers such as RDW, MPV and platelet count may improve the predictive ability of inflammatory markers in clinical disease activity of patients with ulcerative colitis.
Keywords: ulcerative colitis, RDW, MPV, hs-CRP

726. The Effects of Some Isoquinoline and Non Isoquinoline Alkaloids on Oxidative Burst and IL1β Secretion in Human Neutrophils

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Background: Alkaloids are natural products found in different parts of most plants. These compounds have many immunological and pharmacological effects. The aim of this study was to investigate the effects of four alkaloids; noscapine, berberine (isoquinoline), morphine and codeine (phenantheren) on IL1β secretion and respiratory burst as important aspects of innate immunity in human neutrophils. These processes are involved in a variety of pathological conditions such as infections, cancers, ischemia, and trauma. Materials and Methods: Neutrophils were isolated from peripheral blood of healthy donors and incubated with various doses of drugs (10^{-9}- 10^{-5}M) in cell culture condition. After addition of NBT solution, the amount of produced formazan was measured spectrophotometrically at 630 nm. The concentration of IL-1β was measured using ELISA method in 450nm. Results: The results showed that, noscapine and berberine suppressed the oxidative burst and IL1β secretion in a dose dependent manner in human neutrophils, while morphine and codeine had stimulatory effects on oxidative burst and IL1β secretion. Conclusion: In conclusion, common drugs like these natural products that can influence the activity of innate immunity may be favored in treatment of many diseases.
Keyword: IL1β, oxidative burst, berberine, noscapine, morphine, codeine

727. The Effects of Antimicrobial Peptides Extracted From Skin Secretions of *Rana Ridibunda* on Pro-Inflammatory Cytokine Gene Expression in the Human Epithelial Cell Line A549 by Semi-Quantitative RT-PCR

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Background:A large group of low molecular weight natural compounds that exhibit antimicrobial activity have been isolated from animals and plants. Among them, cationic peptides are the most widespread. In addition to direct antimicrobial activity, these peptides play an important role in innate immunity and inflammation by modifying the production of key defensive molecules (cytokines and chemokines). In this study, we sought to evaluate the effects of **antimicrobial peptides extracted from skin secretions of *Rana ridibunda* on** the expression levels of pro-inflammatory cytokines (IL-6 and IL-8) in A549 cells by semi-quantitative RT-PCR in dose and time-dependent manner. Materials and Methods: Initially, antimicrobial peptides were isolated from *Rana ridibunda*. Different concentrations of peptides were added to A549 cells at different time intervals. After 6, 12 and 24-hour post-treatments, total RNA was extracted and cDNA was synthesized .Finally, the levels of gene expression of IL-6 andIL-8 were studied in comparison with internal control gene GAPDH by semi-quantitative RT-PCR. Results: Our results demonstrated that antimicrobial peptides up-regulate the IL-6 and IL-8 expression levels in A549 cells in a dose and time-dependent manner. Conclusion: Antimicrobial peptidesstimulate the production and release of pro-inflammatory and anti-inflammatory molecules. Aye Aye Khine et al. (2006) reported that human neutrophil peptide was able to selectively increase the expression of IL-8 among 10 pro- and anti-inflammatory cytokines examined on human lung epithelial cells (A549). Jie Yu et al (2007) showed that IL-1β and LL-37 induced the synergistic production of IL-10 and IL-6 and chemokines in human peripheral blood mononuclear cells (PBMC), indicatingtheir roles in enhancing certain innate immune responses. Our results based on the increased expression levels of IL-6 and IL-8 in A549 human lung cancer cell lines confirmed the role of antimicrobial peptides in increasing some special inflammation processes.
Keywords: Antimicrobial Peptides, *Rana Ridibunda*, Epithelial Cell Line A549, Semi-Quantitative RT-PCR

Poster Presentation

728. Evaluation of Inflammatory Process in Experimentally Induced Endometriosis in Rats

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Background: Endometriosis is a common gynecologic disorder that presents with chronic pelvic pain or infertility in women. It is defined as the presence of endometrial tissue outside of the uterus. Peritoneal fluid examination of endometriosis patients contains significantly increased amount of inflammatory process and macrophages presence compared to healthy samples. Materials and Methods: In the present study thirty adult female rats were used. The surgical technique consisted to median laparotomy and resection of a 1cm segment of the right uterine horn. After endometrial detachment from myometrium, a 0.25cm^2 flap was removed and sutured to the abdominal muscle in peritoneum. The rats were randomly divided into 3 groups. Group 1 (n=10) was only transplanted with endometrial fragment. Endometriosis was induced in 10 ovariectomised rats in group 2, and animals in group 3 (n=10) administrated Atrovastatin daily after endometrial segments implantation. 3weeks after endometriosis induction, implants were evaluated microscopic analysis with histochemical staining of Sudan Black B and blood samples

have been collected to determine serum ALP concentrations. Results: The results indicated that the implants developed significantly in group 1 compared to groups 2 and 3. Also there was a significant decrease in the weights of rats in group 3. The presence of macrophages and serum ALP concentrations in group 3 decreased significantly in comparison with 2 other groups. Conclusion: In conclusion, Atrovastatin as an anti-inflammatory agent could be an effective inhibitor for inflammation and so endometriosis development.
Keywords: Inflammatory Process, Endometriosis, Rats

MEDICAL ETHICS in IMMUNOLOGICAL RESEARCH

Oral Presentation

729. Medical Ethics: Dos and Don`ts
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On account of new advances in the science of medicine, the necessity of noticing to medical ethics is increasingly revealed. Medical ethics in education, research and clinic includes wide domains. The relation of this cognitive field with immunology as a science and its application covers all above-mentioned domains and is of special importance. Although ethics is essential in all professions, ethics in medical sciences has an outstanding role because its subject is human, the most honorable creature in the world. The society expects the persons who are dealing somehow with human health to have understanding and knowledge of ethics and act accordingly. Medical ethics has a trusted association with philosophy, law, religion and issues related with special fields of medicine. The collection of philosophical, legal, religious consideration and different technical issues of medicine can help to prepare applied guidelines in medical ethics which includes all dos and don`ts. Nowadays, by the virtue of glorious revolution of the Islamic Republic of Iran, our country is advancing in all scientific fields, particularly medical sciences and has achieved invaluable successes. Therefore, expectation is existed that medical doctors, professors and researchers of our country to be pioneer in medical ethics by benefiting from the instructions of enlightening religion of Islam.
Keywords: Medical Ethics, Dos, Don`ts

730. Ethical Encounter with Error of Colleagues and Laboratory
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Human is to err. Therefore, the occurrence of error in medicine and health care services is inevitable. One of the health care centers which have a significant role in the occurrence error is medical diagnostic laboratories. Laboratory errors which include errors prior to testing, during the test, and after testing are mainly related to human and instrument errors and they can lead to diagnostic and treatment errors and consequently cause emotional, physical and financial damage to the patients. Regardless of various definitions of error, it is sometimes defined as negligence and sometimes as preventable adverse medical events .What needs to be taken into consideration is the ethical duty of health care providers to occurrence oferrors. Ethical management of error based on principles of autonomy, beneficence, nonmaleficence and justice considers disclosure of error to patient and health system as one of absolute rights of patients and as a requirement to trust medical profession in community. Besides it considers error disclosure as a measure in prevention and decrease of future error. It also considers nondisclosure of error as deception, threatening professionalism and potential risk for patients. One of the main issues of ethical encounter with errors is which error and by whom, where, when, and how the error must be disclosed. On the other hand, colleague's error is sometimes recognized in providing health care. In these cases, ethical encounter with error includes identification of the error, discussion with the person who commits the error, disclosure of error to patient and health system respecting the colleague, keeping patient's trust, and patient's confidentiality. The present article intends to explain the significance of error disclosure based on the principles of honor, integrity, and commitment to truth. It also attempts to provide a practical guideline to disclose medical error. In this guideline details of way of explaining the error and also the body language used in disclosing error are explained. Finally, it is proposed to encourage physicians to report error to the error management and patient safety committees by winning physicians' trust and supporting them. This will enable health system to develop a database of medical errors in the country and to employ them in training health professionals and also use them in error prevention plans to improve health care quality and patient safety.

731. Patient's Rights Charter in Iran
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Health system needs efficient participation and relationship between health care providers and health care recipients. Health care centers should respect health care recipients, their families, physicians and other health care providers and respect their rights. With this in mind and at the aim of explaining the rights of health care recipients and upgrading ethical observance in the field of treatment-the most important field of health care- Patient's Rights Charter was compiled in three years. After approval by the Council of Policy Making of Ministry of Health and Medical Education, the Patient's Rights Charter was declared to all medical universities in September 2009(2). Iranian patient's rights charter has been formulated in the framework of 5 chapters including: right of receiving suitable services, right to access to desirable and enough information, right to choose and to decide freely about receiving healthcare, right to privacy and confidentiality and finally right to access efficient system of dealing with complaints which have been explained in 14,9,7, 4 and 3 articles, respectively. It must be noted that a serious challenge is how to implement the observing of patient's rights in practice in our healthcare system in Iran.

732. Establishment of Professional Code of Ethics in Medical Organization
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There are two approaches to integrating medical ethics: The *organization-based approach* and the *person-based approach*. The person based approach reduce. Medical ethics to ethics of physicians, nurses and related individuals but the organization based approach. Is intended to serve as guide to the everyday professional conduct of medical system organizational strategies include the development of code of ethics and ethical policy statement. Purposes of code of ethics in medical organization are to promote high standards of practice, to establish a framework for professional behavior, responsibilities and predictibities. To succeed, a code must be result of the following conditions:

1. It must be owned by all who are affected by it.
2. It must be backed by a program of staff development and training that is ongoing and that opens the code up to amendment in the light of experience.
3. It must be supported by philosophical and critical thinking.

Keywords: code of ethics, Medical ethics, organizational ethics, organization-based approach, person-based approach

Poster Presentation

733. The Implementation Study of Ethical Criteria Related to the Students in School of Medicine, Tehran University of Medical Sciences with Universities of Selected Countries

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Background: The purpose of this study was the implementation of ethical standards and the mode of conveying information in the Tehran University of Medical Sciences with many of relevant universities in Asia, Australia, Canada, Europe, and USA. Materials and Methods:The comparative method was performed via searching internet to research at the syllabuses, outlines, green sheets, of laboratory immunology as well as handbooks and guidelines of medical universities (from Asian 4, American 12, Australian 4, Canadian 5,and European 7, universities respectively) then Implementation them with interested criteria of Tehran university of Medical Sciences. Results: Conduct Code, Code of Student Behaviour, Honore Code, Academic Standard of Integrity, USD Academic Integrity Policy, Office of Judicial Affairs, code of Practice on assessment are the terms that were considered for informing of the ethical code to the students of above mentioned universities. The Chinese university of Hong Kong had referenced the ethical code from its handbooks for their students. The most number in providing students with the moral code were demonstrated in USA universities and colleges syllabuses. Australia (3 universities) and Canada (3 Universities) also had definitions and links about this criteria. There were information about related subjects in the handbooks and guidelines of Liverpool and Zurich universities. Conclusion: Nowadays many of world universities as well as Tehran University of medical sciences have especial attention to the criteria of ethics. Finding relevant items and guidelines for informing students will contribute the trust among them and integrity of the university. Suggestions: Have deeper study, launch "Code of Student Behavior Website "and student committee next to the student disciplinary committee on issues related.

Keywords: Implementation Study, Ethical Criteria

MEDICINAL PLANTS & IMMUNOLOGY

Oral Presentation

734. The Sesquiterpene Teuclatriol Isolated from *Salvia Mirzayanii* Exhibits Anti-Inflammatory Action *via* Diminished NF-κB Activity

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Background: Compounds derived from medicinal plants represent an important source of new inhibitors of inflammatory processes. Aim of this study was to determine anti-inflammatory effect and the underlying mechanisms of teuclatriol, an active compound with immunomodulatory effects isolated from *Salvia mirzayanii*, a plant traditionally being used in Iran for the treatment of a variety of inflammation-related diseases. Materials and Methods: We isolated a highly active compound from *Salvia mirzayanii*. This compound was identified as guaiane sesquiterpene teuclatriol by NMR spectroscopic studies. Effects of teuclatriol on viability of several cell types were determined by MTT test. To screen for anti-inflammatory actions of non-toxic doses of teuclatriol, tumour necrosis factor-α(TNF- α)secretion by macrophage-like THP-1 cells was quantified by murine fibroblast-like L929 bioassay. NF-κB DNA binding activity was determined in teuclatriol-treated THP-1 cells stimulated with lipopolysaccharide (LPS) by gel shift assays. The expression of monocyte chemoattractant protein (MCP)-1 and toll-like receptor (TLR)-2 in human umbilical vein endothelial cells (HUVEC) was measured by real time-PCR. Results
Teuclatriol slightly reduced viability of HUVEC and THP-1 cells (at concentrations beginning from 40 μg/ml and 100 μg/ml, respectively). Contrarily, there was a slight stimulatory effect on L929 cells (starting at 80 μg/ml), pointing to cell-specific effects. Teuclatriol significantly reduced the LPS-induced production of TNF- α. Concordantly, NF-κB-DNA binding activity was significantly reduced in teuclatriol-treated THP-1 cells stimulated with LPS. NF-κB activation in HUVEC contributes to an increased expression of MCP-1 and TLR2. TNF- αinduced expression of MCP-1 and TLR2 mRNA in HUVEC was significantly decreased by teuclatriol. Conclusion:
These data suggest that *Salvia mirzayanii* exerts anti-inflammatory actions *via* teuclatriol, which inhibits the production of specific inflammatory molecules. This guaiane sesquiterpene may be a promising structure with NF-kB-pathway inhibiting capacity that is different from the well known anti-inflammatory properties of lactone moieties.

Keywords: *Salvia Mirzayanii*, Anti-Inflammatory, NF-κB

735. A Review on Medicinal Plants with Immunosuppressive Effects

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Medicinal plants have been used for centuries to treat various illnesses. Among over 20,000 herbal medicines known to be in human use, a limited number have been sufficiently studied and numerous remained to be investigated for their efficacy in treating human diseases. A number of herbal drugs are in use for their immunosuppressive effects.This capacity of medicinal herbs may have useful applications in immune-mediated disorders including autoimmune diseases and organ transplant rejection. Plants such as *Salvia miltiorrhiza* and *Tripterygium wilfordii Hook F* has been shown to reduce inflammatory cytokines and mediators, indicating their value in the treatment of acute graft rejections and autoimmunity. *Tanacetum parthenium* inhibits the release of pro-inflammatory mediatorsfrom macrophages and lymphocytes and *Curcuma longa* down regulates the expression of cytokines and chemokines, most likely through inactivation of the transcription factor NF-kappaB**.** In a search through the "Canon of medicine" the epochal work of Avicenna, the great Persian scientist of the middle age, we also looked for information about medicinal plants that have been used to cure inflammatory illnesses. This review focuses on the plants that have recently received more attention regarding their influence on the immune system, and those are also used in Iranian traditional medicine and have been reported to be immunoinhibitory agents.

Keywords:Review, Medicinal Plants, Immunosuppressive

736. Glycyrrhizin Induces Dendritic Cells Maturation and IL-12 Production

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Background: The roots and rhizomes of *Glycyrrhiza glabra* (Leguminosae) have long been used worldwide for liver ailments, dyspepsia, gastric ulcers, sore throats, asthma and bronchitis. Glycyrrhizin is a triterpenoid saponin extracted from the roots of *G. glabra*. Dendritic cells (DCs) represent a heterogeneous population of professional antigen-presenting cells that specialize in the processing and presentation of antigens to effectors cells and play a central role in immunity and tolerance. The aim of this study was to investigate the effect of glycyrrhizin on DCs maturation and function. Materials and Methods: DCs were isolated from mouse spleen using a three- step purification technique including

collagen digestion of tissue, selection of low-density cells using nycoprep and plastic adherence. The effect of glycyrrhizin on the viability of DCs was determined using MTT assay. Surface expression of CD11c, CD86, CD40 and MHCII molecules on DCs was determined by flow cytometry. The proliferation of allogenic T cells in mixed-lymphocyte reaction (MLR) in the presence of glycyrrhizin-treated DCs was measured by BrdU incorporation assay. Result: Study of the role of glycyrrhizin on phenotypic and functional maturation of DCs indicated that glycyrrhizin could up regulate the expression of CD40, CD86, and MHC class II molecules. Cytokine measurement in the culture of glycyrrhizin-treated DCs showed an increase in IL-12 production compared to that in untreated DCs. In MLR, glycyrrhizin induced T cells proliferation. Moreover, an increase in the secretion of IFN-γ and a reduction in IL-4 release by T cells in MLR were observed. Conclusion: The results of this study demonstrated that glycyrrhizin had the ability to up-regulate maturation markers on DCs and activate them to stimulate T cell proliferation. The levels of cytokines secreted by DCs and allogenic T cells showed a Thelper1 cells activation pattern.

Keywords: Glycyrrhizin, Dendritic Cells Maturation, IL-12

Poster Discussion Presentation

737. The Role of *Zataria Multiflora* Essence (Iranian herb) on Innate Immunity of Animal Model

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Background: Herbal medicines have been used since ancient times for treatment of a range of diseases and have represented stimulatory effects on the function of innate immunity. The purpose of this study was to evaluate the effects of *Zataria multiflora* (*Z. multiflora*) on the function of innate immunity including phagocytic activity and TNF.α secretion in animal model. Materials and Methods: Eight BALB/c mice were divided into two equal groups. In group A, *Z. multiflora* essence was injected intraperitoneally to the mice, in group B, distilled water was injected. Blood was obtained from 4 mice in each group, 4 and 7 days following injection. The amounts of phagocytosis (respiratory burst) and TNF.α secretion were assessed by chemiluminescence and ELISA methods, respectively. Results: Significant increase in phagocytosis and TNF-α secretion was observed in group A compared with the control group at days 4 and 7. Conclusions: *Z. multiflora* essence can remarkably stimulate innate immunity function and it may be used to immunize individuals alone or in combination with other immunostimulatory agents.

Keyword: *Zataria Multiflora*, essence, Phagocytosis, TNF-α

738. In vitroCytotoxic and Cell Cycle Effect of Metabolic Extract of Echinophora platyloba on Cancer Cell Lines WEHI-164 and PC3

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Background: medical plant Echinophora platyloba from Apiaceae family used by indigenous areas of Iran. Extensive studies on Echinophora compounds indicated anti-microbial and anti-oxidant activities. In the present study the growth inhibitory effects of methanolic extract of Echinophora platyloba and inducing apoptosis on prostate cancer cell (PC3), fibrosarcoma cell line (WEHI-164)and normal mouse fibroblast cell line (L929) was investigated.Materials and Methods: 3-(4,5-dimethyl thiazol-2y1)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay used for measuring the cytotoxicity cell and viability at 24 and 36 and 48 hours in 50,100,200,300,400,500µg/ml concentrations of methanolic extracts. Also, ELISA method was used to measure apoptosis in different concentrations within 24 hours was also performed and morphologic changes were evaluated.Flow cytometry method were used to measure apoptosis in different concentrations within 24 hours was also performed and morphologic changes were evaluated.Results: Results showed that the methanolic extract of Echinophora platyloba changed morphology of cancer cell lines in to apoptotic cells. This extract had ability growth inhibitory and cytotoxic effect in PC3 and WEHI-164 cells in all three times with IC~300,260, 200, 150 and 90 µg/ml respectively but there was no significant effect on L929 viability and characteristics. This extract induced mostly apoptosis but Flocytometry showed a little necrosis in cell death.Conclusion: Increased concentration of extract and time reduced in cell viability. Also, methanol extract induce apoptosis in these cell lines. Generally these effects depends on concentration methanolic extract of Echinophora platyloba ($P < 0.01$)

Keywords: Echinophora platyloba, cytotoxic activity, apoptosis, tumor cell lines

739. Dichloromethane Extract of Scrophularia Oxysepala Induces Apoptosis in MCF-7 Human Breast Cancer Cells

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Background: Currently, breast cancer is the most common cause of cancer death in women world wide. Therefore, there is an urgent need to identify and develop therapeutic strategies against this deadly disease. The aim of the current study was to investigate the cytotoxic activity and the mechanism of cell death of dichloromethane extract of Scrophularia oxysepala in MCF-7 cells. Materials and Methods: MTT and Trypan-blue assays were performed in human breast carcinoma cell line MCF-7 to analyze the cytotoxic activity of the dichloromethane extract of Scrophularia oxysepala. Further, the apoptosis inducing action of the extract was determined by TUNEL and cell death assays. Results: dichloromethane extract of Scrophularia oxysepala exhibited the most significant cytotoxic properties with the IC50 values of 75, 43 and 20 µg/ml after 12, 24 and 48h treatment, respectively. TUNEL and cell death assays showed that after the treatment of human breast cancer cells with 105 µg/ml the extract for 12 h, the apoptosis induced as large as 50%. Conclusion: Our studies demonstrate that dichloromethane extract of Scrophularia oxysepala is effective in apoptosis inducing in MCF-7 cells, which may be beneficial in anti-cancer therapy.

Keywords: Dichloromethane, Scrophularia Oxysepala, Apoptosis, MCF-7, Breast Cancer

740. Use of the Alcoholic Extract of Alfalfa Root Instead of SLO for ASO Diagnostic Test

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Background: Alfalfa (*Medicago sativa*) has been used as an herbal medicine for over 1,500 years.Streptolysin O (SLO) is one of the several toxic immunogenic exoenzyme produced by group A β-hemolytic Streptococci. Anti sterptolysin O (ASO) is a routine test for the diagnosis and management of acute rheumatice fever and acute glomerulonephritis. This study explored the application of this extract instead of SLO. Materials and Methods: Roots of extraction of Alfalfa was prepared using the maceration method. At frist we determained hemolysis effect of the extract on Sheep Red Blood Cell (SRBC) and Human Red Blood Cell (HRBC). This extract was used instead of SLO antigen in neutralization test .The effect of extract was evaluated on reaction between SLO latex and ASO in slide method. Nonetheless the antigenic properties were determined by percipitation test. Results:The Alfalfa Extract exhibited considerable hemolysis activity on SRBC and hRBC. The ASO was inhibited hemolysis activity of The extract in a specific concentration. The extract decreased 3+ ASO titre to 1+ in agglutination test and showed precipitation by ASO on agarous gel.Conclusion: There is significant similarity of antigenic property in both Alfalfa extract and SLO. Thus purified extract can be used for ASO test instead of SLO.

Keyword : Alcohlic exctract , Alfalfa , Streptolysin O

741. Inhibitory Effect of Aqueous Extract of Saffron (*Crocus Sativus* L.) on Adjuvant-Induced Arthritis in Wistar Rat

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Background: *Crocus sativus L.* (Saffron) is a plant of the iris family (Iridaceae). Saffron flower contains various chemical constituents. Saffron has been long used in folk medicine for treating some disease conditions. Its stigma contains crocin, anthocyanin, carotene and lycopene which are known to have pharmacological effects on various illnesses. Materials and Methods: We have investigated the effects of saffron stigma aqueous extract (SAE) in complete Freund's adjuvant (CFA) induced arthritis rat model. So, Male Wistar rats weighed 120-150 g were used. Animals were injected with 100 µl of CFA (100 mg/ml) into the right tibiotarsal joint. After complete establishment of arthritis on day 14, animals were placed in 6 groups (five animals in each); groups II, III, IV and V AIA rats were injected intraperitoneally with 100, 200, 400 and 800 mg/kg of SAE, respectively. Groups I and VI were injected with equal amounts of normal saline and dexamethasone (mg/kg body weight) as a standard reference, respectively. Saffron, normal saline and dexamethasone injections were repeated four more times every other day (days 2, 4, 6 and 8). Measurement of the paws footpad and tibiotarsal joints diameters on the injected and not-injected paws were performed every three days. An arthritis index was assigned to each animal based on footpad paw diameter, ankle joint diameter and hyperalgesia. Results: Our results show that SAE at 400 mg/ml has the best effect on reducing the footpad and tibiotarsal joint diameters, arthritis indexes and limitations of movement in CFA induced arthritis rats compared to non treated group. However this reduction is not as significant as dexamethasone treated group. Conclusion: It seems that aqueous extract of saffron stigma can alleviate the inflammatory condition of CFA induced arthritis. We showed the effect of saffron aqueous extract on improving disease condition in adjuvant-induced arthritis in rats.

Keywords: Saffron, Arthritis, Wistar Rat

742. *Achillea millefolium* Hydro-Alcoholic Extract Downregultes IL-1B Gene Expression in Streptozotocine-Induced Diabetic Rats

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Background: Type 1 diabetes mellitus is an autoimmune disease that causes selective destruction of insulin producing β-cells. Interleukin-1 (IL-1β) has been implicated as an effector molecule of β-cell destruction in autoimmune diabetes. IL-1β inhibits insulin secretion from pancreatic β-cells by stimulating the expression of inducible nitricoxide synthase (iNOS) that generates the free radical nitric oxide. Protective effect of *Achillea millefolium* extract which is traditionally used as a hypoglycemic agent may be the result of antioxidant action of its flavonoid content. The effect of *Achillea millefolium* extract on IL-1β gene expression in STZ-induced diabetic rats was investigated. Materials and Methods: The rats were divided into normal, diabetes mellitus (DM), and *Achillea millefolium*-treated groups (each group, n=7). Diabetes was induced by intraperitoneal injection of streptozotocin into wistar rats. Streptozotocin-induced diabetic rats were treated with IP injection of *Achillea millefolium* extract (100mg/kg) for 14 days. The expression level of IL-1β was examined in rat pancreas using real-time RT-PCR. Results and conclusion: In diabetic rats, elevated fasting blood glucose concentration was dramatically ($p<0.05$) reduced following administration of *Achillea millefolium* extract. The mRNA expression level of IL-1β gene was significantly ($p<0.05$) increased in diabetic rats. Treatment with extract caused a significant ($p<0.05$) reduction in IL-1β gene expression. In conclusion, *Achillea millefolium* extract showed a beneficial effect on β-cells at least in part due to its antioxidative effect through which it down regulates the IL-1β gene expression.

Keywords: *Achillea millefolium*, IL-1B, Streptozotocine-induced Diabetic Rats

743. *Achillea millefolium* Inflorescence Aqueous Extract Attenuates Cyclophosphamide-induced Oxidative Apoptosis in Rat Testis: Immunohistochemical Evidence

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Background: Cyclophosphamide (CP) is a commonly used agent in cancer chemotherapy and immunosuppression. However, CP-induced apoptosis in spermatogenic cells may result in oligospermia and azoospermia, which limits CP clinical application. This study was conducted to assess the possible protective efficacy of *Achillea millefolium*, a medicinal plant with anti-oxidant property, on CP-induced apoptotic effects in rat testes. Materials and Methods: Male Wistar rats were categorized into four groups. Two groups of rats were administered CP at a dose of 5 mg in 5 ml saline/kg per day for 28 days by oral gavages. One of these groups received *Achillea millefolium* Inflorescences aqueous extract at a dose of 1.2 g/kg body weight/day orally four hours after CP administration.A vehicle treated control group and an *Achillea* control group were also included.The immunohistochemistry was used to determine the expression of caspase-3 in testicular tissue samples. Results: After 28 days, rats treated with CP alone displayed increase of cleaved caspase-3 abundance, while *Achillea* aqueous extract coadministration could effectively prevent nearly this abnormality. Conclusion: These findings provide evidence that *Achillea*would offset the apoptotic impact imposed by CP, and may attenuate the testicular toxicity of CPin clinical practice.

Keyword: *Achillea millefolium*, Cyclophosphamide, Apoptosis, Testis

744. Preparation of Turmeric Ointment Based on Traditional Iranian Medicine References and Investigation of its Efficacy on Rheumatoid Arthritis in Rat

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Background: Rheumatoid arthritis is inflammation of one or more joints which causes joint pain, swelling, stiffness and limited movement. Corticosteroids and NSAIDs are usually used for treatment but they have some limitations including lack of efficacy and high incidence of side effects. In traditional medicine, *Curcuma longa* (turmeric) is used as a powerful anti-inflammatory agent in different inflammatory disorders. In traditional Iranian medicine, combination of turmeric and egg yolk is used for inflammations due to trauma and strains. The major component of egg yolk is lecithin. In the present study, in order to evaluate the efficacy of turmeric and egg yolk combination in treatment of inflammation, the effect of turmeric ointment with or without lecithin on Rheumatoid arthritis has been studied in rat. Materials and Methods: Powder of turmeric was extracted by using methanol 80% and maceration method. Two types of ointments were prepared by using vaseline , eucerine, paraffin and bees wax. The first type contained turmeric extract alone and second one was prepared by turmeric extract and lecithin. Inflammation was induced by Freund's adjuvant subcutaneous injection in right tibiotarsal joint in rats. The ointments were applied on the joints for 20 days. Arthritis index and joint diameter were recorded before and after of 20 day's treatment and compared with control group. Serum TNF-α was measured at the last day of treatment. **Results:** The results showed the variations in the results of joint diameter and arthritis index which cannot be interpreted. While, the results of TNF-α measurement established that all products had anti-inflammatory activity with no significant differences between turmeric alone and turmeric with lecithin groups ($P>0.05$). Moreover, no differences were observed between 2.5% and 5% turmeric formulations. **Conclusion**: It is concluded that turmeric ointment has reasonable anti-inflammatory activity in rheumatoid arthritis model in rat. Since, lecithin had no effect on anti-inflammatory activity of turmeric and 2.5% and 5% turmeric groups showed the same activity, therefore, the product containing 2.5% turmeric extract is suggested as the best formulation among the tested products.

Keywords: Topical dosage form, *Curcuma longa*, Rheumatoid arthritis, Traditional medicine

Poster Presentation

745. Suppressive Effect of Peppermint Extract on IL-13 Production

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Background: Medicinal plants have been broadly used in treatment of various diseases. Mints are a group of plants belonging to Labiatae family have anti-bacterial, anti-tumoral and anti-inflammatory effects. Peppermint is a mint specious extensively used in therapy of several disorders such as common cold and bronchitis. The anti-bronchospasmodic and anti-allergic effects of peppermint have also been shown. Elevation of interleukin-13 (IL-13) (a Th2 cytokine profile) level is a well known indicator of allergy. In the present study the effect of aqueous extract of peppermint on IL-13 production in human peripheral blood mononuclear cells (hPBMCs) has been assessed in vitro. Materials and Methods: The hPBMCs were isolated from the venous blood of healthy volunteers by ficoll-hypaque-gradiant centrifugation. Then the PBMCs were cultured in complete RPMI medium. The cells at logarhytmic growth phase, were incubated with different concentrations of aqueous extract of peppermint leaves (0.01-10 mg/ml) in triplicate for 24 hours. Afterward the cell culture supernats were collected and the IL-13 concentration was measured using a standard ELISA kit. Results:Peppermint aqueous extract significantly decreased the IL-13 production in hPBMCs dose-dependently.Conclusions: The results of this study indicate that peppermint aqueous extract down-regulates the production of IL-13 in hPBMCs. Regarding the important role of IL-13 in atopic allergy, the anti-allergic activity of peppermint and also its inhibitorty effect on bronchospasm (a symptom of respiratory allergy), may be partly due to its inhibitory effects on IL-13 production.
Keywords: Peppermint, IL-13, hPBMCs

746. Recombinant Production of an Anti-HIV Lectin, Griffithsin, with Two Leader Peptide in Soybean

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Background:Plant bioreactor, also called molecular farming, has enormous potential to produce recombinantproteins infinitely. Plant bioreactor could be a safe, economic and convenient production system, and can been widely applied in industries and agricultures, especially in the life science and pharmaceutical industry. The application of transgenic plant in the production of vaccines, antibodies and pharmaceutical proteins has become a hot point in the plant genetic engineering in recent years. Griffithsin (GRFT) is a anti-HIV lectin which isolated from red alga, *Griffithia sp*. The potent anti-viral activity of GRFT against both laboratory and primary isolates of HIV at picomolar concentrations makes this protein an attractive candidate microbicide to prevent sexual transmission of HIV. Materials andMethods:Codon optimization of GRFT based on soybean's genome was done. Three vectors were designed, one used KDEL sequence after for protein targeting in ER, second extensin sequence before GRFT sequence for protein targeting in apoplastic area and third without any leader peptide. For both RNA extraction and cDNA synthesis Biozol method was used. The level of transcription and protein expression was obtained by real time PCR and ELIZA method respectively.Results:The results of real time PCR confirmed that recombinant GRFT was expressed 11.819, 7.807 and 67.72 times more than what is expressed in control plant respectively in KDEL construction, extensin construction and in no leader peptide construction. Also, ELIZA results showed respectively 32 and 29 times in extensin and KDEL leader peptide more protein retention than when no leader peptide was used. Conclusion:Our results suggested that although the level of expression between KDEL and extensin construction is statistically significant but protein retention in both ER and apoplastic area is same. On the basis of the results of this work and similar studies we could propose ER as a safe place for this protein retention.
Keywords: Recombinant, Griffithsin, Soybean

747. Inhibition of Pro-inflammatory CytokinesProduction by Ethyl Acetate Extract of *Scrophulariastriata*

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Background:The aim of this study was to investigate theeffect of ethyl acetate (EA) extract of *Scrophulariastriata* (*S. striata*) on the production of pro-inflammatory cytokines. Materials and Methods*:* Mouse peritoneal macrophages cultured with/ without different concentrations(1, 10, 50, 100 and 200 µg/ml)of EA extract of *S. striata* and 2 µg/ml lipopolysaccaride (LPS) for 24 h. In order to recognize chemical components of the extract, Thin Layer Chromatocheraphy (TLC) was used. Production of pro-inflammatory cytokines including Interleukin 1β (IL-1β) and Tumor necrosis factor - α (TNF-α) were examined using ELISA. Results*:* Phytochemical assay showed main components; including phenyl propanoids, phenolic compounds and flavonoids were presented in the extract. EA extract in concentrations of (10- 200 µg/ml) significantly inhibited pro-inflammatory cytokines (IL-1β and TNF-α) production by LPS stimulated peritoneal macrophages. Conclusion*:* The effect of EA extract of *S. striata* on pro-inflammatory cytokines may lead to improving inflammatory diseases, and possibly acts as anti-inflammatory agent via immunomodulatory effect.
Keywords:Pro-inflammatory,Ethyl acetate extract, *Scrophulariastriata*

748. The Effect of Garlic Consumption on Blood Total Antioxidant Status and Some Biochemical/Hematological Parameters in Rat

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Background: The effect of garlic supplementation on blood serum total antioxidant status (TAS), nitrate and routine biochemical/hematological parameters were investigated in rats. Materials and Methods: A total of 30 rats were randomly divided into two groups. Each of fifteen rats of garlic group was fed 600mg/kg garlic solution in water and controls received distilled water by gavage. After garlic consumption for one month, serum total antioxidant, nitrate and some routine biochemical/hematological tests including serum lipids parameters, blood sugar, complete blood count (CBC), Hemoglobin and so on were performed. Results: the mean levels of TAS (1.18±0.11mmol/L) and nitrate (1.44±0.27µmol/L) in the blood serum of rats which treated with garlic significantly ($p<0.01$), ($p<0.05$), increased in compare with the controls (0.77±0.10 mmol/L), (0.78±0.06µmol/L) respectively. But there were no meaningful differences with regard to the routine biochemical/hematological parameters. Conclusion: this investigation confirms that garlic has antioxidant property and may have no effect on lipids profile and total blood cell counts.
Keywords:Garlic, Blood Serum Total Antioxidant Status, Rat

749. *In vitro* Cytotoxic Effect of *Cuscuta chinensis* whole extract on Human Acute Lymphoblastic Leukemia (ALL) Cell Line

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Background; the study of bioactivity of natural products is one of the major researches for drug discovery and the aim of this study was to investigate whether the aqueous whole extract of *Cuscuta chinensis* Lam., which is a traditional medicinal herb commonly used in Iran and other oriental countries, could induce cytotoxic effect in the human caucasian acute lmphoblastic leukemia (CCRF-CEM) cell line in compare with the normal human lymphocyte (JM) cell line. Materials, Methods and Results; *In vitro* cytotoxic screening with various concentrations (0, 0.1, 1, 10, 25 and 50 µg/ml) of the extract with microscope and methyl tetrazolium bromide test (MTT) showed the minimum concentration for the efficient effect of the plant extract was 1 µg/ml, and increasing the dose to 10 µg/ml induced stronger effect. The extract had inhibitory concentration 50% (IC50) about 3 µg/ml in 24 hours and 2.5 µg/ml in 48 h against CCRF. In contrast, the extract was not able to have cytotoxic effect in these doses for the normal cells. Conclusion; the results suggest that *C. chinensis* may play an important role in the treatment of tumor cells.
Keywords: *Cuscuta chinensis*, Human Acute Lymphoblastic Leukemia, MTT assay

750. Humoral Immune Response of Broiler Chickens Fed *Zingiber officinale* and *Zingiber* zerumbet during Acute Heat Stress Period

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Background: Heat stress is a worldwide problem in modern poultry industry and causes big economic losses every year. Heat stress causes suppressive result on immune system. It has been reported that heat stress results in decease of weights of both primary and secondary lymphoid organs, profiles of circulating leukocytes, T cell in the blood, and antibody response against Newcastle disease [1-3]. Then, the aim of this research was to evaluate the effects of *Zingiber officinale* and *Zingiber zerumbet* as anti-stress herbal plants on humoral immune response in broiler chickens. Materials and Methods: A total of 300 one-day-old male broiler chicks (Cobb 500) in a factorial scheme (5x2) were randomly assigned in group of 5 to 60 battery cages for 42 days. All chickens were vaccinated against Newcastle disease (ND) on d 7and 21. Commencing from d 28, equal numbers of chicks were randomly assigned to one of the following five diets: 1) Basal diet (control), 2 and 3) Basal diet supplemented with 1 and 2% *Zingiber zerumbet*4 and 5) Basal diet supplemented with 1 and 2% *Zingiber officinale*. At 35 days of age, half of the chickens from each group were maintained at 23±1°C while remaining birds were exposed to 38±1°C for 2 h daily in another environmentally controlled chamber. On d 37, 10 chicks from each group were randomly selected and their blood samples were used for antibody activity titre against Newcastle disease (ELISA method) and heterophils (H) and lymphocyte (L) count as indicators of stress. Results and Conclusion: Following 2 days of heat treatment H count and H/L ratio were significantly elevated while L count decreased. Feeding regimes had no significant affect on those parameters. ND titer not influenced by Feeding regimes and heat treatment.
Keywords: *Zingiber officinale*, *Zingiber* zerumbet, Acute Heat Stress

751. *In vitro* Viability of Mice Splenocyte following the Use of Aqueous and Alcoholic Extracts of *Sambucus ebulus* and its R100 Fraction

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Background: *Sambucus ebulus* (Dwarf Elder) is used in folk medicine as an ingredient for anti allergy, weight reduction and improve rheumatism. The drug also is used for constipation and as an emetic and to treat edema and kidney disease.Recently, a number of scientific survey carries out its influence on immune system responses like inflammation as well as its contents like Non-toxic type 2 ribosome-inactivating proteins (RIPs). The plant is found from southern Sweden throughout central and southern Europe, in northern Africa, in North America, and in western Asia as far as Iran.In this study, we evaluated the efficacy of herb extracts on viability of mice splenocyte. Materials and Methods: The plant extract was obtained in two aqueous and alcoholic forms, and its R100 was separated using ultra-filtration method. Splenocytes were isolated from inbred Balb/c mice aged 8-10 weeks obtained from the animal laboratory, Shahed University. Then derived splenocytes cultured (in the absence and presence of mitogen) exposed to different doses of these extracts and cell viability was evaluated by MTT assay 48 and 72 hours after culture. Results: The splenocytes cell viability significantly decreased 48 hours after exposure to all doses of aqueous and alcoholic extracts. In 72 hrs cultures, the cells viability decreased after exposure to the aqueous extract, and increased after exposure to high dilutions of alcoholic extract. The cell viability was also decreased 48 hours after exposure to R100 fraction of aqueous and alcoholic extracts, increased 72 hours after exposure to R100 fraction of aqueous extract and decreased 72 hours after exposure to R100 fraction of alcoholic extract.Conclusion: The results of this study show that aqueous and alcoholic extracts of *Sambucus ebulus* and their R100 fraction affect the balb/c micesplenocytes cell viability dose and time dependently and this is probably the explanation for its clinical effects.
Keyword: *Sambucus ebulus*, splenocyte, viability

752. The Effect of R100 Fraction of The Sumbucus ebulus L. on the Macrophage Viability and NO Production

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Background: The plant Sumbucus ebulus L. is one of the invaluable herbal medicine growing in north Iran as well. it is traditionally used in Iran and other parts of the world in treating and preventing infectious and inflammation diseases, such as arthritis rheumatoid, and sore throat. Traditional consumption of this plant shows that one of its effects is immunomodulation. Macrophages are the one of important cells in inflammation.
In this study, we explore the effect of R100 fraction of the extract of the plant Sumbucus ebulus L on the macrophages cell viability and it's NO producing in an in vitro condition. Materials and Methods: Peritoneal exudate cells were obtained from inbred Balb/c mice aged 8_10 weeks obtained from the animal laboratory, Shahed University. The plant extract was obtained in water and alcohol forms, and its R100 was separated by ultra-filtration method. Then the effect of R100 fraction was measured on the viability of macrophages using MTT method upon 24 hours and NO production using NO test following 16 hours from the time of culturing. Results: The viability of macrophages treated with R100 fraction isolated from Sumbucus ebulus L. in dilutions 1/2, 1/10, and 1/1000 has significantly increased in comparison to the control group, and enhanced significantly subsequent to R100 fraction of the alcohol extract in dilutions 1/2, 1/10, and 1/100 comparision to the control group. As the results show, cells that treated with R100 fraction of plant's water extract in dilutions 1/10, 1/1000 has significantly increased in NO production in comparision to the control group, and enhanced significantly in NO production subsequent to treated with R100 fraction of the alcohol extract in dilutions 1/10, 1/100, and 1/1000 comparision the control group. Conclusion: The obtained results suggest that the substances over 100 KD separated from the water and alcohol extracts of Sumbucus ebulus L. have had anti- inflammatory effects dose dependently, and even had increasing cell viability and NO production in low dose. It is recommended that further studies on other fractions containing substances with lower molecular weight be carried out.
Keyword: Sumbucus ebulus L; cell viability; macrophage; NO production; herbal medicines

753. Study on Analgesic and Anti-Inflammatory Properties of *Cordia myxa* Fruit Alcoholic Extract

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Background: *Cordia myxa* is a plant which is used in tropical regions worldwide. Analgesic and anti-inflammatory effect of fruit of this medicinal plant in mice was investigated. Materials and Methods: Alcoholic extract was prepared by maceration method. In six groups of mice (6 animals in each group) acid acetic test and in another six groups (of 6 mice) formalin test was conducted. Groups one to sex in each test were administered normal saline, oral indomethacine, intraperitoneal teramadol, 100 mg/kg oral extract, 200 mg/kg oral extract, 100 mg/kg intraperitoneal extract. Acetic acid-induced writhings were counted within 10 minutes in the first group. In formalin administered group however numbers of foot lickings was calculated within minutes 0 to 5 (acute phase) and 15 to 25 (chronic phase). Results: The results showed alcoholic extract of *Cordia myxa* fruit was considerably effective in formalin test. Conclusion: analgesic and anti-inflammatory properties of this plant's fruit in both acute and chronic phase is comparable with experimental colitis.
Keywords: Analgesia, anti-inflammation, mice, *Cordia myxa* fruit extract

754. Ethyl Acetate Fraction of Shallot (*Allium ascalonicum*) Induces Delayted Type Hyper Sensitivity in Balb/c Mouse

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Background: cell-mediated immunity is essential against intracellular microbes, viruses and tumors. DTH is a reaction mediated by T cells thatis associatedwithactivation ofmacrophagesandinflammation.The medical uses of shallot have been known for centuries. In the present study, we studied the effect of shallot extract and its ethyl
acetate fraction on delayed type hypersensitivity (DTH) in Balb/c mouse. Material and Methods: After preparation of the extract of shallot bulbs with 50% ethanol, the extract was successively fractionated in to n-hexane, ethyl acetate,n-butanol and aqueous fractions. DTH evaluated by priming balb/c mice with sRBC injected subcutaneously.The sensitized animals were challenged with shallot extract intra pritoenly and sRBC subcutaneously on the left hind footpad on day 5. The increase in the footpad thickness was measured at 24, 48h by colis. Results: The results indicated that shallot extract at a dose of 100mg/kg caused a significant ($p< 0.05$) increase in DTH response within 24 h compared to the control group.The results also showed the 0.1, 0.5 mg/kgethyl acetate fraction could increase DTH ($p<0.05$). Conclusion: Our findinges showed that shallot extract and its ethyl acetate fraction caused a significant increase in DTH response in mouse.We can suggest that the shallot contains potential component (s) that can be used for immunomodulation against intracellular microbs and tumor, however further studies is needed to clarify the responsible component (s) and its mechanisms.
Keywords: Immunodeficiency; shallot extract; Delayed type hypersensitivity.

755. *In vitro* Effect of Shallot (*Allium ascalonicum*) Extract and Fractions on Proliferation of Lymphocytes

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Background: T lymphocytes, the cells of cell-mediated immunity, have various functions against intracellular microbes,tumors.To date There are few clinical reports about pharmacological properties of shallot. In the present study, effect of shallot extract and its fractions on lymphocyte proliferation was investigated. Material and Methods: After preparation of extract from shallot bulbs with 50% (v/v) ethanol, the extract was successively fractionated in to n-hexane, ethyl acetate,n-butanol and aqueous fractions. Lymphocyteisolated of mouse spleen were cultured in RPMI-1640 containing 10%FBS, 5µg/ml conA, in an incubator (5% CO2, 37°C). After 24h different doses of shallot and its fractions were added to the respective wells and after 48h, cell proliferation was assayed by MTT.Finally, the absorbance of produced formazan dye by viable cells (significantly in comparison to control) was measured at 570 nm. Results:The results indicated that shallot extract at 10µg/ml shallot increase lymphocytes proliferation ($p<0.05$).The ethyl- acetate, which had the highest activity among shallot fractions, could induce the proliferation of lymphocytes at 100,300ng/ml significantly ($p<0.05$). Conclusion: The results of the presentinvestigation showed that shallot extract and itsfractions especially the ethyl acetate fraction, can induce lymphocytes proliferation in vitro.We can suggest that shallot extract contain component(s) with potential immunomodulatory effect considered for further studies on modulation of immune response.
Keywords: Shallot, Immunomodulator, lymphocyte proliferation, MTT assay

756. Apoptotic Effect of *Melissa officinalis* Extract on Tumor Cell Lines

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Background: Medicinal plants have been effective in cancer chemoprevention. The present study investigated the apoptotic effect of *Melissa officinalis* extracts on tumor cell lines. Materials and Methods: Different extracts of *Melissa officinalis* at various concentrations was used to induce apoptosis in K562 and Jurkat cell lines. The viability of K562 and Jurkat cells in the presence of the extracts was determined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)colorimetric assay. AnnexinV /PI flow cytometry assay was used to determine apoptosis induction in the cell lines. Results: Dicholoromethane extract of *Melissa officinalis* showed the strongest inhibitory effect on the growth of Jurkat and K562 cell lines. The 50% inhibitory concentration (IC50) of this extract at two time points, 24 and 48 hours were as follows. Jurkat cell line; 45.79 µg/ml after 24 hours culture and 37.4 µg/ml after 48 hours. K562 cell line; 46.56 µg/ml after 24 hours and13.21 µg/ml after 48 hours. This extract induced apoptosis in both cell lines after 24 hours treatment in a dose dependent manner. The percentage of apoptotic cells at concentration of 50 µg/ml of the extract was 85.66±4.98 and 85.93±5 in Jurkat and K562 cell lines, respectively. Conclusion: *Melissa officinalis* showed apoptosis inducing property on human tumor cells. Whether the Intrinsic or the extrinsic pathways of apoptosis are involved in the induction of cell death by this extract is under study.
Keywords: *Melissa officinalis*, apoptosis, Tumor Cell Lines

757. The Effect of Satureja Khozestanica Extract on the Proliferation of J774A.1 Macrophage Cell Line

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Background: Satureja Khozestanica a native plant grows in Khuzestan area of Iran. Limited studies showed that Satureja Khozestanica have anti-inflammatory effect. The aim of this study is to determined the effect of Satureja Khozestanica extract on the proliferation of J774A.1 cell line. Materials and Methods:J774A.1 cells 1×10^6 cell ml were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. J774A.1 cells were treated with Satureja Khozestanica ethanol extract (0.001, 0.002, 0.004, 0.008%) for 12h and 24h and 48 h. The proliferation of cell was examined by MTT colorimetric assay. Results: MTT assay showed Satureja Khozestanica extract could induced the proliferation of J774 A.1 macrophage cell line in a dose- and time- dependent manner. Conclusion: Satureja Khozestanica extract have a potential for proliferation of J774 A.1 macrophage cell line and may be effective in treatment of infection diseases.
Keywords: Satureja Khozestanica extract , J774 A.1 cell line, Proliferation

758. Growth Arrest and Induction of Apoptotic by Ethanolic Extract of Alpinia Galanga Rhizhome in Human Breast Carcinoma Cell Line

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Background: Alpinia galanga L. Rhizomes have been used as traditional medicine in Southeast Asian countries. We investigated the potential of ethanolic extract of galangal rhizomes to induce cytotoxic and apoptotic effects in cultured human breast carcinoma cell line, (MCF-7) in compare to non-malignant (MRC-5) cells. Materials and Methods: Both cells were cultured in DMEM medium and treated with the ethanolic extract of galangal rhizomes at various concentrations for three concecutive days. Our study resulted in sequences of events marked by apoptosis, such as loss of cell viability, morphology changes which were evaluated by MTT assay and invert- microscope respectively. The percentage of apoptotic cells was determined by flow cytometry using Annexin-V fluorescein isothiocyanate. Results: The results showed that ethanolic extract of galangal rhizomes decreased cell viability in malignant cells as a concentration- and time- dependent manner. The IC_{50} values against MCF-7 were determined 400±11.7 and 170±5.9 µg/ml after 48 and 48h respectively. Morphology of MCF7 cells treated with ethanolic extract confirmed the MTT results. Ethanolic extract of Alpinia galanga induced apoptosis in MCF-7 cells, as determined by flow cytometry. Conclusion: We also showed that the extract of Alpinia galanga exerts proapoptotic effects in a breast cancer-derived cell line and could be considered as a potential chemotherapeutic agent in breast cancer.

Keywords: Alpinia galanga L., Cytotoxicity, MCF-7, MRC-5, MTT

759. Preventive Effects of Saffron Extract against Oxidative Damage in Aged Diabetic Rat Brain

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Background: The present study was established to examine the effects of aging and/or diabetes mellitus on oxidative stress and the protective effect of vitamin E in the bladder. It was proposed that the balance between oxidant and antioxidant species is important regarding the aging process and prevention of diabetic complications. Materials and Methods: Young and aged rats were randomly allotted into six experimental groups: aged control, aged diabetic, aged diabetic and saffron extract-treated, young control, young diabetic, young diabetic and saffron extract - treated. Diabetes was induced by streptozotocin. Saffron extract was administered to the treated groups. Malondialdehyde and reduced glutathione levels were measured in all rat brain, and histological changes were examined by electron microscopy. Results: We found increased malondialdehyde and decreased glutathione levels in the young and aged diabetic groups compared with the nondiabetic control groups. Elevated malondialdehyde and reduced glutathione levels were observed in the aged compared with the young control groups. There were no significant differences in the malondialdehyde and glutathione levels between young and aged diabetic saffron extract-treated groups compared with the related control groups. Degeneration was greatest in the aged diabetic group. The protective effects of saffron extract were seen in young and aged diabetic groups, especially in the young diabetic group. Conclusions: Our results suggest that saffron extract supplementation prevents free radical damage in brain of young and aged diabetic rats.

Keywords: Saffron, Oxidative Damage,Aged Diabetic Rat Brain

760. Anti-Diabetic Effects of Cichorium Intybus in Streptozotocin-Induced Diabetic Rats

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Background: Herbal medicine widely used in treatment of disease like diabetes mellitus. The present study was designed to investigate the hypoglycemic and hypolipidemic properties of an aqueous extract of *Cichorium intybus* (CIE) which is widely used in India as a traditional treatment for diabetes mellitus. The objective of this study was to investigate the effects of *Cichorium intybus* (CIE) aqueous extract on plasma glucose concentration and lipidprofile in normal and streptozotocin-induced diabetic rats. Materials and Methods: Male Sprague–Dawley rats aged 9 weeks (160–200g) wereadministered with streptozotocin (STZ, 80 mg/kg) intraperitoneally to induce experimental diabetes. The Cichorium intybus leaves were exhaustively extracted (aqueous), concentrated at 40 ◦C using a rotavapor and freeze dried to get powder. Aqueous extract were administered (i.p) to diabetic and normal rats for 28 days. Blood serum glucose, triglycerides, cholesterol, low –density lipoprotein cholesterol, high-density lipoprotein-cholesterol levels, atherogenic index (AI) levels, body weights and food intake were monitored at 0, 7.14 and 28 days after induction of diabetes. Results: In spite of the fact that diabetes elevated blood lipids in all rats after 14 days, *Cichorium intybus* (CIE) aqueous extract significantly decreased serum concentration of cholesterol, triacylglicerols and LDL-C and AI. The most significant result in this assay was the reduction of blood glucose in diabetic rats treated with *Cichorium intybus* (CIE) aqueous extract after 28 days. *Cichorium intybus* (CIE) aqueous extract promoted a general improvement in the condition of the diabetic rats, in body weight and food intake in compare to non-treated rats. Conclusion: The results of this research suggest that *Cichorium intybus* (CIE) aqueous extract, should be effective in the treatment of hyperlipidemia and diabetes in humans.

Keywords*: Cichorium intybus*, Blood glucose level, Streptozotocin, Triglycerides, Total cholesterol

761. Antineoplastic and Apoptosis Activity of Chrysin in Human Breast Adenocarcinoma Epithelial Cell Line

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Background: The Antioxidants have several preventative effects against different diseases, such as neurologic degeneration, aging and cancer, has led to the search for food rich in antioxidants. Honey is reported to contain various compounds such as phenols, vitamins and antioxidants. Materials and Methods: In the present study the anticancer potential of a constituent of honey (Chrysin) in the human breast adenocarcinoma epithelial cell line (MCF-7); as well as in human lung fibroblast cell line (MRC-5) was investigated. Both cells were cultured in RPMI medium and treated with the increasing doses of Chrysin for up to 72 h. The viability of MCF-7 and MRC-5 cells were determined by the MTT assay. A fluorescence-activated cell sorting analysis with annexin-V/propidium iodide was performed to determine cell apoptosis. Results: The results revealed that the cell viability decreased in a concentration and time- dependent manner in the malignant cells treated with Chrysin in comparison with non-malignant cells. Reactivity with annexin V fluorescence antibody and propidium iodide showed that apoptosis occurred in MCF-7 cells. Conclusion: This study shows that Chrysin could also be considered as a promising chemotherapeutic agent and anticancer activity in breast cancer treatment in future.

Keyword: Annexin V, apoptosis, breast cancer, Chrysin, MTT assay

762. Apoptotic Activity of Honey against Human Lung Cancer Cell Line

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Background: Recently it has been highlighted that antioxidants have preventive effects against different diseases. Therefore, that it has led to the search for food rich in antioxidants. Honey has long been used in medicine. Only recently, however, its antioxidant property and preventive effects against different diseases, such as cancer, have been concentrated. In this study, we investigated the anti-proliferative role, apoptosis and the antitumor activity of local honey (Gavan) on human lung adenocarcinoma cell line (A549) and non-malignant cell line (MRC-5). Materials and Methods: The cells were cultured in RPMI with 10% fetal bovine serum treated with different concentrations of honey (0,5, 10, 15 and 20%) for three consecutive days. Cell viability was quantitated by MTT assay. Apoptotic cells were determined using Annexin-V-FITC by flow cytometry. Results: The results of the MTT assay showed that honey was able to induce an anti-proliferative effect on lung breast carcinoma cell line in a dose- and time-dependent manner. Honey showed anti- proliferative activity with the IC 50 value, 15 and 8% after 48 and 72h respectively in A549 cell line. Honey induced apoptosis of the A549 cells in a concentration- dependent manner, as determined by flow

cytometry histogram of treated cells which induced apoptotic cell death is involved in the toxicity of honey. Conclusion: It might be concluded that honey may cause cell death in human lung adenocarcinoma cell line, in which apoptosis plays an important role.
Keyword: Annexin V, Apoptosis, Lung Cancer, Honey, MTT assay

763. The Impact of *Zataria multiflora* Boiss Extract on Human Lymphocyte's Th_1/Th_2 Cytokine (IFN-γ/IL-4) Balance

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Background: The effect of macerated extract of *Zataria multiflora* Boiss (*Z. multiflora*) on cell cytokines gene expression of stimulated peripheral blood mononuclear cells (PBMCs) by phytohemagglutinin(PHA) and non-stimulated cells were examined. Materials and Methods: In both non-stimulated and stimulated cells with PHA, RNA was extracted from the PBMCs of treated and untreated cells to make cDNA for relative quantification. Then, real-time PCR carried out to evaluate relative quantities of IFN-γ and IL-4 mRNA concentrations. Results: In both non-stimulated and stimulated cells with PHA, dexamethasone showed significant inhibitory effect on IFN-γ gene expression (P<0.01 and P<0.001, respectively). Different concentrations of the extract did not show significant effect on this gene in non-stimulated cells but in stimulated cells, the last concentration (200 µg/ml) showed significant stimulatory effect on IFN-γ gene expression (P<0.05). However, the effects of the extract on IFN-γ gene expression were significantly higher than those of dexamethasone in both non-stimulated and stimulated cells (P<0.01 to P<0.001). IL-4 gene expression was significantly decreased due to dexamethasone and different concentrations of the extract in both non-stimulated and stimulated cells (P<0.05 to P<0.01). Cell treatment with dexamethasone and different concentrations of the extract lead to significant increase in the proportion of IFN-γ to IL-4 (Th_1/Th_2 balance) (P<0.05 to P<0.001). The effect of all concentrations of the extract on IFN-γ and IL-4 gene expression and the last concentration on Th_1/Th_2 balance were significantly higher in stimulated than non-stimulated cells (P<0.05 to P<0.001). **Conclusion:** These results indicated that the extract of *Z. multiflora* leads to increase in IFN-γ and decrease in IL-4 gene expression and enhancement of the ratio of IFN-γ to IL-4 (Th_1/Th_2 balance). Therefore, the extract of *Z. multiflora* could be of therapeutic value in inflammatory diseases associated with decreased Th_1/Th_2 balance such as asthma.
Keywords: *Zataria multiflora* Boiss, PBMCs, IFN-γ/IL-4 Balance

764. Inhibitory Effects of Two Satureja Species on Human Lymphocyte Proliferation

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Background: Medicinal plants have been used as a remedy for various diseases in folk medicine. In previous studies the antioxidant activity of Satureja bachtiarica and anti-inflammatory effect of Satureja hortensis on reduction of edema caused by carrageenan have been shown. In the present study the effects of these two species of Lamiaceae family on the proliferation of human lymphocytes was investigated. Materials and Methods: Methanolic extract of the plants were prepared. Peripheral blood lymphocytes separated from healthy individuals were stimulated with phytohemagglutinin and cultured with different concentrations of the extracts. Proliferation of the lymphocytes was measured by BrdU incorporation assay. The extracts with noticeable effects were further fractionated into hexane, dichloromethane, water and butanol and re-examined by proliferation assay. Results: The methanolic extract of both plants showed an inhibitory effect on the growth of mitogen-stimulated lymphocytes. After fractionation, it was appeared that all the fractions except water fraction caused a decrease in lymphocyte proliferation examined by BrdU assay. Among the fractions, the hexane fraction showed the strongest effect on the growth of cells. IC50 values of this fraction for Satureja bachtiarica and for Satureja hortensis were 3.1 and 37.2 µg/ml, respectively. Conclusion: The results of this study showed the presence of immunomodulatory components in the hexane fraction of Satureja bachtiarica and Satureja hortensis. Further studies are needed to identify the compound(s) responsible for this effect.
Keywords: Satureja bachtiarica, Satureja hortensis, Human Lymphocyte Proliferation

765. Analysis of Nitric Oxide Production in the Presence of Several Medicinal Plants of Labiatea Family

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Background: One of the known groups of plants in Iran is Labiatae family with 46 genera consisted of 410 species and subspecies. About 18% of these species are being used for medicinal purposes. Nitric oxide (NO)is an important cellular signaling moleculewhich is produced in acute and chronic inflammatory conditions. The aim of this study was to investigate the effect of several species ofthe Labiateafamilyon the inhibition ofnitric oxide production. These plants included *Dracocophalum kotschyii, Echium amoenum, Foeniculon vulgare, Linum persicum, melilotus officinalis, Mentha longifolia, Portulaca olerace* and *Saturja hortensis.* Materials and Methods: Methanolic extracts of the plants were prepared and studied for NO production by J774A.1 macrophage cell line. The concentrations without cytotoxicity effect on the cells were determined by colorimetric assay. Lipopolysaccharide-activated J74A.1 macrophage cell line was treated with various concentrations of the extracts and then accumulated NO in culture medium was measured using a colorimetric assay based on the Griess reaction. Those extracts with noticeable inhibitory effects were further fractionated into hexane, ethyl acetate, dichloromethane and butanol and re-examined for inhibition of NO production. Results: The methanolic extracts were non cytotoxic against J7 cell line at concentrations of less than; 1 µg/ml for *Foeniculon vulgare*,100 µg/ml for *Linum persicum,* 200 µg/ml for *melilotus officinalis*,500 µg/ml for *Mentha longifolia* and 200 µg/ml for *Portulaca oleracea*. Among the various methanolic extracts, four of them showed a dose-dependent decrease on NO production. After fractionation and determination of $IC_{50,}$ it was appeared that the hexane fraction of*Dracocophalum kotschyii* at100-500 µg/ml, *Mentha longifolia* at1-200 µg/ml and *Saturja hortensis* at10-200 µg/ml had the ability to decrease NO secretion from the stimulated cells. With respect to *Echium amoenum,* the active fraction was the ethyl acetate with an inhibitory effect at 50-200µg/ml.Conclusion:The results of this study showed the presence of anti-inflammatory components in the hexane and ethyl acetate fractions of *Dracocophalum kotschyii, Mentha longifolia* and *Saturja hortensis* and *Echium amoenum* plants. Further studies are needed to identify the compound(s) responsible for this effect.
Keywords: Nitric Oxide, Methanolic extracts, Labiatea Family

766. Purification of Bowman–Birk Inhibitor (BBI) from Soybean Using Single Step Ion-exchang Chromatography

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Background: Soybean Bowman-Birk inhibitor (BBI), a single chain protein with 70 amino acids, has special tendency for trypsin and chymotrypsin enzymes. BBI has anti-inflammatory and anti-cancer activity *in vitro* and *in vivo.* The purification of BBI has always been difficult and a time taking process that involved several steps and various purification columns. This study was focused on a simple method for purification of BBI in order to experimental allergic encephalomyelities. Materials and Methods: Flour soybean was deffated with methanol in two time and reminder was extracted with 60% ethanol. Ethanolic extraction dissolved insodium acetate buffer and was subjected to70% ammonium sulphate fractionation. BBI riched fraction was then dissolved inTris-Hcl buffer and loaded on to DEAE – sephacel column. After washing unbounded proteins, BBI was eluted from the column by linear NaCl gradient. Purity and activity of the inhibitor (BBI) was assessed by SDS-PAGE and a spectrophotometric method, respectively. Results:SDS-PAGE showed a single protein band with molecular weight of 8KD and purity of more than 90 percent under reducing conditions. Activity of the purified inhibitor protein was 555 CTIU/mg. The results showed the

developed method has proper yield and purity. Conclusion:The proposed method is simple and efficient for preparation of large amounts of pure BBI. The pure BBI can be used for study of BBI on various models.
Keywords: BBI, Soybean, allergic encephalomyelitis, Ion-exchang chromatography

767. Purification of SporaminA from Sweet Potato (*Ipomoea batatas*(L.) Lam) in an Ion-exchang Chromatography
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Background: Sporamin accounts for about 60% to 80% of total soluble protein in sweet potato tubers, and share the amino acid sequence identity with some Kunitz-type trypsin inhibitors. sporamin possesses a dual role in sweet potato tuber, one as a somatic storage protein and the other as a natural defense agent, i.e.as a trypsin inhibitor. The aim of this study was to purity of sporamin A from sweet potato using a simple and efficient method in order to assess anti- inflammatory effect of the peptide on experimental allergic encephalomyelitis. Materials and Methods: Initially crude extract was prepared from sweet potato with Tris-HCl buffer with 0.1% NaHSO3, and then subjected to 60% solid of ammonium sulphate fractionation. The obtained precipitation was dissolved in and then loadea on to a column of DEAE – sephcel, equilibrated with sample baffer. Sporamin eluted by (0.1- 0.3) Mlinear NaCl gradient. Purity and activity of the inhibitor was assessed by SDS-PAGE and a spectrophotometric method, respectively. Results*:* SDS-PAGE showed a hemogenous protein band with molecular weight of 31KDa and purity of more than 95 percent under reducing conditions.Trypsin inhibitory activity of the purified sporamin was 707 TIU/mg. The results showed that lineare NaCl gradient (0.1- 0.3) M increased the purity. Conclusion: The proposed method is simple and efficient for purification of sporaminA. In addition, this method does not need any further steps such affinity and gel- filtration columns.
Keywords: SporaminA, allergic encephalomyelitis, Sweet Potato, Ion-exchang Chromatography.

768. Effect of Raphanus sativus Feeding on Regression of Experimental atherosclerosis in Guinea pigs
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Background: Radish (Raphanus sativus) has been used as a dietary agent through-out the world Atherosclerosis is one of the Autoimmune-like diseases and is the cause of many complications in the human societies. In this study, the effect of Radish feeding were studied on lipid profile and atherosclerotic plaques formation in hyperlipidemic guina pigs. Materials and Methods: twenty-five guinea pigs were fed cholesterol (0.5 g per kg. body weight) for an initial period of 4 weeks. Cholesterol was then discontinued and they were divided into two groups. Group-I (n=7) was fed stock diet while group-II (n=18) was given 2 gm per kg body weight of Raphanus sativus daily for 4 weeks. fasting blood samples were collected at onset of study, at 4 weeks duration and finally at the end of study (8 weeks period), for estimation of serum cholesterol, serum triglycerides, LDL-C. HDL-C, VLDL-C and atherogenic index. At 8 weeks duration all the animals were sacrificed for grading of atherosclerotic lesions. Results: In present study Raphanus sativus showed significant hypolipidemic activity as it reduced serum cholesterol, triglyceride, LDL-C and atherogenic index in hyperlipidemic guineapigs ($p<0.01$). The significant rise in HDL-C level was not observed. Conclusion: It can be concluded that Raphanus sativus species of radish is a potent hypolipidemic and antiatherosclerotic agent. It may be related to its antioxidant and anti-inflammatory effects. The validity of these points in humans needs further investigations.
Keywords:Raphanus sativus, atherosclerosis, Guineapigs

769. Ethanol Extract of Satureja Khozestanica Inhibit Nitric Oxide Production in Lipopolysachride Stimulated-J774A.1 Macrophage Cell Line
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Background: Satureja Khozestanica originates from Lorestan and Khozestan, Iran and grows in tropical areas. Limited studies showed that Satureja Khozestanica have anti-inflammatory effect. However, the mechanism underlying the extract of Satureja Khozestanica has not been fully defined. The aim of this study is to determined the effect of ethanol extract of Satureja Khozestanica on the nitric oxide (NO) production in LPS stimulated- J774A.1 macrophage cell line. Materials and Methods: J774A.1 cells 1×10^6 cell ml were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. J774A.1 cells were treated with ethanol extract of Satureja Khozestanica (0.001, 0.002, 0.004, 0.008, 0.016%) for 12h, 24h and 48h in presence or absence of LPS (1µg/mL). NO production was determined by Griess reaction. Results: Results showed that Satureja Khozestanica extract inhibit NO production in J774 A.1 macrophage cell line in a dose- and time- dependent manner. Conclusion: Satureja Khozestanica extract by inhibiting of NO production may be effective in reduce of inflammation.
Keywords: Satureja Khozestanica extract , J774 A.1 cell line, nitric oxide, inflammation

770. Cytotoxic Effects of *Quercus infectoria* and *Terminalia chebula* on Some Cell Lines
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Background: *Quercus infectoria* and *Terminalia chebula* are natural flora of Iran and the Middle East which has been used for the treatment of some diseases. Although some benefits of *Quercus infectoria* and *Terminalia chebula* have been shown by researcher, very few studies have investigated the cytotoxic effects of this herb. Materials and Methods: In this current work, the cytotoxic effects of tannin and non-tannin methanolic extracts for two plants so-called *Quercus infectoria* and *Terminalia chebula* are investigated in four concentrations (1,10,50 and 100 µg/mL) on two cell lines, A549 and PC12 by two methods of trypan blue and wst-1. Moreover, tannin and non-tannin extracts cytotoxic effects are compared with each other. Results: The results showed that methanolic extracts *Quercus infectoria* and *Terminalia chebula* inhibited cell lines growth. Furthermore, IC_{50} values for each cell line were calculated for A549 (*Quercus infectoria* (tannin:80 µg/mL, non-tannin:102 µg/mL),*Terminalia chebula*(tannin:104 µg/mL, non-tannin:111 µg/mL)) and for PC12 (*Quercus infectoria* (tannin:68 µg/mL, non-tannin:103 µg/mL), *Terminalia chebula*(tannin:80 µg/mL, non-tannin:129 µg/mL)). Conclusion The gained data indicated that both plans *Quercus infectoria* and *Terminalia chebula* could be appropriate candidate for therapeutic use and should be study further.
Keywords: *Quercus infectoria*, *Terminalia chebula* , A549, PC12, tripan blue, wst-1, cytotoxicity

771. The Cytotoxic Effects of *Cuscatae chinesis, organum vulgare, Allium sativum, and Aloe vera* Extracts on Raji Cell Line of Lymphoma
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Background: Besides their nutritional usage herbs have wide range of therapeutic properties and have been used as a traditional drug since ancient time. Recent studies have proved that the herbal extracts are potential agents for cancer therapy. In this study we examined the effects of 4 herbal extract, Oregano (organum *vulgare*), Garlic (Allium *sativum*), Cuscuta (Cuscatae *chinesis*) and Aloe (Aleo vera) on Raji lymphoma cell line. Materials and Methods:For preparation of Cuscuta and Oregano, 100 g of the aerial parts were put into boiling water, the solutions were filtrated and dried extracts were used. Aloe fresh leaves were cut and inside gel were taken. The gel was mixed with sterile stilled water and then refrigerated. Peeled garlic was mixed in ratio of 1 g of garlic to 1 mL of distilled water. To assess cells viability, MTT [3-(4, 5-Dimethylthiazol-

2-yl)-2,5-diphenyltetrazoliumbromide] reduction assay was used. Results: different herbal extracts cytotoxicity on Raji cell line was evaluated; garlic and Aleo vera extract had no significant effect in clinicaly appropriate dosages. In contrast, Oregano and Cuscuta in almost all concentrations had remarkable effect on Raji cell line. Oregano at 72h was the most efficient extract (average inhibitory effect: 83.7%) and the optimum suppressive effect was in 0.2 mg/ml with 88% cytotoxicity. likewise, it can considerably reduce the viability of Raji cells in all concentrations at 48h and the optimum inhibition was observed in 5 mg/ml (79.2%). Although, Cuscuta extract decreased cell viability in some concentrations at 24 h, 48h and 72h; the most reduction was detected in 5 mg/ml, 2 mg/ml and 1 mg/ml with 80.6%, 65.5% and 56.1% respectively. Concluion: Oregano is a potential candidate for Raji lymphoma therapy. More evaluations on animal models and also biochemical isolations of the effective material is needed.
Keywords: Garlic, cytotoxicity, Raji cell line, lymphoma, herbal medicine

772. Topical Application of Semelil (ANGIPARS™) in Treatment of Ulcers Caused by *Leishmania major*
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Background: Cutaneous leishmaniasis is endemic in 88 different countries. Melilotus officinalis has beenintroduced as new drug named Semilil (ANGIPARS™) and studies have approved its safety and beneficial effects such as improvement of blood circulation, reduction of inflammation, improvement of lymphedema, and immune system and its safety and efficacy in human diabetic foot ulcer and can be used in treatment of ulcers caused by *Leishmania major*. Materials and Methods: For evaluation of effects of *ANGIPARS*™ on the periods of healing of scars produced by the *Leishmania major*, promastigotes of *Leishmania major* injected to the base of tails of forty BALB/c mice. After appearing the lesions, treatment began according to the below: 10 mice treated with Glucantime®, 10 mice treated with Glucantime®+ topical *ANGIPARS*™ and 10 mice treated with topical *ANGIPARS*™ alone .10 mice were reserved as positive control.Results: In comparison, the period of healing of Glucantime®+ ANGIPARS™ group were shorter than Glucantime® or ANGIPARS™ groups. It seems that the ANGIPARS™ increase the rate of angiogenesis and progress of healing. ANGIPARS™ alone could not decrease the progress of infection. Conclusion: *ANGIPARS*™ is a new and highly effective component for treatment of chronic ulcers especially diabetic foot ulcer and bed sore and acceleration of wound healing. The action mechanisms of it might be related to stimulation of proliferation of endothelial cell and by angiogenic activity. Coumarin which is the main component of this drug is known for its anti-inflammatory and antioxidant activity and reducing synthesis of NO in the phagocytes as an anti-inflammatory action. Cutaneous leishmaniasis is an increasingly prevalent disease causing ulcerative lesions that are often disfiguring and can leave permanent scars for which drug therapy is largely inadequate. Topical treatment offers few adverse effects, better compliance, reduced costs and is feasible for a rural setting.
Keywords: ANGIPARS™, Treatment, Ulcers, *Leishmania major*

773. Immunomodulatory effects of Aloe Vera on mice macrophages response in the presence of the fungus Candida Albicans
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Background: Natural products are important resources in herbal medicines and have been long used for prevention and treatment of many diseases. Aloe vera is one of these plants with medicines properties. Aloe vera has been shown to modulate the immune response. Candidiasis is one of the most common fungal infections and Candida albicans has become the fourth most common cause of hospital infections. Macrophages have been shown to play an essential role as the first line of defense against invading pathogen. In this article the effects of Aloe vera gel extract has been evaluated on macrophage activation. Materials and methods: 5 groups of the balb/c mice were infected with Candida Albicans and then allow the Candida to activated in one week. The Aloe Vera extract has injected to peritonea of the mice. Intraperitoneal macrophages were isolated and MTT assay was performed in order to evaluate viability of intraperitoneal macrophages.Findings: In vivo results show that all doses of the Aloe Vera extract 100 ،50 ،20،10 mg/kg significantly increased cell viability in presence of mitogen but all doses of the Aloe Vera extract does not have an influence in viability of macrophages in absence of mitogen. Results: This study showed Aloe Vera extracts in the In vivo in presence of immune stimulator has an effective role in stimulating the immune system. more studies, such as isolation and purification of aloe vera components, are necessary to clarify the modulatory effects of aloe vera on macrophage function.
Keywords:aloe vera, Immunomodulator, Candida Albicans, Macrophage

774. The Effect of Dried, Aqueous and Alcoholic Extract of the Sumbucus Ebulus on the Macrophage Viability and Nitric Oxide Production
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Background: Sumbucus ebulusL , from the family Caprifoliaceae, extensively grows in the northern regions of Iran. In Iranian folk medicine, the leaves and rhizomes of this plant have been used topically for curing inflammatory joint diseases,sore throat, snake bites and wounds. There are several reports concerning the anti-inflammatory, and antinociceptive effects of the plant *S. ebulus* in Iranian traditional medicine. Macrophages are one of the important cells in innate immunity and inflammation. In the present study, we explore the effect of dried, aqueous and alcoholic extract of Sumbucus ebulus on the macrophages cell viability and it's NO producing in an in vitro condition. **Materials and Methods:** Peritoneal exudate cells were obtained from 3 inbred Balb/c mice aged 8-10 weeks obtained from the animal laboratory, Shahed University**.** The plantextract was obtained in dried, water and alcohol forms. Then the effect of dried, aqueous and alcoholic extract of plant (in serial concentrations including 5, 2, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 mg/ml) on the viability of macrophages was measured using MTT method and NO production using Greess method. **Results:** There was no considerable difference between all concentration of extracts and control group except for 0.01 and 0.001 concentrations of dried extract in which the viability of macrophages was significantly increased as compared to the control group. As the results show, almost all cells that treated with plant's dried extract has significantly increased in NO production in comparison to the control group, and enhanced significantly in NO production subsequent to treatment with the alcohol extract in dilutions 5 , 2 , 0.5 and in the water extract in dilution 1 in comparison with control group. **Conclusion:** The obtained results suggest that the different dilutions of dried, water and alcohol extracts of Sumbucus ebulus L. have had anti- inflammatory effects dose dependently, and had increasing cell viability in some dilutions and NO production in different doses. It is recommended that further studies are required to confirming the anti inflammatory effect of S.ebulus L.
Keyword: Sumbucus ebulus L , Cell viability, NO , Macrophage

775. Tehranolide Modulates the Immune Response
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Background: *Artemisia diffusa* contains a new type of sesquiterpene lactone with an endoperoxide group (Tehranolide). Due to the existing similarity between the structures of Tehranolide and Artemisinin, it was hypothesized that Tehranolide would have similar effects as Artemisinin. In this study, the immunotherapeutic effectiveness of Tehranolide was investigated by direct intra-tumoral injection. Materials and Methods: Tehranolide was purified from *Artemisia diffusa*, and its effect on the tumor volume was investigated. The splenocyte proliferation, shifting of cytokine profile, and the presence of naturally-occurring CD4+CD25+Foxp3+ Treg cells were assessed to describe the anti-tumor immune response. Results: Analysis of immune response showed that, intra-tumoral injection of Tehranolide decreased the rate of tumor growth

compared to control group. Furthermore, the proliferative response of mice treated with Tehranolide was enhanced. In comparison with the control group, decrease in the level of IL-4, increase in the level of IFN-γ in the animals treated with Tehranolide. The results indicated a decrease in tumor CD4+CD25+Foxp3+ T lym-phocytes in the Tehranolide-treated group compared to the control group.
Conclusion: Treatment of tumors with Tehranolide attenuated CD4+CD25+Foxp3+ Treg cell-mediated immune suppression and elicited a persistent anti-tumor immunity against cancer. The measurement of splenic CD4+CD25+Foxp3+ T lymphocytes indicated that Tehranolide significantly ($p < 0.05$) decreased the number of these lymphocytes. These findings show that the use of Tehranolide molecule represents a novel strategy with major suggestions for cancer therapy approachesIn according with our findings, it is suggested that a possible mechanism of antitumor activity of Tehranolide may be due to its involving in modulation of the immune responses. Lastly, it is concluded that Tehranolide has antitumor properties superior to Artemisinin, and can be recommended for further studies to improve its antitumor activity. Thus, in short, Tehranolide can greatly enhance the antitumor potency of a novel form of immunotherapy.
Keywords: Tehranolide, Direct intra-tumoral injection, Immune Response

776. Dihydroartemisinin Modulates the Treg Intra-tumoral Infiltrated Cell and Inhibits Tumor Growth *In vivo*

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Background: Some investigators have been found that Artemisinin and its derivates have inhibitory effect on growth of cancer cells. Among these derivatives, Dihydroartemisinin (DHA) is well known as a semi-synthetic one. In this research, we assessed the effects of DHA on tumor cell growth inhibition *in vitro* by MTT assay and in vivo by intratumoral injection of DHA against breast cancer. Materials and Methods: In this study, the effect of intratumoral administration of DHA on the number of splenic CD4+CD25+Foxp3+ regulatory T lymphocytes was measured with flow cytometry. The levels of IL-4 and IFN-γ were determined by a ELISA system. Results: The results showed that the IC50 values of DHA for RIN pancreatic tumor cell line were 30 μM and significant decrease in the tumor size *in vivo*. Our results demonstrated that a significant decrease in the level of IL-4, increase in the level of IFN-γ in the animals treated with DHA and significant decreased in the level of splenic CD4+CD25+ Foxp3+ T regulatory cells. Conclusions: This study was undertaken to assess the approach of identifying and characterizing novel anti-tumoral activity of DHA compound. Then, a shift was revealed in immune responses towards Th1, besides reduction in tumor size. In view of the above observations and analyses, we assumed that, through using an appropriate does of DHA, it will elucidate more the new therapeutic aspects of this medicine in cancer therapy by reducing Treg and also tumor size as an immuno- stimulant object.
Keywords: Dihydroartemisinin, Tumor Growth, breast cancer, *In vivo*

777. Sclareol Shift the Immune Response Towards Th1

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Background: In this study, we assessed the effects of Sclareol on tumor cell growth inhibition in vitro by MTT assay and in vivo by intra-tumoral injection of Sclareol against breast cancer. Materials and Methods: In this study, the number of splenic CD4+CD25+Foxp3+ regulatory T lymphocytes was measured with flow cytometry. The levels of IL-4 and IFN-γ were determined by a ELISA system. Results: The results of tumor volume in the group with intra-tumoral injection of Sclareol indicated that this injection significantly decreased the rate of tumor growth compared to the control group. The results obtained from spleen cells, which were collected after intra-tumoral injection of Sclareol and were re-stimulated with the lysate antigen, indicated that proliferative response of mice treated with Sclareol enhanced. Based upon the results, Sclareol showed a significantly higher level of IFN-γ producing ability. On the other hand, significantly lower levels of IL-4 production were observed in the Sclareol group. Thus, Sclareol is responsible for immuno-modulatory activity and shift in cytokine pattern in Balb/c mice and the outcome of the immune response with regard to Th1. Measurement of the tumor-infiltrated CD4+CD25+ Foxp3+ T lymphocytes also indicated that intra-tumoral injection of Sclareol significantly ($p< 0.05$) decreased the level of CD4+ CD25+Foxp3+ lymphocytes compared to control group. Conclusions: we demonstrated that local injection of Sclareol can be safely used to treat subcutaneous tumor and it is possible that with a higher local dose of Sclareol we would generate a stronger local effect and may induce a systemic anti-tumoral effect. Therefore, the direct intra-tumoral injection of Sclareol may make it a desirable candidate for cancer treatment.
Keywords: Sclareol, Tumor cell growth inhibition, MTT assay, Intra-tumoral injection, Breast cancer

778. Effect of Hydro-alcoholic Extract of *Lavandula Officinalis* on the Levels of Interleukin-1β and Interleukin-6 inBrain Tissue of Morphine Dependence Male Mice

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Background: Opioids such as morphine alter immune function and decrease host resistance to microbes in experimental animal models. On the other hand, Lavandula Officinalis (L.O) have been used for sterilization and wound recovery in traditional medicine. In this paper we studied amount of interleukin-1β and interleukin-6 in brain tissue of morphine dependence male mice and the effects of (L.O) extract on these factors. Materials and Methods: Morphine dependence, was induced by injection of gradually increasing doses during 15 doses within 8 days. Animals divided into 2 classes: In the 1st class, lavandula extract was administrated 30 min before the last injection of morphine and in the 2nd class, it was done 30 min prior to each morphine injection. Naloxone was injected 2 hrs after the last morphine injection on the 8th day. On the 9th day, the animals were anesthetized by ether and sacrified and then their brains were removed. The brains were homogenized and centrifuged after washing by cold saline and adding triss buffer. Then supernatant was used for measuring of factors.Results: Interleukin-1β increased and interleukin-6 decreased in morphine dependence mice significantly. Chronic administration of (L.O) extract (200 mg/kg), significantly decreased amount of interleukin-1β to the base level, but acute utilization of (L.O) (200, 400mg/kg) slightly attenuated interleukin-6 level. Likewise, chronic administration of (L.O) extract (200 mg/kg) significantly potentiated the effect of morphine reduction on interleukin-6 level, whereas acute administration of it had no significant effect. Conclusion: morphine dependence changed the level of interleukin-1β and interleukin-6 in brain tissue and (L.O) extract can modulated them, dose dependently.
Keywords: Lavandula Officinalis, dependence, morphine, mice, interleukin1β, interleukin6

PSYCHONEUROIMMUNOLOGY

Oral Presentation

779. The Effects Of Isoproterenol And Propranolol on the Cytokine Profile Secretion by Cultured Tumor-Infiltrating Lymphocytes Derived from Colorectal Cancer Patients

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Background: Anti-tumor immunity and cytokine profiles have important roles in the development of cancer. Norepinephrine (NE) release due to sympathetic activation leads to a Th2 deviation via the beta-2 adrenergic receptor (β2AR) and could increase cancer progression. The purpose of

study was to determine the effects of isoproterenol (beta-agonist) and propranolol (beta-antagonist) on the production of IFN-ɣ, IL-4 and IL-17. Cytokine levels were examined in the tumor-infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs) of patients with colorectal cancer. The β2AR expression on lymphocyte subsets was also assessed. Materials and Methods: TILs were isolated from fresh colorectal cancer tissue, and patient's PBMCs were obtained just before surgery. The cells were cultured in medium for 72 hours. Concomitantly, the cells were stimulated with 10 µg/ml phytohemagglotinin alone or in the presence of either 1 µmol/L of propranolol or 1 µmol/L isoproterenol. Cytokines' level was measured in the supernatants by ELISA. Three-color flow cytometry was used to determine the expression of β2AR on the lymphocyte subsets. Results: Level of IFN-ɣ, IL-4 and IL-17 were elevated after PHA-stimulation of PBMCs and TILs. However, the elevation of IFN-ɣ and IL-17 production by TILs in response to PHA was significantly lower than PBMCs. In the presence of ISO, the IFN-ɣ/IL-4 ratio reduced in all groups, but this reduction was very low in TILs. Interestingly, the effects of PRO on cytokine production were, at least partially, comparable to those of ISO. Conclusion: This study demonstrated the effects of isoproterenol and propranolol on cytokine production by TILs and determined β2AR expression on these cells. ISO failed to induce a shift toward the expected Th2 cytokine profile in colorectal cancer patients' TILs, which might be due to downregulation of β2AR expression on TILs. It was also demonstrated that PRO induced a shift to a Th2 profile in PBMCs.

Keywords: Isoproterenol, Propranolol, Beta-2 adrenergic receptor (β2AR), Tumor-infiltrating lymphocytes, Colorectal cancer

780. Interleukin-27 Ameliorates Experimental Autoimmune Encephalomyelitis in C57BL/6 Mice

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Background: Interleukin-27 (IL-27), a member of IL-12 cytokines family, has anti-inflammatory properties. In this study, we evaluated the effect of IL-27 plasmid on some neuroimmunological parameters, in experimental autoimmune encephalomyelitis (EAE), a murine model of human multiple sclerosis (MS), in C57BL/6 mice.Materials andMethods: IL-27 gene was subcloned in p240 plasmid. The recombinant plasmid (p240-mIL27) was injected to EAE mice (test group), two times. At these times, control group received only p240 plasmid. A week after the last injection, both groups were sacrificed and ELISA test was performed for measurement of IL-4, IFN-γ and IL-17. Also using MTT test, spleen cells proliferation response against MOG_{35-55} challenge was performed in vitro. Moreover, pathological exams were performed on the brainstem of mice, to demonstrate cellular infiltration.
Results: the results showed that p240-mIL27 plasmid could improve neurological scores in EAE mice. Also in test group, the p240-mIL27 plasmid diminished the production of IFN-γ and IL-17 and enhanced the production of IL-4 from the spleen cells of EAE mice. In MTT test, the spleen cells of test group showed less proliferative response than control group. Finally, less cellular infiltration was seen in the brainstem of EAE mice treated with p240-mIL27 plasmid.Conclusion: IL-27, having anti-inflammatory properties, could be a suitable candidate for the treatment of inflammatory diseases such as MS.

Keywords:Interleukin-27, experimental autoimmune encephalomyelitis

781. Influence of IL1RN intron 2 Variable Number of Tandem Repeats (VNTR) Polymorphism on Bipolar Disorder

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Background*;* Several lines of evidence point to the role of neurobiological mechanisms and genetic background in bipolar disorder (BD). The interleukin-1 receptor antagonist (IL-1Ra) is the principal regulator of IL-1α and IL-1β bioactivities. This study aimed to investigate the potential role of the variable number of tandem repeats (VNTR) polymorphisms of the IL-1Ra gene (*IL1RN*) in conferring susceptibility to BD. Materials and Methods: Totally, 217 patients meeting DSM-IV-TR criteria for BD and 212 controls were recruited for the study. DNA was extracted from all patients, and the genotypes of *IL1RN* were determined by polymerase chain reaction amplification of VNTR of 86 base pairs in intron 2 of *IL1RN*. Results*:* Genotype distribution of*IL1RN* polymorphism was significantly different between BD patients and controls. *IL1RN*1/2* genotype was more prevalent in BD patients than controls (44.2% vs. 30.2%, P=0.003). Multiple logistic regression analysis demonstrated that *IL1RN*1/2* heterozygotes had a significantly higher risk for BD (OR, 1.83; 95% CI (1.22- 2.74), p= 0.003). Further stratification of the BD patients into *IL1RN*2* allele carrier and non-carrier subgroups revealed a strong association between *IL1RN*2* carriage and prolongation of the disease (P=0.02). Conclusion: These findings suggest that the *IL1RN*-VNTR polymorphism might be a genetic susceptibility risk factor for BD.

Keywords: *IL1RN*, polymorphism, Bipolar disorder, VNTR, IL-1Ra,

782. Study of Cell Death In Sensory Neurons of Dorsal Root Ganglia in Adult Mouse

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Background: Despite the considerable ability of sensory neurons in dorsal root ganglia (DRGs) to survive and regenerate, these neurons display cell death in vitro. The aim of this research in order to culture dorsal root ganglia in adult mouse is to investigate sensory neuron death and mechanism participated in the death of these neurons. Materials and Methods: The L5 DRGs were dissected and incubated for 24, 48, 72 and 96h in culture medium. After fixation, DRGs were cut into 10 -µm- thick sections using a cryostat. TUNEL method was used to investigate biochemical feature of cell death and to investigate the role of caspases, general caspase inhibitor (Z-VAD.fmk, 100 µM) and activated caspase-3 antibody were used. Results: After 24, 48, 72 and 96h in culture, the sensory neurons were also TUNEL positive. The application of Z-VAD.fmk could considerably inhibit apoptosis in these neurons. At these time periods, intense activated caspase-3 immunoreactivity was found in the nucleus and the cytoplasm of these neurons. Conclusion: biochemical study of cell death in sensory neurons showed the occurrence of apoptosis in such neurons. Application of general caspase inhibitor and activated caspase-3 antibody, revealed a caspase-dependent apoptosis in the neurons.

Keywords: DRG, Sensory neurons, Apoptosis, Caspase, Adult mouse.

Poster Discussion Presentation

783. Association between CTLA-4 Polymorphism and Bipolar Disorder

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Background: Bipolar disorder is a severe and relapsing disease with complex etiology such as genetic,biological and psychiatric.the immune responses change in the patients with bipolar disorder. T-lymphocyte plays a major role in pathogenesis of bipolar I disorder.cytotoxic T-lymphocyte (CTLA-4) has the critical role in balance of immune tolerance.polymorphisms of the CTLA-4 gene activate the self reactive T-cells and effect on immune response. This study evaluate the association between polymorphism (promoter-318(C/T) CTLA-4) and bipolar I disorder, if this event could have effect on severity of bipolar disorder?. Materials and Methods: This study was involved 149 patients with DSM-IV diagnosis of BD type I and 168 age- and gender-matched controls.severity of BD was evaluated with standard question sheets YMRS for manic phase and HDRS for depression phase. Polymorphism of CTLA-4 gene was detected with specific primer pair and SSCP-PCR. Results: polymorphisms of CTLA-4 gene at -318(C/T) promoter, showed significant association between patients with bipolar disorder and control group. C/T genotype frequency in patients with BD was 25/5% but in control group was 19/6% (P=0/02). Allelic tests of association showed an increased frequency of the T allele in the patients (P=0/006). Evaluation of this polymorphism in variable phases, showed increased frequency of the hetrozygote genotype -318(C/T) in manic phase (P=0.04). The T allele increase risk of mania in BD (OR=3/6). The hetrozygote genotype -

318(C/T) and T allele have major effect in BD disorder severity. Conclusion: This findings show significant association between polymorphism at position -318(C/T) promoter and BD type I. This polymorphism is a genetic risk factor in pathogenesis of BD. Therefore, its evaluation as genetic marker are helping in prediction of results of BD. Detection of genetic markers that influence in this disorder, provide early detection of high risk persons and it help to select therapeutic methods for individual.
Keywords: Polymorphism, Bipolar disorder (type I), Mania, Depression, CTLA-4

784. Toll Like Receptor-4 Gene Variant is Associated with Migraine?
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Background: Migraine is one of the most common and expensive disorders in the health system which has approximately affected 12 % of worldwide population. Considering to congenital immunity, gene polymorphism of Toll Like Receptors (especially TLR4) probably have role in pathogenesis of migraine. We investigated the effect of Asp299Gly gene polymorphism on the measure of afflicting with migraine. Materials and Methods : In this study we compared 170 patient with migraine by the mean age of 32.4±11.22 with 170 healthy controls. After extracting the genomic DNA, we investigated the gene polymorphism ASP299Gly by Tetra-ARMS method. The effect of TLR_4 gene variant on migraine was assessed by using multivariable logistic regression analysis. Results: We found that a significant difference in distribution of frequency of genotype Asp299Gly polymorphism of TLR4between patients with migraine and healthy people (p=0.00002) so that the frequency of allele G in patients vs healthy people was 15% against 4.7 %, which regression analysis showed. Our results showed the the existence of the allele increases the risk of disease up to 3.75 times (95%, CI; 1.9-6.4) and also existence of heterozygote genotype increase the probability of migraine more than 3.78 times (95% CI; 2.52-7.4, P=0.00002). Conclusion: The results of this study showed that ASP299Gly polymorphism in TLR4can be considered as a genetic risk factor for migraine. This situation is more apparent in
female patients than that the males.
Keywords:Migraine, TLR_4, Polymorphism

785. Preliminary Evaluation of Cellular Immunity in Military Pilots
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Background: Flight can be a stressful environment for human .Military pilots can suffer from great anxiety during air travels. Apart from stress, microgravity, cosmic radiations and low oxygen pressure in high altitude can affect the physiological systems of human body, specially the immune system. The purpose of this study is to investigate the immune system specially cellular immunity in military pilots of the Islamic Republic of Iran. Materials and Methods: Pilots were asked to complete a questionnaire and their blood sample was collected and examined for white blood cell count and differentiation and cell surface markers CD_3 ,CD_4,CD_8 and CD_{56} using flow cytometry technique. IgM and IgG levels against herpes simplex virus 1,2(HSV-1,2), Cytomegalovirus (CMV) and Epstein –Barr virus (EBV) were also analyzed by ELISA method. Results: T-test was used to analyse the data. The results of this study showed that peripheral blood neutrophil cells percentage , cytotoxic T cells(CD_3^+, CD_8^+) and natural killer cells(CD_{56}^+), were significantly higher in pilots compared to the control group ($p < 0.05$). In contrary, total peripheral blood lymphocytes percentage and helper T cells (CD_3^+ ,CD_4^+) were significantly lower in pilots (($p < 0.05$) . Conclusion: This preliminary study indicated that aviation can change pilots immune system specially their cellular immunity.
Keywords: Cellular Immunity, Military Pilots, flow cytometry

786. The Effect of Wet Cupping on Transforming Growth Factor-*β* in Patients with Migraine and Sciatica
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Background: Bleeding and cupping or 'wet cupping' have been used in medicine since ancient times in the treatment of inflammatory disorders. Recent studies show its useful effects on migraine and sciatica. Some researches show TGF-*β* involvement in immuno-regulation of inflammatory diseases. Aim of this study was to show if wet cupping have any effect on serum TGF-β concentration of patients with migraine and sciatica. Materials and Methods: To measure serum TGF-β concentration, serum of twenty patients and twelve healthy controls was taken and frozen before and one week after the 3rd treatment. The TGF-β concentration was measured by ELISA method. Results: We found significant changes in serum TGF-β concentration of patients after wet cupping in comparison with control volunteers. Conclusion: This ongoing study could help to show the effect of wet cupping on the immune system.
Keywords: Wet Cupping, TGF-β, Migraine, Sciatica

787. Cytomegalovirus and Toxoplasma Gondii Antibodies in Schizophrenic Patients: Findings in an Iranian Sample
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Background: Environmental factors have been suggested as etiology of schizophrenia. Some studies have shown the relationship between negative symptoms in schizophrenia and virus, protozoan infection, such as CMV and toxoplasmosis. The aim of our study was to evaluate the relationship between CMV, toxoplasmosis and schizophrenia disorder in Iranian patients. Materials and Methods: 253 patients (18-65 years), hospitalized in Ebn_e Sina and Hejazi, Mashhad, Iran with Diagnosis of schizophrenias was based on the DSM_IV_tR criteria. The negative and positive symptoms were assessed by PANSS test. Toxoplasmosis and cytomegalovirus antibodies were measured by ELISA. We assess the association between deficit status and seropositivityfor toxoplasmosis and CMV IgG antibodies. Results: Toxoplasmosis antibodies were positive in 53.3% of schizophrenic patients. And seropositivity for CMV was 100% .There was no specific relationship between negative symptoms of schizophrenia with either toxoplasmosis (p=0.25) or CMV antibodies.(p= 0.4). Conclusion: In schizophrenic patients the prevalence of toxoplasmosis infection was higher than general population, but there was no relationship between Deficit status of schizophrenia and toxoplasmosis infection. Although in all of the chronic schizophrenic patients cytomegalovirus infection was positive, considering high incidence of positive cytomegalovirus infection in our society, we cannot suggest the effectiveness of CMV infection on deficit status of schizophrenic patients in Iranian population.
Keywords: Toxoplasmosis, Cytomegalovirus, schizophrenia

788. Effect of Experimental Autoimmune Encephalomyelitis on RatHippocampal Long-Term Potentiation
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Background: Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis distinguished by infiltration of leukocyte into the central nervous system. Multiple sclerosis patients show deficits in learning and memory function, and the mechanisms underlying these impairment remain poorly understood. Long-term potentiation (LTP) is widely considered as one of the major cellular mechanisms that underlies

learning and memory. In this study for first time, we evaluated the effects of EAE on hippocampal LTP. Materials and Methods: In this experimental study, EAE was induced by immunization of male Wistarrats with MBP (myelin basic protein) emulsified in complete Freund's adjuant(CFA) and pertussis toxin . After onset ofsymptoms, rats anaesthetized with urethane and placed in the stereotaxic instrument. The stimulating and recording electrodes were placed at the shaffer collateral and CA1 region of hippocampus and synaptic transmition and LTP was recorded. Results*:* Theresults showed that the EAE disease completely blocked TP in the rats(96.66±8.73) as compared to control group(129.68±17.23), (p=0.005.Conclusion*:* It can be concluded that LTP was blocked in EAE disease. However, learning and memory might be affected in multiple sclerosis patients.
Keywords: Experimental autoimmune encephalomyelitis, Multiple sclerosis, Long-term potentioation, Hippocampus

789. Relationship between Sleep Disorders with TH1/TH2 Cytokines 20 Years after Sulfur Mustard Exposure: Sardasht-Iran Cohort Study

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Background: Recently, there has been demonstrated a relationship between sleep and cytokines and a great effort has been done to understand the importance of cytokines in the regulation of mechanisms that control sleep. This study aimed to assess the relationship between sleep disorder with IFN-γ and IL-4 cytokines in sulfur mustard (SM) exposed people 20 years after SM exposure. Materials and Methods: In a historical cohort study, Sardasht-Iran Cohort Study (SICS), 372 SM exposed participants were studied. The Pittsburgh sleep quality index (PSQI) was used to obtain a self-reported measure of sleep disorders. Cytokine was assessed by Elisa quantitative kits. Results:
There were significant relationships between sleep Disorderswith TH1 and TH2 cytokines (P=0.040, P= 0.024 respectively). Conclusion: Since there has been previously reported a shift in the Th1/Th2 cytokine balance towards increased TH1 by sleep, the correlation of TH1 and TH2 with sleep disorders in SM exposed people indicates different response in this population.
Keywords: sleep disorder, TH1/TH2, cytokines, sulfur mustard

790. Relationship between Poorer Sleep Quality with IL-β Cytokine in Chemical Victims: Sardasht-Iran Cohort Study

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Background: Sleep problems in chemical victims are frequently overlooked despite negative impact on patients' perceived health- related quality of life. Sleep deprivation induces significant elevation of serum IL-1 β in healthy people. Furthermore, IL-1β and TNF-α are considered as sleep regulatory cytokines. The aim of this study was to evaluate the relationship between poorer sleep disorder with IL-β cytokine in sulfur mustard exposed people. Materials and Methods: In a historical cohort study, Sardasht-Iran Cohort Study (SICS), 372 SM exposed participants were studied 20 years after exposure. The Pittsburgh sleep quality index (PSQI) was used to obtain a self-reported measure of sleep quality. Cytokine was assessed by ELISA quantitative kit. Results: Based on the result of this study, there is a significant relationship between IL-β cytokine with sleep quality (P= 0.01). Conclusion: Further insight into the functional role of cytokines on the sleep disorder of SM exposed individuals may result in the identification of novel therapeutic perspectives.
Keywords: Cytokines, Sulfur Mustard, IL-β,sleepdisorder

791. Effect of Acupuncture on the Immune Function in Female Rat

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Background: Acupuncture is an ancient therapeutic intervention widely practiced in Eastern countries for thousands of years, owing to the favorable results obtained in the treatment of many diseases and pain relief. Acupuncture provides the possibility to influence the immune system through its effects on the nervous system. The purpose of this study was to assess the effect of acupuncture on the immunological response like anti-sheep red blood cells and (SRBC) plaque-forming cells (PFC). Materials and Methods: 30 adult female rats separated in 2 groups: controul and treatments. Treated by auricular acupuncture for 6 weeks and then serum interleukin-2 (IL-2) and IL-6 contents and splenic lymphocyte transformation rate were compared among the groups. Results: In normal non-immunized rat, enhancement of PFC was seen after a single acupuncture treatment when spleen cells from stimulated rats were cultured with SRBC in vitro. Serum obtained from rat 1h after acupuncture stimulation enhanced the PFC of normal spleen cells in vitro, but the enhancement was abolished by the addition of propranolol. After treatment by auricular acupuncture the serum IL-2 level and the splenic lymphocyte transformation rate significantly increased, and the IL-6 level significantly decreased as compared with the model group.Conclusion: According to this study suggested that acupuncture modulates the immune response on female rats.
Keywords: Acupuncture, Immunological response, Female Rat

792. The Effect of Prenatal Stress on Susceptibility in Mice Model of Multiple Sclerosis

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Background: There is a growing evidence to suggest that the antecedents of some adult diseases can be traced back to their fetal origins. Despite extensive research on such diseases, to our knowledge there has been no research investigating the relationship between the origins of multiple sclerosis disease and maternal infections. The aim of this study was to examine the role of prenatal exposure to endotoxin in fetal programming with respect to induction of susceptibility/vulnerability to multiple sclerosis. Materials and Methods: The pregnant dams were administered a single intraperitoneal injection of Lipopolysaccharide in gestational day 10. The male offspring were weighed and examined for clinical signs of Experimental autoimmune encephalomyelitis in a blinded fashion within 36 days after immunization (postnatal day 63-98). Results: Our data provide the evidence showing that prenatal exposures to higher doses of Lipopolysaccharide resulted in an earlier onset of the disease, an augmentation of its clinical signs also lower body weight in C57BL/6 mice with prenatally Lipopolysaccharide -treated after immunization. Conclusion: Therefore, the present research can provide evidence that prenatal stress may play a role in enhancing the clinical symptoms of experimental autoimmune encephalomyelitis/multiple sclerosis.
Keywords:Experimental autoimmune encephalomyelitis, Fetal programming, Lipopolysaccharide, Multiple Sclerosis, Prenatal stress

REPRODUCTIVE IMMUNOLOGY

Oral Presentation

793. Ovarian Stimulation Affects the Frequency and Localization of Mouse Uterine NK Cells

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Problem: The influence of ovarian stimulation on endometrial mouse NK cell population at implantation sites and their intervals was assessed.Materials and Methods: Tissue samples were prepared from implantation sites and intervals of implantation sites of the uterine horn and spleen of the ovarian stimulated and non-stimulated pregnant mice on 7th day of pregnancy. Serum 17-β estradiol and progesterone were measured in the same time. The tissue cryosections were prepared and double stained for CD 161 and CD3 markers and their cells populations were analyzed. Results: Relative frequency of NK cells was significantly lower at decidua and myometrium of uterus in hyperstimulated mice compared with control. However, no difference was seen in percentage of NK cells in spleen, myometrium and decidua of implantation intervals between the two groups. Conclusion: It seems that ovarian stimulation influences the localization and frequency of NK cells at implantation site and may affect the embryo implantation.
Keywords: NK Cells, 17-β estradiol, CD 16, CD3

794. The Study of TLR3 Gene Expression in Endometrium of Women with Unexplained Recurrent Spontaneous Abortion

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Background:Recurrent spontaneous abortion (RSA) is usually defined as three or more consecutive pregnancy losses before 20th week of gestation. RSA is multifactorial but in some cases, the cause of RSA remains unknownwhich called unexplained RSA. Immunological factors are suggested asetiologicalfactors in unexplained RSA. Toll like receptors (TLRs) as one of pattern recognition receptors (PRRs) play critical roles in innate immunity .TLRs consist of 10 functional receptors in human and recognize wide range of pathogen associated molecular patterns.Recently, some endogenous ligands are known for TLRs. TLR3 recognizes nucleic acids derived viral pathogens and play important role in viral infections. Previous studies indicate that TLRs are implicated in inflammatory and immune disorders. In addition,the existence of TLRs isshown at different parts of female reproductive tract.In present study, the expression of TLR3 gene was investigated in endometrium of patients with unexplained RSA in comparison to endometrium of normal women.Materials andMethod: Endometrial samples were obtainedbetween day 19th and 24th of menstrual cycle (window of implantation) from 10 women with unexplained RSA and6 fertilewomen (having at least one successful pregnancy).TLR3 gene expression was studied by RT-PCR and then quantified byreal time PCR. Beta actin was used as housekeeping gene.Results:TLR3 gene expression was detected in endometrium of patients with unexplained RSA andnormalwomen.The mean relative expression of TLR3 gene was significantly higher in endometrium of women with unexplained RSA in compare to normal ones. Conclusion:Alteration in expression of TLR3 gene might play important role in the pathogenesis of unexplained RSA. Our further studies are directed towards studying the expression of TLR3 protein and also gene expression of other TLRs.
Keywords: TLR3, RT-PCR, Real time PCR, RSA

795. The Effect of Ovarian Stimulation on Uterus Dendritic Cells

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Background: Ovarian stimulation is widely used in IVF clinics. Following ovarian stimulation, hormonal secretions of ovary, particularly estradiol and progesterone increases dramatically. Dendritic cells (DCS) as most important antigen presenting cells possess receptors for the estradiol and progesterone. DCs play an important role in appropriate implantation and successful pregnancy. Increase in estradiol and progesterone concentrations can affect the recruitment, frequency, phenotype and maturation of immune cells particularly dendritic cells. This study was done to explore the above issue. Materials and Methods: On the seventh day of pregnancy blood was collected from two groups of pregnant mice (without ovarian stimulation and ovarian stimulated) and estradiol and progesterone were measured in their sera by ELISA method. To study the systemic and local changes of dendritic cells, their frequency, phenotype and maturation in spleen and decidua were investigated by two colors immunohistochemistry using anti- CD11c antibody and one of the anti CD11b, CD8α, CD86, CD40 and MHC-II antibodies. Results: The result of this study showed multiple fold increase in progesterone and estrogen concentration as well as the decrease in frequency and maturation of dendritic cells in hyper-stimulated group compared to control group. The lymphoid dendritic cells (CD8α+) were the dominant subpopulation in hyper stimulated group compared to controls where myeloid cells (CD11b+) were more frequent. These changes were observed in both tissues (spleen and decidua). However the changes in spleen were not statistically significant. Conclusion: Due to our information, this is the first report of dendritic cell changes following hyper-stimulation. Regarding the accepted role of dendritic cells in embryo implantation and regulation of maternal immune response, it seems that their changes in implantation site can influence the pregnancy rate after IVF.
Keywords: Dendritic Cells, IVF, ELISA

796. Expression of CD69, CD161 and CD94 Receptors on NK Cells and Their Correlation with NK Cell Cytotoxicity in Women with Recurrent Spontaneous Abortion and in Vitro Fertilization Failure

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Background: NK cells have an important role in success or failure of implantation. The purpose of this study was to compare the percentage of circulating NK cells expressing activation and inhibition markers between RSA and IVF failure patients with healthy control women, and to determine the correlation between these markers and the NK cell cytotoxicity assay. Materials and Methods: In this case-control study peripheral blood samples from 43 patients including 23 women with RSA and 20 IVF failure women, and 43 healthy control women including 36 normal multiparous and 7 successful IVF women were collected. The expression of CD69, CD94 and CD161 on NK cells was detected using specific monoclonal antibodies, and analyzed by flow cytometry. NK cell cytotoxicity detected by lactate dehydrogenas (LDH) release assay. Results: RSA and IVF failure patients had significantly higher expression of NK cell activation markers of CD69+ and CD161+ than control women. The expression of CD94, NK cell inhibition marker, showed significantly an increase in RSA and IVF failure patients in comparison with control group. The level of NK cell cytotoxicity assay in the patients was interestingly higher than control group. However, no correlation was detected between the expression of CD69, CD94, CD161 and the NK cytotoxicity assay in RSA and IVF failure patients. Conclusion: Activation markers including CD161+ and CD69+ expressed on NK cells as well as NK cell cytotoxicity can be added to the previously reported risk factors for immunologic implantation failure such as increase of NK cell. Although NK activation markers and cytotoxicity in RSA and IVF failure patients increase, it seems NK cell cytotoxicity and peripheral blood NK cell receptors be different aspects of NK cells and do not correlate, which required further studies.
Keywords: NK Cells, CD69, CD94, CD161, Cytotoxicity, RSA, IVF failure

797. The Study of Anti-tumor and Immunomodulatory Activity of Vitamin D3 in Endometrioma

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Background: Endometriosis is a chronic inflammatory disease characterized by the growth of endometrial tissue outside the uterine cavity. In patients with ovarian endometriosis (endometrioma), the risk of ovarian cancer increases fivefold. Regarding its anti-tumor andanti-inflammatory

action, the effect of vitamin D3 on pre-cancerous state and anti- and pro-inflammatory cytokines production by endometrioma cells were investigated in this study. Materials and Methods: Stromal cells were prepared though enzymatic digestion of eutopic and ectopic endometrial tissues from 10 endometriotic patients. Endometrial stromal cells of 10 non-endometriotic patients served as control. Following cell characterization through immunocytochemisty and flowcytometry, stromal cells were cultured in the presence or absence of the active form of vitamin D3. Cultured cells were analyzed for proliferation, adhesion to extracellular matrix and invasion to matrigel. Additionally, the levels of IL-6 ،IL-8 ،IL-17 ،TGF-β ،TNF-α and IFN-γ in culture supernatants was determined using ELISA. Results: In all groups, vitamin D3 treatment resulted in a significant increase of attachment ($P<0.05$), while decreased cell invasion to matrigel ($P<0.05$). Such treatment, however, had no significant effect on the proliferative capacity of these cells. Ectopic and eutopic endometrial cells from patients secreted higher levels of IL-6 and IL-8 compared to the control group in the absence of vitamin D3 ($P<0.05$), whereas, vitamin D3 treatment resulted in a significant decrease in IL-6 production by patient ectopic cells ($P<0.05$). Stromal cells from all groups, showed no detectable secretion of other cytokines. Conclusion: With regard to the reducedinvasivenessandincreasedadhesionofstromalcellsin the presence ofvitaminD3, it seemsthatthishormonecan effectively be used in inhibition of disease spreading or reducing cancer risk. The decreased production of IL-6, as a pro-inflammatory cytokine, by ectopic stromal cells following vitamin D3 treatment, implies anti-inflammatory action of this hormone which could be viewed as its potential beneficial effect over disease course.

Keywords: Vitamin D3, Endometriosis, Inflammation, Ovarian cancer

798. Presence of Autoantibody against Two Placental Proteins, Peroxiredoxin 3 (Prx3) & Peroxiredoxin 4 (Prx4) in Sera of Recurrent Pregnancy Loss (RPL) Patients

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Background: Recurrent Pregnancy Loss (RPL) is defined as two or more consecutive abortion before the twentieth week of gestation.Several evidences support the involvement of the immune responses in the etiology of RPL. Autoimmune diseases increase the risk and accounts for at least 20% of RPL.Placenta is a pregnancy unique tissue and proper formation of placenta is key phenomenal for either success of a pregnancy or occurrence of PRL. The aim of the present study was investigation of the placental proteins that may act as antibody targets in RPL patients. Materials and methods: Total placental proteins were extracted and separated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technique.After 2D-PAGE, separated protein spots were transferred on PVDF membrane. Western blot was performed on PVDF membranes using pooled sera from twenty RPL patients and compared with membranes blotted with twenty normal pooled women sera. Different blotted spots were identified by MALDI TOF/TOF mass spectrometry technique. Results of the mass analysis also were checked by western blot using mAb and RT-PCR technique. Results: The results indicated that RPL women may produce antibody against Peroxiredoxin 3 and Peroxiredoxin 4. Expression of Peroxiredoxin 3 and Peroxiredoxin 4 in placenta was also confirmed at RNA and protein level using RT-PCR and western blot, using mAbs, technique respectively. Conclusion: Our results candidate two placental proteins, Prx3 & Prx4, as new placental immune targets. Considering the role of antioxidant defense in the protection of placenta from oxidative stress, anti-peroxiredoxin antibodies may enhance the oxidative stress by inhibiting antioxidant enzymatic activity. Production of antibodies against peroxiredoxin 3 and 4 may introduce a new autoimmune hypothesis in RPL, which is needed to be test in the future works.

Keywords: Autoantibody, RPL, Prx4, Prx3

799. Relationship between IL-17 -197 G/A and IL-6 -174 C/G Gen Polymorphisms with Recurrent Spontaneous Abortion in Iranian Women

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Background: Spontaneous abortion is one of the common complications of pregnancy. The aim of this study was to survey the frequency of the interleukin 17 (IL-17) -197 G/A and IL-6 -174 C/G gene promoter polymorphisms in Iranian women with recurrent spontaneous abortion (RSA). Materials and Methods: In this case – control study, 200 women with at least three RSA as the case and 100 healthy women with a history of two successful deliveries, without any pregnancy complecations, as the control groups, were selected. Blood samples were recruited from Avicenna Infertility Clinic, Tehran,Iran. Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) were performed to assess the frequency of the two mentioned promoter gene polymorphisms. The frequencies of the were calculated and compared between the case and the control groups. Results: The frequencies homozygotes and heterozygotes polymorphisms of IL-17 -197 G/A promoter gene were 8.5% and 48.5 %, in the case group and 8 % and 50% in the control group, respectively. In addition, the frequencies of homozygote and hetrozygote polymorphisms of IL-6 -174C/G promoter gene were 47% and 48.5% in the case group and 41 % and 52% in the control group, respectively. Conclusion: The data showed that There were not any significant differences in the frequencies of IL-17 -197 G/A and IL-6 -174 C/G polymorphisms between normal women and those suffering from RSA.

Keywords: Abortion, polymorphisms, Interlekeuin 17, Interlekeuin 6

800. Vitamin D3 Inhibits Inflammatory Cascade in Endometrial Cells through Down Regulation of TLR Signaling

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Background: Elevated levels of pro-inflammatory cytokines at the feto-maternal interface are associated with increased risk of miscarriage, preterm labor and preeclampsia. Bacterial lipopolysaccharide (LPS) and lipoteichoic acid (LTA) are among pathogen-associated molecular patterns (PAMP) which induce TLR signaling leading to release of pro-inflammatory cytokines. Based on the immunomodulatory action of 1,25(OH)2 vitamin D3 (VD3) over a broad range of cells, we assessed here the effects of this hormone on production of inflammatory cytokines and expression of TLR2, TLR4 and MyD88 in endometrial cells in response to LPS and LTA stimulation. Materials and Methods: Endometrial tissues were obtained from cycling women undergoing operations for benign gynecological conditions. Whole endometrial cells (WECs) and purified endometrial stromal cells (ESCs) were treated with different concentrations of LPS and LTA in the presence or absence of vitamin D3. Expression of TLR2, TLR4 and MyD88 and production of IL-6, TNF-α and IL-8 were then assessed by RT-PCR and ELISA, respectively. Results:VD3 significantly decreased expression of MyD88 gene in ESCs in both LPS-treated and control cells. ($P<0.05$), but did not have significant effect on TLR-2 and 4 expression. Vitamin D3 treatment resulted in substantial decrease of TNF-α and IL-6 production by LPS- and LTA- stimulated cells, whereas increased IL-8 production. Conclusion:Based on the results presented here, it seems that vitamin D3 could control adverse effects associated with female reproductive tract infection through modulation of MyD88 expression, an adaptor protein which play essential role in TLR signaling cascade. Based on these findings, we propose vitamin D3 as a novel natural therapeutic compound for threatened pregnancies due to genital tract infections.

Keywords: Vitamin D3, Toll like receptor, Signaling, LPS, LTA, Pregnancy, Infection

Poster Discussion Presentation

801. Comparative Immunophenotypic Profiles of Menstrual and Peripheral Blood Leukocytes in Fertile Women

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Background: Successful pregnancy requires balanced regulation of immune cells and immunomodulators at the feto-maternal interface. Although, such regulations could be demonstrated at the systemic level, systemic monitoring of the immune system could not precisely highlight the local immune status in the uterus, where the fetal antigens are in close proximity with maternal immune system. In this study, the endometrial immune milieu was investigated by a non-invasive method through immunophenotyping of immune cells in menstrual blood (MB) and the results were compared with those of the peripheral blood (PB). Materials and Methods: Peripheral and menstrual blood of healthy fertile women with no previous history of abortion and at least one healthy live birth was collected in the second day of their menstrual cycle. Peripheral and menstrual blood mononuclear cells were isolated by gradient centrifugation and the frequency of different subpopulations of leukocytes was enumerated by flow cytometry. Results: The frequencies of total T cells $CD3^+$(%66±8 and %60±9), $CD4^+$ (%45±5 and %40±8) and $CD8^+$ (%24±6 and 24±7) subsets were similarly represented in PB and MB, respectively. However, NK cells ($CD56^+$, $CD16^-$) and NKT cells ($CD3^+$, $CD56^+$) were significantly higher in MB (10.1%±4.6, and %4.4±4.6, respectively) compared to those in PB (%3.2±2.3 and %1.6±1.6, respectively) (p=0.01 and p=0.06, respectively). Conclusion: Our findings indicate that distribution of the immune cells in MB is very similar to that reported in the endometrium. Since all previously reported studies on endometrial immune cell populations have been conducted on biopsy samples, our results are novel and present a new and non-invasive approach on immunological investigation of endometrial immune system.
Keywords:Immunophenotyping, menstrual blood, flow cytometry, fertility, endometrium, leukocyte

802. Circulating Endothelial Cells (CECS) and E-Selectin; Predictors of PeeclampsiaEarly in the Third Trimester

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Background: Circulating endothelial cells (CECs) and E-selectin known as sensitive and specific markers of endothelial dysfunction. This study investigated whether CECs and E-selectin are surrogate biomarkers of preeclampsia and if measurement of CECs and E-selectin, early in third trimester could be a means of predicting preeclampsia. Materials and Methods: In this prospective, descriptive-analytic study, 483 pregnant women were tested for roll over test during 28-30 weeks of gestation. CECs were measured with anti-CD 146–driven immunomagnetic isolation in women with positive roll over test and they follow up prospectively until delivery without any active intervention. Women with and without preeclampsia were determined. The number of CECs and level of E-selectin were compared in two studied groups.Results: 41 women with positive roll over test out of 483 ones were selected and follow up. From which 19 pregnant women diagnosed with preeclapmsia and reminder were normotensive. The numbers of CECs was significantly higher in preeclampsic women (median 24.7cells/mL) than normal pregnancies (median 13 cells/mL).The best cut off point for CECs number was 6.5 with a 78.9% and69.1% sensitivity and specificity, respectively. The level of E-selectin was significantly higher in mothers with preeclampsia ($P<0.05$). Conclusion: Considering that mean level of CECs and E-selectin in preeclamptic women were significantly higher than normotensive pregnant women and the predictive value of CECs for preeclapmsia early in the third trimester, our results support the endothelial involvment of the disease. It could be concluded that the determining and correction of the endothelial malfunction using CECs and E-selectin early in pregnancy would help us to prevent obstetrics complications related to preeclampsia.
Keywords: Preeclampsia, Endothelial dysfunction, CECs, E-Selectin

803. The Comparison of TLR1,2,6,10 Gene Expression in Ectopic, Eutopic and Normal Endometrium

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Background: Endometriosis is a benign condition which is characterized by the presence of endometrial glandular and stromal cells in areas outside of the uterine. Endometriosis is a common condition with a varied phenotype that is mainly associated with pain including dysmenorrhea and deep dyspareunia as well as problems with fertility. There are relationships between the female immune system and the occurrence of endometriosis. Toll-like receptors (TLRs) are essential receptors of the innate immune system that stimulate numerous inflammatory pathways and harmonize systemic defense against pathogens. TLR2 is essential in the recognition of microbial lipopeptides and peptidoglycan derived from Gram-positive bacteria. Also TLR1 and TLR6 recognize components of gram-positive bacteria which cooperate with TLR2 to discriminate subtle differences between triacyl and diacyl lipopeptides, respectively.The objective of this study is to clarify the expression of TLR 1, 2, 6 and 10 in the ectopic and eutopic endometrium of women with endometriosis and compare with normal endometrium of healthy women. Materials and Methods: Normal and eutopic endometrium obtained with piple from endometrium of women without and with endometriosis, respectively. Ectopic samples obtained with laparoscopic procedure from patients with endometriosis. Five samples in each group were studied by reverse transcriptase polymerase chain reaction (RT-PCR) to show the expression of TLR1, 2, 6 and 10 genes. Results: RT-PCR showed the expression of TLR1, 2, 6 and TLR10 genes in normal endometrium but variable expression of these genes were seen in ectopic and eutopic samples. Conclusion: The different expression of TLR1, 2, 6 and10 in these three types of endometrial tissues may be a strong evidence of critical role of innate immune system in out break of endometriosis. Further study need to be done for quantitative assay by Real time PCR.
Keywords: TLRs, RT-PCR, Real time PCR

804. Effect of High-Dose Dexamethasone in Early Pregnancy on Pregnancy Outcome

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Background: Embryo implantation, which is an absolute requirement for reproduction, starts with blastocyst apposition to the uterine endometrium. Implantation can only take place in a receptive uterus. It is increasingly clear that inflammation-like events are necessary for the successful embryo implantation. In the present study, we aimed to evaluate the anti-inflammatory effects of high-dose dexamethasone in early pregnancy on pregnancy outcome. Materials and Methods: Pregnant BALB/c mice were treated daily with dexamethasone (4 µg/g body weight per injection) as experimental group or an equivalent volume of saline as control group on days 0.5-4.5 of pregnancy. On day 13.5 of pregnancy, the pregnant BALB/c mice were sacrificed and placenta, decidua and blood were collected. Serum levels of progesterone, 17-β estadiole and ferritin was determined by ELISA. The effect of decidual cell supernatants (DS) and placenta cell supernatants (PS) on PHA or LPS-induced lymphocyte proliferation was investigated by MTT assay. Results: Results of this study showed a statistically significant higher abortion rate in experimental group compared to control group. Experimental group showed increased amounts of progesterone and decreased amounts of 17-β estadiole, but we didn't see a significant difference in ferritin concentration between two groups. MTT assay results showed that DS from

experimental group significantly suppressed ($P < 0.05$) LPS and PHA-stimulated splenocyte proliferation compared with control group. PS from experimental group compared to control group showed no significant effect on LPS and PHA-stimulated splenocyte proliferation. Conclusion: The presented results demonstrate that maternal High-dose dexamethasone application in early pregnancy has pronounced effect on levels of hormones and immune state of decidua and finally on fetus resorption.
Keywords: Dexamethasone, BALB/c, MTT assay

Poster Presentation

805. Is the Endometrial Cytokine Profile of Women with Repeated Implantation Failure Similar to Fertile Women?
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Background: Repeated Implantation Failure (RIF) is one of the most complex obstacles in assisted reproduction. The cytokine and chemokine content of uterine cavity seems to be involved in the appropriate contact of endometrium and embryo during implantation process. The aim of this study was to compare the cytokine profile in the endometrium of normal fertile women and those with repeated implantation failure. Materials and Methods: Endometrial tissues were digested enzymatically under sterile condition. Whole endometrial cells and endometrial stromal cells which were obtained from RIF and normal fertile women were cultivated and stimulated for cytokine secretion. The levels of IL-10, TGF-β, IFNγ, IL-6, IL-8 and IL-17 in culture supernatants of two groups were assayed by ELISA and compared. Results: Endometrial stromal cells and whole endometrial cells of normal fertile women produced higher levels of IL-6, IL-8 and TGF-β compared to RIF group. This difference was statistically significant only in endometrial stromal cells (P value<0.05). Moreover, endometrial stromal cells of normal fertile women produced lower levels of IL-10, as ananti-inflammatory cytokine, in comparison with RIF group (P value<0.05) Conclusion: Disturbances in cytokine production at the feto-maternal interface could be a leading cause of implantation failure. A pro-inflammatory cytokine profile seems to be necessary for successful implantation.
Keywords: Repeated Implantation Failure, Endometrium, Cytokine

806. ImmunoregulatoryEffects of 1, 25$(OH)_2$vitaminD_3 on Cytokine Production by Endometrial Cells of Women with Repeated Implantation Failure
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Background: Repeated implantation failure (RIF) constrains a great number of problems onpatients and health care systems. Vitamin D3 has been proposed to have positive effects during pregnancy.The aim of present study was to compare the immunomodulatory effects of $1,25(OH)_2$vitaminD_3 on cytokine production by endometrial cells of women with RIF and healthy fertile controls.Materials and Methods: Whole endometrial cells (WECs) and endometrial stromal cells (ESCs) from RIF and normal fertile women were treated with $1,25(OH)_2D_3$. The levels of IL-10, TGF-β, IFNγ, Il-6, IL-8 and IL-17 in culture supernatants were measured by ELISA. Ability of the cells from both groups to produce $1,25(OH)_2D_3$ was evaluated and compared.Results: $1,25(OH)_2D_3$ down-regulated cytokine production in WECs from both groups except for IL-8 which was elevated. Similar trends were also seen in ESCs except up-regulation of TGF-β in RIF group. Endometrial cells of both groups had comparable capacity to produce 1, $25(OH)_2D_3$.Conclusion: Based on our results, vitamin D3 showed minimal differential immunoregulatoryeffects on endometrial cells from RIF and control women.
Keywords: 1,25-dihydroxy-vitamin D_3, Cytokine, Endometrium, Immunomodulation, Implantation Failure

807. The Comparative Study of Anti-B2gp1 Autoantibody Level in Serum of Pregnant Women with History of Recurrent Miscarriage and Pre-Eclampsia with Healthy Subjects in Gorgan
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Background: Recurrent miscarriage (RM) is generally considered to be the loss of three or more pregnancies before viability. Preeclampsia (PE) is amongst the major causes of mortality and morbidity during pregnancy and childbirth. Pre-eclampsia is usually defined as the presence of proteinuria and hypertension after 20 weeks gestation. Antiphospholipid antibodies are recognized as a treatable cause of recurrent pregnancy loss and risk factor of preeclampsia. Beta2 glycoprotein I (*β*2GPI) being one of the most important antigenic targets for these auto antibodies. This study was assumed in order to determine the frequency of anti-*β*2GPI in pregnant women with RM history or PE and normal pregnant. Materials and Methods: A case-control, cross sectional study was conducted on 150 pregnant women divided in three groups: first study group of 50 pregnant women with RM , second study group of 50 pregnant women with PE and control group of 50 pregnant without RM and PE history. Seroprevalence was assessed by both commercial ELISA and multiparametric processor with single dose ready-to-use tests measuring IgM and IgG auto antibodies to β2GP1 antigen. Results: Our results indicated that among recurrent miscarriage patients, % 7.5 was seropositive for Anti-β2GP1IgG, and % 5 were seropositive for Anti-β2GP1 IgM. Our finding show that among normal pregnant controls % 7.5 were seropositive for Anti-β2GP IgG and % 7.5 were seropositive for Anti-β2GP1 IgM. Conclusion: Our finding show that among pregnant women with Preeclampsia % 12.5 were seropositive for Anti-β2GP1IgG, and % 7.5 were seropositive for Anti-β2GP1 IgM. These data suggest that occurrence of Anti-β2GP1 auto antibodies in population of pregnant women with RM and PE has not case-control significance differences ($P > 0.05$).
Keywords: Autoantibody, Antiphospholipid syndrome, Recurrent Miscarriage, Pre-eclampsia

808. The Study of Relationship between Serum Cytokines and Th17 and Treg Cell Populations in Endometriotic *vs.* Non-endometriotic Patients
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Background: Endometriosis is a common, painful and chronic gynecological disease characterized by the growth of endometrial tissue outside the uterine cavity. It has been suggested that IL-17 producing T cells (Th17 cells) and regulatory T cells (Tregs) are involved in the pathogenesis of endometriosis. Bases on the key role and mutually exclusive action of different cytokines on differentiation of naïve T cells toward each aforesaid T cell populations, we studied the relationship between serum cytokines and Th17 and Treg cell populations in endometriotic *vs.* non-endometriotic patients. Materials and Methods: The studied groups comprised of 35 women with endometriosis and 15 women without endometriosis diagnosed during laparoscopy. The percentage of peripheral Th17 and Tregs cells was evaluated by flow-cytometry with a panel of

fluorophore-conjugated monoclonal antibodies specific for CD3/CD4/IL-17A and CD4/CD25/FoxP3, respectively. Serum levels of IL-6, IL-8, IL-17, TGF-β, TNF-α, and IFN-γ were measured using ELISA sets. Results: Frequency of peripheral Th17 and Treg cells showed a statistically significant increase in women with endometriosis in comparison to controls (P=0.039, 0.003, respectively). Patient with endometriosis had significantly higher levels of serum IL-6 and TGF-β compared to non-endometriotic patients ($p<0.05$). The levels of other cytokines had no significant difference in two groups. Conclusion: Increased levels of peripheral Th17 cells in endometriotic patients may highlight the inflammatory nature of the disease with overflow mechanisms leading to sustained higher levels of systemic IL-17 producing cells. This result is supported by the higher levels of serum IL-6 and TGF-β, the two key cytokines responsible for the differentiation of Th17 cells from naïve T cells. More importantly, this inflammatory response was accompanied with higher levels of regulatory T cells which may be a part of counter-regulatory mechanisms for controlling the inflammatory process mainly ensued by Th17 cells. In opposite point of view, this cell population may suppress T cells capable of targeting ectopic endometriotic tissues.

Keywords: Endometriosis, Th17, Treg, Inflammation, Cytokine

809. Presence of autoantibody against two placental proteins, Annexin A1 & Vitamin D binding protein, in sera of women with pre-eclampsia

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Background: Pre-eclampsia (PE) is one of the most complex and life-threatening pregnancy disorders. PE is characterized by maternal hypertension and proteinuria. PE affects 2-8% of all pregnancies worldwide. However, the exact etiology of PE is not well known but, combination of several factors such as environmental, racial, genetic and immunologic factors could predispose pregnant women to PE. Several evidences support an immunologic etiology for PE and auto-immunity is a risk factor for PE. Considering the major role of the placenta in the pathogenesis of PE, the aim of the present study was investigation of placental proteins which may act as a target for auto-antibodies in PE patients. Material and methods: Total placental proteins were extracted and separated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technique. After 2D-PAGE, separated protein spots were transferred on the PVDF. Western blot was performed on PVDF membranes using pooled sera from twenty PE patients and compared with membranes blotted with twenty normal pooled women sera. Different blotted spots were identified by MALDI TOF/TOF mass spectrometry technique. Results of the mass analysis also were checked by western blot using mAb and RT-PCR technique. Results: The results indicated that PE women may produce antibody against Annexin 1 and Vitamin D binding protein (VDBP). Placental expression of Annexin 1 and VDBP was also confirmed at RNA and protein level using RT-PCR and western blot, using mAbs, technique respectively. Conclusion: The present study candidate two new placental proteins, Annexin 1 and VDBP, as placental immune targets. Considering the relation between vitamin D deficiency and increasing risk of pre-eclampsia and the role of annexin1 in the resolution of inflammation, production of antibody against Annexin 1 and VDBP may account as a new autoimmune hypothesis in pre-eclampsia which is called for further investigations in the future works.

Keywords: Annexin A1, Vitamin D binding protein, 2D-PAGE

810. Assessment of effect of melatonin on vitrification –thawing immature mouse testicular tissue

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Background: In the children subjected to cancer, preserve their fertility after chemotherapy and radiotherapy should be considered. One of options in the children for preserve fertility after treatment is cryopreservation testis tissue samplesbefore chemotherapy or radiotherapy. Optimization of cryopreservation media with anti-oxidants such as melatoni for reduction of injury to cells is necessary.Melatonin secreted from pineal gland and other organs and represents an exceptional multiplicity of actions such as anti-oxidative, anti-apoptotic and antibiotic properties. Materials and Methods: Testicular tissue were harvested from neonate pup mouse than vitrified with 0.5 molar sucrose, 15% DMSO, 15% ethylene glycol, 100 µg/ml melatonin and 20% FBS. Add 100µg/ml melatonin to thawing solution.We immersed samples in 0.5, 0.25 and 0.125 molar sucrose, respectively. Samples digested with 1mg/ml collagenase, 200-500 µg/ml DNase I and 1ml trypsin-EDTA. Cell suspension was analyzed for viability and apoptosis with cytotoxicity kit and Flowcytometry. Results: the results are presented as Mean±SD. Of the untreated cells of the neonate testicular tissue, 1.56±0.62, showed apoptosis, whereas supplementation media with melatonin, this proportion reached 5.2±0.47.Testes tissue vitrified-thawed without melatonin, 8.39±0.76, showed viability, whereas in the supplemented media with melatonin, this proportion decreased to 4.78±0.46. Cytotoxicity was calculated as 9% for melatonin. Conclusions: supplemented frozen-thawed media with 100 µg/ml melatonin don't protected testicular tissue from damaged induced in the process vitrification and thawing. In the future dosimetry melatonin and applied another cryoprotectants in the matching with melatonin should be attention.

Keywords: Melatonin, vitrification

811. Anti -Cytomegalovirus Avidity Index in Women with and withoutRecurrent Pregnancy Loss

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Background: Some evidence has shown a relationship between human cytomegalovirus (CMV) infection and pregnancy loss. However, whether recurrent or latent CMV infection or altered immune response to CMV may relate to recurrent pregnancy loss (RPL) is unclear and few data are available in this regard. We evaluated CMV infection and humoral immunological response to CMV in women with RPL and compared it to women without any history of abortion. Materials and Methods: This cross-sectional, observational study was conducted in Clinical Immunology outpatient clinic, Alzahra University Hospital, Isfahan, Iran, between 2008 and 2010. Cases were 43 women with RPL referred by Obstetric and Gynecology outpatient clinic and controls were randomly selected from healthy age match multiparous women without history of abortion. Inclusion criteria were at least 3 recurrent spontaneous abortions, and no history of any diagnosed underlying diseases as etiology of RP L . Blood samples were obtained from patients and controls to evaluate CMV IgG and IgM antibodies and IgG avidity index by the Enzyme Linked Immunosorbant Assay Method (ELISA). Data were analyzed with SPSS version 16.0, using Student's t-test, chi-squared test, and multivariate analyses. Results: One case (2.3%) of positive IgM in each group of RPL's and controls was detected. Also, there were 39 (90.6%) and 30 (69.8%) cases of positive IgG in RPL's and controls, $P<0.05$. Patients and controls were similar regarding serum IgM titer ($P>0.05$). But, IgG titer was significantly higher in RPL group, $P<0.05$. No differences were found between the two groups in IgG avidity index ($P>0.05$). In multivariate analyses, only IgG positivity was related to RPL ($P<0.05$) and avidity was not related to RPL (P=0.277). Also, IgG positivity (P=0.159), IgM positivity (P=0.856), or avidity index (P=0.440) were not related to the number of abortions in RPL patients. Conclusions: Previous exposure to CMV detected by a positive IgG antibody is significantly related to RPL in the present study. However, we found no relationship between IgG avidity index and RPL .Whether Latent CMV infection starting an indirect process of autoimmune etiology for RPL or women with RPL had recurrent or reactivation of CMV infection but avidity index is not the best index to detect it, mostly because of altered immune function to CMV in RPL patients need further investigations.

Keywords: CMV, RPL, IgG

812. Effects of LPS and LTA in production of inflammatory cytokines and expression of TLR2, TLR4 & MyD88 by human endometrial cells

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Background: Toll-like receptors (TLR) have been appeared as important upstream mediators of inflammation at many human tissues. TLR-mediated inflammatory processes has been suggested to be involved in pathophysiology of such pregnancy-related disorders as spontaneous abortion, preterm labor, preeclampsia, and intrauterine growth restriction. The aim of the present study was to investigate expression TLR2, TLR4, and MyD88, as the main adaptor of TLR signaling pathway, and production of inflammatory cytokines by lipopolysaccharide (LPS)- and lipoteichoic acid (LTA)-activated endometrial cells. Materials and Methods: Endometrial tissues were obtained from cycling women undergoing operations for benign gynecological conditions. Whole endometrial (WECs) and purified endometrial stromal cells (ESCs) were examined for expression of TLR2, TLR4 and MyD88 genes in LPS- and LTA-stimulated or non-stimulated conditions at both gene and protein levels by semi-quantitative RT-PCR, western blotting and flow cytometry, respectively. TLR stimulation was functionally assessed by measurement of TNF-α, IL-6, and IL-8 production in cell culture supernatants by ELISA. Results: Isolated ESCs exhibited high expression of CD10 and vimentin, but failed to express cytokeratin indicating their high purity. Flow cytometry analysis demonstrated that ESCs do not express TLR2 and TLR4 at their surface, but they expressed TLR4 intracellularly as judged by western blotting. Both WECs and ESCs expressed TLR4 and MyD88 transcripts. In contrary to ESCs, whole endometrial cells also expressed substantial amounts of TLR4. LPS stimulation increased TLR4 gene expression in both cell types, but this was not statistically significant, whereas treatment with LPS after 8 hours significantly increased MyD88 gene expression. Also, LTA increased TLR2 and MyD88 gene expression, but this was not reached to statistically significant level. LPS and LTA increased production of TNF-α, IL-6 and IL-8 in ESCs and WECs in a dose-dependent manner. Conclusion: These findings imply that TLRs are among the first defense mechanism of innate immune system at the female reproductive tract. In the same time, triggering TLRs during pregnancy by ascending bacterial infections may mediate inflammatory cytokine release which could potentially be harmful to developing fetus.
Key words: Endometrium, Toll like receptor, LPS, LTA, pregnancy

813. The Involvement of TLR5 and Ectopic Pregnancy

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Background: The Toll-like receptors (TLRs) are conserved family of transmembranereceptors, which play an important role in innate immunity.TLRs recognize distinctPathogen-associated molecular patterns (PAMPs) produced by various bacterial, fungal, and viral pathogens. To date, 10 functional members of these receptors have been identified in human. Among these TLRs, in mammals the recognition of bacterial flagellin is mediated by TLR5.Constitutive expression of TLR5 has been reported in female genital tract. It is likely that pregnancy disorders associated with variation in TLRs expression. So our objective in this study is investigation TLR5 expression in an Ectopic Pregnancy (EP). EPis an abnormal pregnancy that occurs outside the uterus, most often in the Fallopian Tube (FT).Materials and Methods: In this study, Biopsies from Infundibulum, Ampulla and Isthmus of FT were obtained from caseand control groups.Case and control groups werewomen who underwent salpingectomy and hysterectomy respectively. Human chorionic gonadotropin (hCG) was injected in14 days leading up to hysterectomy to produce a state of pseudo-pregnancy. RT-PCR was used to show the existence of TLR5 gene in these sections. FTs of ectopic pregnant and pseudo-pregnant were compared with Q-PCR for TLR5 gene.Results: RT-PCR has been shown the existence of TLR5 gene in all regions of the FT incases and control groups. Q-PCR was shown relative TLR5 expression in Infundibulum, Ampulla and Isthmus of FT in case group is lowerthan FT in control group.Conclusion:This study was performedtoassess the immunological importance of EP.
Reduction of TLR5 expression in Infundibulum, Ampulla and Isthmus of FT in case group indicates that TLR5as key components of the innate immune system have important role in outbreak of EP.
Keywords: TLR5, Ectopic Pregnancy, RT-PCR

814. Expression and Function of Toll-Like Receptor 1, 2, 6, 10 in the Fallopian Tube Carrying Ectopic Pregnancy

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Background:*Chlamydiatrachomatis and Neisseria gonorrhoeae*infections are thought to be the main global causes of tubal infertility and tubal ectopic pregnancy (tEP). tEP occurs when an embryo implants in the Fallopian tube (FT). There is increasing evidence that many interactions between the immune and reproductive systems involve the Toll-like receptors (TLRs).TLRs are a family of pattern recognition receptors.Among TLRs family,TLR2 forms heterodimers with TLRs 1 and 6 and recognizes a broad range of microbial products from Gram-positive bacteria while no specific ligand has yet been identified for TLR10. During microbial infection in the female genital tract, TLR2 is considered to recognize the PGN of *C. Trachomatis*, LPS and fragments of PGN of *Neisseria gonorrhoeae*.Thus our objective in this study was to clarify TLR2, 4, 6, 10 genes expressions in FTs carrying EP. Materials and Method:Biopsies from Infundibulum, Ampulla and Isthmus of FT were obtained from case and control groups. Case and control groupswere women who underwent salpingectomy for EP and hysterectomy respectively. Human chorionic gonadotropin (hCG) was injected in 14 days leading up to hysterectomy to produce a state of pseudo-pregnancy in control group. In this study, RT-PCR was used to show the expression of TLR1, 2, 4, 10 genes. FTsfrom case and control groups compared with Q-PCR for these genes. Results: RT-PCR has shown TLR1, 2, 6, 10 genesexpression in infundibulum, Ampulla and Isthmus from case and control group.Q-PCR has demonstrated, TLR1, 2, 6, 10genes expressionin all parts of FT from case group werelower than control group. Conclusion: Infection and inflammation can have devastating consequences to fertility and pregnancy. It is possible that increasing of infection in fallopian tube following decline of TLR1, 2, 6, 10genesexpression in FT can result in tubal ectopic pregnancy.
Keywords: tEP, TLRs, hCG

815. Investigation of antiviral TLRs gene expression in Ectopic Pregnancy

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Background:Ectopic Pregnancy (EP) is a complication of pregnancy in which the embryo implants outside the uterine cavity, usually in Fallopian Tube (FT). Various factors are associated with EP including: pelvic inflammatory disease,injuryandinfection ofthe FT. **Recently, it has been proposed that immunological factors are involved in the occurrenceof EP.** Toll like receptors (TLRs) constitute a major part of the innate immune system and recognize pathogen-associated molecular patterns (PAMPs) in various pathogens. Antiviral TLRs, TLR3, 7, 8 and 9 are expressed on endosomal membranes. TLR3 and TLR7- 8 recognize double-stranded and single-stranded RNA, respectively, whereas TLR9 recognizes both bacterial and viral CpG DNA motifs. It is likely that EP associated with variation in TLRs expression, so the aim of this study was to investigate the gene expression of antiviral TLRs in EP. Materials and Method: FT tissues were obtained from two groups: case and control. Case group consists of women with EP who underwent tubectomy. As control group, human chorionic gonadotropin (hCG) was injected to women who underwent abdominal hysterectomy for benign gynaecological conditions to produce a state of pseudo-pregnancy.In both groups,

RT-PCR was used to show the existence of antiviral TLR genes in Infundibulum, Ampulla and Isthmus of FTs while Q-PCR investigates the relative expression of these genes in these mentioned sections. Results: RT-PCR has been shown the expression of antiviral TLR genes in all regions of the FT in both case and control groups . Q-PCR has confirmed that the relative expression of these genes in Infundibulum, Ampulla and Isthmus of case group is lower than control group. Conclusion: The lower expression of antiviral TLRs gene in Infundibulum, Ampulla and Isthmus of FT in women with EP could suggest that variation in expression of these TLRs gene may influence on implantation of embryo in FT.
Keywords: TLRs, PAMPs, RT-PCR

816. Role of TLR4 to Prevent Gram Negative Bacterial Infections in Tubal Ectopic Pregnancy

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Background:Ectopic pregnancy is a complication of pregnancy in which the embryo implants outside the uterine cavity. *Chlamydia trachomatis* infection can cause tubal ectopic pregnancy (EP) due to adhesions, damage, or occlusion of fallopian tube (FT). The mechanisms by which these occur are believed to be primarily immunologically mediated and not a direct consequence of tissue destruction by the organism. Although, recent evidence does support a cytotoxic effect of *Ch. trachomatis* on ciliated epithelium. *Ch. trachomatis* has been shown to initiate innate immune responses by ligating members of the Toll-like receptor (TLR) family of pattern recognition receptors, in particular TLR4. TLR4 recognizes lipopolysaccharide of Gram negative bacteria such as *Ch. trachomatis*.TLR4 have higher expression in FT compared with other regions of female reproductive tract that suggests a critical role of TLR4 in FT.The aim of this study is to clarify relation between TLR4 expression level and EP. Materials and Methods: Our samples were divided into two groups: Case and control groups. Case group was women underwent salpingectomy forEP. Control group was women with healthy tube underwent hysterectomy. For control group, Women were injected with human chorionic gonadotropin (hCG) in 14 days leading up to hysterectomy to produce state of pseudo-pregnancy. In this investigation TLR4 expression was survey with RT-PCR. Also Q-PCR was used to compare quantitative expression of TLR4 between two groups. Results: Using RT-PCR shown that TLR4 was expressed in infundibulum, ampulla and isthmus of control group, but in case group was not expressed in infundibulum and ampulla. Whiles, very low expression in isthmus was detected. Using Q-PCR shown that, In all regions of case group TLR4 level expression was significantly lower than control group. Conclusion: Decreasing in the expression of TLR4 in fallopian tube may have predisposing role for infections such as *Chlamydia* trachomatis *and could increase risk of EP*.
Keywords: *Ch. Trachomatis, EP,* RT-PCR

817. Viral TLRs expression in Endometriosis

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Background: Endometriosis is a complex disease that profoundly affects the quality of life in many women. This disease affects roughly one in ten women of reproductive age. Endometriosis induces a variable amount of inflammatory reaction in pelvic environment. An active immune system needs to recognize these inflammatory agents. Rapid innate immune system defenses against infections involve the recognition of invading viral and bacterial pathogens, by the family of Toll Like Receptors (TLRs). Among TLRs family only TLR3, 7, 8 & 9 that expressed in the intracellular endosomal compartments, which can detect viral infections.TLR3 distinguishes double strand RNA viral motifs. TLR 7/8 are specific for single strand RNA. While TLR9 recognizes unmethylated CPG DNA of viruses. The objective of this study is to clarify the expression of antiviral TLRs in the woman with endometriosis. Materials and Methods: In this study three groups were examined. Ectopic biopsies were obtained with laparoscopic procedure from patient with endometriosis. Eutopic and control biopsies were gained with piple from endometrium of women with and without endometriosis. Reverse transcriptase polymerase chain reaction (RT-PCR) for 5 samples of each groups used to show the existence of TLR3, 7, 8 & 9 genes. Results: TLR3, 7, 8 & 9 mRNA were expressed in the each groups but in ectopic and eutopic samples we showed variable expression. Conclusion:The expression of TLR3, 7, 8 and 9 in the women with endometriosis is a strong evidence of critical role of innate immune system against viral infections in this disease. Further study will be done for quantitative assay by Real time PCR.
Keywords: TLRs, Real time PCR, unmethylated CPG

818. The role of TLR4 and 5 in Endometriosis

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Background: Toll-like receptors (TLRs) are a major family of innate immune systems wich recognize specific pathogen associated molecular patterns (PAMPS) in bacteria, fungi, virus and parasites. Human TLRs comprise a large family of 10 different types proteins that are expressed on various immune cells. Among TLRs, TLR4 recognizes Lipopolysaccharid (LPS) of Gram-negative bacteria and TLR5 is a receptor for flagellin of bacterial. Endometriosis is a benign gynecologic disorder characterized by the ectopic growth of misplaced endometrial cells. Endometriosis is involved mainly innate immune System. Whereas TLRs are expressed in the endometrial cells as a result their expression and their regulation might be vital for the pathogenesis of endometrial diseases especially endometriosis. Materials and Methods: This study contains three groups (n=5). Ectopic biopsies were obtained with laparoscopic procedure from patient with endometriosis. Eutopic and control biopsies were gained with piple from endometrium of women with and without endometriosis. The existence of TLR4&5 genes were tested with reverse transcriptase polymerase chain reaction (RT-PCR). Results: TLR4, 5 mRNA were expressed in the each groups but in ectopic and eutopic samples we showed variable expression. Conclusion:The expression of TLR4, 5 in the women with endometriosis is a strong evidence of critical role of innate immune system against bacterial infections in this disease. Further study will be done for quantitative assay by Real time PCR.
Keywords:TLRs, PAMPS, LPS

819. Association of FCRL3 Gene Polymorphisms (-169 A/G and -110C/T) with Susceptibility of Iranian Patients to Preeclampsia

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Background: Pre-eclampsia/eclampsia is a common vascular pregnancy disorder associated with high maternal and infant mortality and morbidity worldwide. Both genetic and environmental risk factors have been identified. The Fc receptor-like (FCRL)-3 gene is one of the genes that possibly have an immunoregulatory role in human diseases such as malignancies, infections and specifically autoimmune disorders. Objective: To determine the possible role of FCRL3-3 (-169C/T) and FCRL3-4 (-110A/G) gene polymorphisms in development of preeclampsia in Iranian patients. Materials and Methods: FCRL3-3 and FCRL3-4 polymorphisms were investigated in 218patients with preeclampsia and a control group consisting of 208 healthy subjects using PCR-RFLP method. Results: The frequencies of GG, AG and AA genotypes at position -110 in patients were 54.2 %, 39.4% and 6.4%, respectively. Corresponding data for healthy controls was 55.3%,36.5 % and 8.2% (P=0.84). In terms of -169 C/T polymorphism, the frequency of CC, CT and TT genotypes in patients were 39.9 %, 45% and15.1% respectively, whereas in healthy controls it was 37.5%,49 % and 13.5% (P=0.7). No significant difference in genotype distribution and allele frequencyfor both

polymorphismsof FCRL3 gene between patients and controls was found.Conclusion: Results of this study showed no association between FCRL3 polymorphism at positions-169C/T and -110A/Gand susceptibility to preeclampsia in Iranian patients.
Keywords: Preeclampsia, polymorphism, FCRL3

820. Barrier Contraception, Sperm Antigen and Preeclampsia

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Background: The immune maladaptation theory suggests that tolerance to paternal antigens, resulting from prolonged exposure to sperm, protects against the development of preeclampsia. Objective: To investigate whether the sperm exposure can reduce the incidence of preeclampsia by increased tolerance of mother to semen antigen
Materials and Methods: This was a retrospective cohort study carried out on prim gravid women hospitalization in Taminegtemay hospitals in Tehran that developed preeclampsia as defined by ante partum systolic blood pressure > or = 140 or diastolic blood pressure > or = 90 plus proteinuria in 2010 (n=120) and health pimgravida (n=240). All women in the cohort were asked about preconception contraception and timing of first sexual intercourse with the spouse before pregnancy. Odd ratio comparing cases with preeclampsia to the rest of the cohort were adjusted for age, and body mass index (BMI).Results: Women using barrier contraception prior to conception were no more likely than women not using barrier contraception to develop preeclampsia (adjusted OR 1.0, 95% CI 0.6-1.6). In unadjusted analyses, a prolonged time to conception was associated with preeclampsia (OR 1.9), however, after adjustment, the association was less prominent (OR 1.6) and after stratification by contraception method, the link between time to conception and preeclampsia was eliminated.Conclusion: These data do not support the sperm immune maladaptation theory of preeclampsia.
Key words:immune, maladaptation, preeclampsia

821. Effect of R10 Fraction of Garlic on Serum Levels of Nitric Oxide in Pregnant Mice

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Introduction: In normal pregnancy, a physiological increase in blood volume caused by dilated blood vessels has been adjusted with the production of NO. Besides the lack of NO production can cause abnormalities in blood pressure such as preeclampsia. Mechanisms responsible for this complication have not yet been determined. In preeclampsia Th1 response is predominant inTh1/Th2 response. R-10 is a immunomodulator of garlic, one of the important effects of this is cytokine shift toward Th1 response. Now, according to the course of the effect seems to be necessary during pregnancy. Methods & Materials: 30 female mice(balb / c) were divided after mating to four groups that receiving a fraction R10 for a week, two weeks and the proper control groups. Fraction injection continued with a dose of 2/0 mg/ kg intraperitoneally till the eighteenth day of pregnancy. In the nineteenth day of pregnancy, all mices were killed and blood samples were obtained and serum was prepared from mices hearts, peritoneal macrophages were isolated and cultured and the supernatant was collected. Nitric oxide production was measured using Greiss method with and without the addition of actonitrile. test results was evaluated with T-test. Results: Serum level of nitric oxide in mices that recived R-10 fraction for a week and those whom recived for two had no significant changes. Level of nitric oxide in peritoneal macrophages in mices receiving a fraction for a week in compared to control group increased and in mices receiving two weeks have been less than the control group, although this difference was not statistically significant ($P>0.05$). LPS stimulation significantly increased nitric oxide production in the group receiving for two weeks ($P<0.05$). Conclusion: On the results can be stated that the injection of fraction immunomodulator of garlic (R10) with a dose of 2/0 mg/ml to pregnant mices for a week and two weeks are not caused a significant change in nitric oxide production in serum and peritoneal macrophages. To confirm the effect of garlic on pregnancy study of other important parameters such as blood pressure and biochemical parameters during pregnancy is essential.
Keywords: garlic immunomodulator fraction R-10, Nitric oxide, pregnancy

RESEARCH & PRODUCTION

Oral Presentation

822. The Construction a Recombinant *Lactococcus Lactis* as a Candidate for Immunotherapy

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Backgerand: Recently with increasing prevalence of type I allergy, active delivery of recombinant molecules to mucosal surfaces by genetically LAB (lactic acid bacteria) is represent a novel and attractive vaccination approach. This study was aimed to construct a recombinant probiotic bacteria producing the major *chenopodium album* pollen allergen (Che a 2), which is one of most important allergen in Iran, as a candidate for oral immunotherapy. Materials & methods: Che a 2 sequence was amplified by RT- PCR method and then the PCR products were cut by *Pst I* and *Sal I*. Subsequently, using T4 DNA ligase the PCR products were inserted to the specific expression vector, PNZ3004, for LAB. The recombinant plasmid was transformed in to the *Lactococcus lactis* as probiotic bacteria. The bacteria that contain recombinant plasmids were grown on MRS media with 5 µg/ml erythromycin. Cell extract of these bacteria was separated by SDS-PAGE and transferred to nitrocellulose membrane by electroblotting according to standard protocol. Finally, immunological properties of recombinant LAB were analyzed by western blotting with a pooled serum from *C. album* allergic patients alongside anti- Che a 2 monoclonal antibodies. Results: Che a 2 sequence was successfully amplified, inserted to PNZ3004 vector and confirmed by DNA sequencing. In this study, the recombinant *Lactobacillus acidophilus La5* was able to produce che a 2.The IgE antibodies in the sera of *chenopodium album* allergic patients were reacted to recombinant LABs that producing Che a 2. In addition, these recombinant bacteria had high potential of immune-reactivity with anti Che a 2 monoclonal antibodies.
Conclusion: Live recombinant LABs are able to produce the major chenopodium pollen allergen, Che a 2, and they could be a suitable candidate for antigens delivering in mucosal routes for allergen specific immunotherapy.
Keywords:Recombinant, *Lactococcus Lactis*, Immunotherapy, *chenopodium album*

823. Cloning, Expression, Purification and Antigenic Evalution of Vaca,. Antigenic Fragments Recombinant Protein of Helicobacter Pylori

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Background: Helicobacter pylori is a gram negative curved bacilli colonized in the human stomach. It causes duodenal ulcer, gastritis and is associated with adenocarinoma. The vacuolating cytotoxin (vacA) is major bacterial factor involved in gastric injury. The aim of this study was to construct a recombinant vector containing antigenic regions of vacA from H. pylori and expressed it in E. coli, as well as determining its antigenicity as a vaccine candidate of H. pylori. Materials and Methods: Firstly the target gene encoding vacA high antigenic regions was amplified from H. pylori chromosome by PCR. After being purified, the target fragment was cloned into plasmid pTZ, and the recombinant plasmid pTZ-vacA was transformed into E. coli DH5α. The sequence of inserted fragment was analyzed. Thirdly, vacA gene from recombinant plasmid pTZ-vacA was digested by EcoRI and XhoI and was inserted into expression vector pET32a which was digested by corresponding restricted endonuclease enzyme. The positive recombinants were transferred into E. coli BL21 (plyss) and identified by restriction enzyme digestion and PCR. Finally, the genetically engineered bacteria including pET32a-vacA plasmids were induced by IPTG, the expression was analyzed by SDS-PAGE, furthermore antigenicity was studied by western blotting after Ni-NTA agarose resin purification. Results: Enzyme digestion analysis, PCR and sequencing showed that the target gene (1233 bp)was inserted correctly into the recombinant vector. The recombinant protein (45kDa) was recognized by the human sera infected with H. pylori. Conclusion: Our results indicates that antigenic regions of recombinant vacA protein from H.pylori was recognized by patient sera, so it is a good candidate for the development of H. pylori vaccine and ELISA kits and diagnostic purpose.
Keywords: Helicobacter pylori, *vacA cytotoxin*, Cloning,antigenic regions

824. Preparation and Evaluation of the Labeled Ag B with Gold Nanoparticles, in Order to Rapid Diagnosis of Hydatid Cyst
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Background: cystic echinococcosis, caused by the metacestode stage of *Echinococcus granulosus*, is one of the most important zoonosis worldwide. In this research examined the using Ag B from hydatid cyst fluid (HCF) and labeled Ag B with golden nanoparticles to rapid diagnosis of hydatid cyst. Materials and Methods: Ag B was purified to homogeneity from hydatid cyst fluid as described by Oriol et al (1971) and then labeled with gold nanoparticles by Frens (1973). Positive sera samples were collected from sheep naturally infected and the tested. Color change not only observed by the naked eye, but also was read at 450 nanometers wavelength by spectrophotometry. The results with indirect ELISA (using Goat anti rabbit HRP) were compared. Results: Color change from red to purple occurred in less than 5 minutes. The results of the sheep serums test in this method were consistent with ELISA which indicated the reliability of the agglutination with gold nanoparticle for rapid detection of hydatidosis. Conclusion: our finding was showed that, agglutination method with labeled Ag B by gold nanoparticles is easy, fast, cost-effective and available method in hydatid distinguish. Positive results were determined by the purple color and there was color difference between the infected and uninfected samples. This rapid test for diagnosis for ovine hydatid cyst can be used as an important tool not only for the rapid diagnosis of hydatidosis, but also for monitoring of disease.
Keywords: Gold Nanoparticles, Hydatid Cyst

Poster Discussion Presentation

825. Purification of Single Chain Variable Fragment (Scfv) Specific to Human Tumor Necrosis Factor Alpha in *Escherichia Coli*
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Background: Peptide tags are mostly used for affinity purification purposes. The immobilized metal ion affinity chromatography (IMAC) is widely used for the purification of pharmaceutical-grade proteins, because of simplicity, ligand stability, high protein loading, mild elution conditions, simple regeneration and low cost. Materials and Methods: The bacterial periplasmic fraction containing the scFv against human TNF-α was loaded onto IMAC that was equilibrated with washing buffer (20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4). The scFv was eluted out with elution buffer (20 mM sodium phosphate, 0.5 M NaCl, 300 mM imidazole). Purity of the protein was assessed by SDS-PAGE. The gel was stained with silver staining. Western blot analysis was performed for functionally assessment of purified ScFv. Results and Conclusion: SDS-PAGE showed that the purity of protein was up to 95% and in high yield. The major band at a molecular weight of approximately 28 kDa was observed. A 17 kDa band visualized by western blot analysis showed that produced ScFv can successfully detect human TNF-α.
Keywords: single chain variable fragment (scFv), TNF-α, *Escherichia coli*

826. Diagnosis of Typhoid Fever by Enzyme-Linked Immunosorbent Assay
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Background*: Salmonella enterica* subspecies enterica serovar Typhi (Salmonella typhi) causes typhoid fever in humans. *S. typhi* has been a major human pathogen for thousands of years, on a global scale, at least 16–20 million cases of typhoid fever occur annually resulting in approximately 600,000 deaths. The laboratory diagnosis of typhoid fever is time consuming and prone to erroneous results. InvH protein can induce antibody production in mice. These reasons suggest that InvH could be a candidate antigen for early diagnosis of typhoid and might be also useful for a vaccine design. Materials and Methods: InvH protein was expressed and purified by nickel–nitrilotriacetic acid (Ni–NTA) affinity chromatography. Recombinant Invh protein was used for ELISA with infected mice (with *S. typhi* and *E. coli*) and sera from typhoid patients. Results: ELISA test results were confirmed antibody response with inoculated mice with *S. typhi* and srea from typhoid patients compared with *invH* deficient bacteria such as *E. coli* were significantly higher than the control group. Conclusion: In this research enzyme-linked immunosorbent assay (ELISA) was used for the detection of typhoid fever. The results indicated that InvH protein is a surface protein and can be employed in diagnostic measures against *Salmonella Typhi*.
Keywords: *S. typhi*, Typhoid Fever, ELISA

827. Cloning and Expression of Human Granulocyte Macrophage–Colony Stimulating factor in *E. coli*
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Background: The granulocyte-macrophage colony-stimulating factor (GM-CSF) is a hematopoietic growth factor mainly responsible for the proliferation of granulocytes and macrophages.Materials and Methods: In this study for cloning of *gm-csf* gene, several cell lines were verified for *gm-csf* mRNA expression using Real Time PCR technique.Among the cell lines tested, Mehr80 cell line (a large cell lung cancer cell line) showed the most quantities of gm-csf mRNA. After RNA isolation and cDNA synthesis, specific primers (GM-CSF F, GM-CSF R) were used for *gm-csf* amplification. The amplified DNA fragment (384 bp) wasconfirmed by its restriction pattern with *Apa*I and *Bgl*I enzymes and then cloned into pUC57 vector. The new construct was confirmed by enzymatic digestion and sequencing and designated as pUCMY7. To express the *gm-csf* gene and production of its recombinant protein in prokaryotic system, pUCMY7 was digested and gel purified fragment was cloned into pET22b (+) expression vector. Recombinant colonies were confirmed with colony PCR, enzymatic digestion and sequencing. The new construct designated as pETME1 and then transferred into *E. coli* BL21 (DE3). Expression of recombinant protein at several conditions was done and SDS-PAGE, Dot blot and Western blot techniques were used for confirmation. Results: Sequencing of recombinant construct showed 100% homology

with NCBI records and confirmed fragment in pET22b (+) was used to express the recombinant GM-CSF. The results of SDS-PAGE was shown no band in expected weight, although Dot blot using GM-CSF monoclonal antibody was shown a few amounts of protein expression. With condition optimization a single band in SDS-PAGE which was also positive on western blot confirmed the recombinant protein expression. Conclusion: Currently recombinant GM-CSF is imported to our country for several clinical applications, Selection of high producing clone of GM-CSF in this study provide an opportunity to set up production of the recombinant GM-CSF domestically.
Keywords:Cloning, GM-SCF, *E. coli*

828. Evalution of Antigenicity for CagA Antigenic Fragments Recombinant Protein of *Helicobacter pylori*

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Background: The gram negative, microaerophilic bacterium *Helicobacter pylori* colonizes the human gastric mucosa and establishes a chronic infection that is tightly associated with atrophic gastritis, peptic ulcer, and gastric carcinoma. The cytotoxin-associated gene A (CagA) isone of themost important virulence factors in *H. pylori*. The aim of the present study was to construct a recombinant protein containing antigenic regions of cagA from *H. pylori* and determining its antigenicity as a vaccine candidate of *H. pylori*. Materials and Methods*:* The target gene encoding cagA high antigenic regions (1245 bp) amplified from *H. pylori* chromosome by PCR, digested by restricted endonuclease enzyme and inserted into the prokaryotic expression vector pET32a which was digested by BamHI and XhoIrestriction endonuclease enzyme. The target protein was expressed in the BL21 (plyss) *E. coli*. Furthermore antigenicity was studied by western blotting after Ni-NTA agarose resin purification. Results: Enzyme digestion analysis, PCR and sequencing showed that the target gene was inserted correctly into the recombinant vector. Plasmid pET32-cagA could express a specific 45kDa protein in E. coli BL21.The recombinant protein was recognized as an antigen by the human sera infected with *H. pylori*.Conclusion: The present data indicate that antigenic regions of recombinant cagAprotein from *H. pylori* were recognized by patient sera, so it is a good candidate for the development of H. pylori vaccine and diagnostic purpose.
Keywords: *Helicobacter pylori*, *cagA* gene, antigenicity, antigenic regions

829. Construction, Expression, Structural and Functional Analysis of Two Novel Variants of Human Interleukin-2 (IL-2)

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Background: Interleukin-2 (IL-2) is a 133 amino acid alpha-helical protein secreted by activated T-cells. This protein possesses three cysteine residues. Cysteines 58 and 105 are two residues involved in forming an intramolecular disulfide bridge, whereas cysteine 125 owns a free sulphydryl group. Formation of this disulfide bond is critical for biological activity of the protein. Materials and Methods: In this study, we constructed two mutant proteins of IL-2 through site-directed mutagenesis. In the mutant C125 A, the free sulphydryl group was eliminated. This change will probably prevent the formation of mispaired disulfide bonds during the refolding process. In the mutant L18C, a new cysteine residue was introduced for formation of disulfide bond with cysteine 125. This mutation was designed to study the effect of an extra disulfide bond on the structure and function of IL-2. These mutant analogs were then expressed isolated as solution phase in *E. coli*-derived pLysS strain. Subsequently, the expressed protein was purified using NI-NTA columns. In order to investigate, the effects of introduced modifications on the structure of the protein, Circular Dichroism (CD) and Fluorescence Spectroscopy were exploited. Results and Conclusion: The data obtained exhibited no alteration in the structure of IL-2 variants. As well, the analysis of biological activity of the mutants is under examination on CTLL-2 cells.
Keywords: Human Interleukin-2, Site-directed Mutagenesis, Disulfide Bond, Biological Activity.

STEM CELLS

Oral Presentation

830. Immunomodulatory Effects of Mesenchymal Stem Cells Conditioned Media in *STZ-induced Autoimmune* Diabetes in C57BL/6 Mice

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Background: Type 1 diabetes (T1D) is a chronic immune-mediated disease which is characterized by selective loss of insulin-producing β-cells in the pancreatic islets in genetically susceptible subjects. Mesenchymal stem cells (MSCs) have been shown to have immunomodulatory and regenerative potential.Materials and Methods: In this study we investigated the effects of injection of mouse adipose tissue-derived mesenchymal stem cells conditioned media in streptozotocin (STZ)-induced diabetic mice. In this study, MSCs culture supernatant wasinjected intraperitoneally, and blood glucose levels, intraperitoneal glucose tolerance test, serum insulin, body weight and islets histology were analyzed. In addition, spleen CD4+CD25+Foxp3+ regulatory T lymphocytes, splenocytes IFN-γ, IL-10, IL-17, IL-4 and TGF-β secretion were assessed to describe in vivo immunomodulatory effects of MSCs.Result: The results demonstrated that injection of mouse adipose tissue-derived MSCs conditioned media could reverse STZ-induced diabetes in mice. Blood glucose levels, intraperitoneal glucose tolerance test, were significantly recovered in the test group. Our findings showed that injection of mouse adipose tissue-derived MSCs conditioned media has resulted in increased CD4+CD25+Foxp3+ regulatory T cells in the spleen, reducedTh17 and induced a Th2-type cytokine shift in splenocytes.Conclusion: In conclusion, our study demonstrated that injection of mouse adipose tissue-derived MSCs conditioned media was therapeutically useful in autoimmune STZ-induced diabetic mice, and the underlying mechanisms may be related with their immunomodulatory potential.
Keywords: Immunomodulatory Effects, Mesenchymal Stem Cells, Type 1 diabetes (T1D),C57BL/6 Mice

831. Modulation of CXCR4 Expression on Cord Blood Stem Cells

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Background: CXCR4 is critical adhesion molecule in homing process. Modulation of its expression on cord blood (CB) $CD34^+$ could overcome delay following cord blood transplantation. Complicated network of growth factors including cytokines and neuropeptides in microenvironment has important role in regulation of this adhesion molecule. So, we aimed to assess the role of two neuropeptidesSubstance P (SP) and Calcitonin gene related peptide (CGRP) in addition to cytokine cocktail on CXCR4 expression.Materials and Methods: $CD34^+$cells purified from CB were cultured in a serum-free liquid culture system. Different concentrations of SP and/or CGRP were used in combination with cytokine cocktail. Protein and genomic levels of CXCR4 was assessed by flowcytometry and real time RT-PCR.Results: Our data show increased $CXCR4^+CD34^+$ cells cultured with SP and/or CGRP by day 7. Concentration 10^{-9}M either SP or CGRP increased the genomic expression of CXCR4 moleculeby

day 11 compare to control group.Conclusions: Our experiment indicates that SP and CGRP induce CXCR4 protein expression in 7 days culture and enhance its genomic expression. Consequently, overexpression of CXCR4 improves engraftment of CB $CD34^+$ cells.
Key word: CXCR4, cord blood, stem cell

832. Effects of CXCR1 and CXCR2 Inhibition on Expansion and Differentiation of Umbilical Cord Blood CD133+ Cells into Megakaryocyte Progenitor Cells

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Background: There have been various reports on the roles of CXC receptors (CXCR) in modulation of hematopoiesis. In the present study, we investigated the effects of CXCR1 and/or CXCR2 inhibition on expansion and differentiation of umbilical cord blood (UCB) CD133(+) cells into megakaryocytic progenitors.
Materials and Methods: Purified UCB CD133(+) cells were cultured in a serum-free liquid culture either in the presence or absence of neutralizing anti-CXCR1 and/or anti-CXCR2 antibodies in combination with a conventional cytokine cocktail for up to 14days. Expression of megakaryocytic lineage markers (CD41 and CD61) and determination of ploidy level were determined by flowcytometry. In addition, colony-forming unit assay was performed using CD133(+) cultures in serum-free collagen-based medium containing the cytokine cocktail plus neutralizing CXCR1 and -R2 antibodies. Colony forming unit-megakaryocyte (CFU-MKs) and non-MKs were counted after immunocytochemistry staining on day 12. Results: We show that while simultaneous inhibition of both CXCR1 and -R2 causes a significant reduction in the fold expansion of UCB CD133(+) cells, it also leads to an increase in percentages of CD61(+), CD41(+), and CFU-MK populations. Conclusion: CXCR1 and CXCR2 play significant roles in the suppression of megakaryopoiesis. We demonstrate that blocking of this suppressive effect by a simultaneous inhibition of both receptors can enhance the differentiation of UCB CD133(+) cells into megakayocytic progenitors.
Keywords: CXCR1, CXCR2, Inhibition, Megakaryopoiesis,Umbilical cord blood (UCB), CD133+ cells

833. Evaluation of Gene Expression and Serum Protein of Pro-And-Anti Inflammatory Cytokines in SPMS Patients Pre- and Post-Mesenchymal Stem Cell Therapy

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Background: Mesenchymal stem cells (MSCs) are currently strong candidates for stem cell therapies.They are potentially effective in the treatment of neurodegenerative disorders. Cytokines has a profound effect on resultant immune responses.To answer the question related to the efficacy of MSC in MS patients: does MSC change the cytokine responses? This study aimed to evaluate the serum level of relevant cytokines and their gene expression in MS patients pre and post MSC therapy. Materials and Methods: In this study blood sample were collected from 25 SPMS patients, who had been treated by MSC.To measure gene expression ofFOXP3, INF-γ, TGF-β, IL-4, IL-10 and IL-6, and their serum protein,samples were collected at five time intervals: day 0 prior to injection and months 1, 3, 6, and 12 after the intrathecal injection of MSC.Gene expression was assayed by real-time PCR and protein values were measured by ELISA.Results: There was statistically significant elevation of FOXP3, IL-6, and INF-γ and non-statistically significant elevation ofIL-10 and TGF-β gene expressionafter stem cell therapy. Result of gene expression of IL-4 was constant.Cytokines protein assay showed increment of INF-γ and IL-6 but the amount of TGF-β and IL-10 were continuously declining.IL-4 protein level in all patientswas not detectable using R&D kit.There is only a significant correlation between increment of IL-6 gene expression and disease progress.Conclusion: Our study showed that unlike past presumption and animal studies, MSCs in human, simultaneously decrease or increase some anti- and pro-inflammatory cytokines. However we can say that MSCs exert their immunomodulatoryeffects by stimulation of $FOXP3^+$ Treg production and prevention of Th17 cell production through reduction of TGF-β and IL-10. Our cytokine variation which has been proved with patients' clinical status showed that MSC protective effects remain until one year and will be decreased after that. This study showed only IL-6 increment is significantly correlated to disease progression.
Keywords: cytokine, MSC, MS

Poster Discussion Presentation

834. In Vitro ImmunomodulatoryEffects of Adipose-Derived Mesenchymal Stem Cells Isolated From Three Inbred Mouse Strains on Splenocytes Proliferation and Cytokine Production

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Background:Over the last decade, there has been increasing evidence about the *in vitro* and *in vivo*immunomodulatory properties of mesenchymal stem cells (MSCs). Recent reports have demonstrated that MSCs have immunomodulatory effects. Materials and Methods:Murine adipose-derived MSCs (AD-MSCs) were obtained from male C57BL/6, BALB/c and DBA mice strains.We purified and expanded MSCs characterized by expression of markers (CD105, CD73, CD29, Sca-1, CD90, CD44, CD11b, CD34 and CD45), and their ability to differentiate into osteogenic, and adipogenic cells.We next analyzed the capacity of AD-MSCs conditioned media from C57BL/6, BALB/c and DBA mice to modulate TGF-β, IFN-γ, IL-4, IL-17 and IL-10 production by splenocytes.Results:The isolated MSCs were positive for CD105, CD73, CD44 CD29, Sca-1, CD90, and negative for CD11b, CD34 and CD45. Our results indicated that the MSCs and its conditioned media significantly suppressed mitogen-induced splenocytes proliferation in vitro in a dose-dependent manner. In addition, culture of splenocytes with AD-MSCs conditioned media from C57BL/6, BALB/c and DBA mice resulted in increased TGF-β, IL-4, and IL-10 production and decreased IFN-γ and IL-17 production by unstimulatedsplenocytes. The results showed that AD-MSCs conditioned media from BALB/c mice significantly decreased IFN-γ and IL-17 production and significantly increased IL-4, and IL-10 production compared to AD-MSCs conditioned media from C57BL/6 and DBA mice. Conclusion:Taken together, these data demonstrate that immunomodulatory or immunosuppressive effects are strain dependent.Moreover, soluble factors derived from MSC, modulate immune responses and suggest that MSC create an immunosuppressive microenvironment.
Keywords:In Vitro, Immunomodulatory Effects, Splenocytes, MSCs

835. The Effect of Substance P on Very Late Antigen -5 (VLA-5) Expression in Cord Blood $CD34^+$Cells

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Background: To facilitate the homing process of cord blood (CB) stem cell, the overexpression of cell adhesion molecules -esp. those involved in migration process- could be helpful. Factors such as microenvironment and released mediators regulate this process. Additionally, according to existing documents concerning the presence of substance P (SP) receptor on CB $CD34^+$cells, we aimed to explore effects of SP on very late antigen (VLA)- 5 expression on CB $CD34^+$ cells. Material and Methods: $CD34^+$cells derived from CB, were cultured in Stemspan culture and were treated with various concentrations of SP in combination with cytokine cocktail of SCF, FL, TPO, IL3 and IL6. Control groups were treated with cytokine cocktail. VLA5 expression was checked by flowcytometry.
Results: Our results show significant raise in percentage of SP treated cells at 10^{-11} M following 7 days cultivation as compared to control group. Additionally median flowcytometric intensity of VLA5 at corresponding culture period and SP concentrations were significantly increased compare to freshly purified $CD34^+$cells at day 0. Conclusions: Our data suggest that using SP neuropeptide help to maintain or increased VLA5 on CB stem cells and this would be beneficial in homing process of cells during transplantation.
Key word: SP, VLA-5, cord blood, stem cell

836. Innate Immunity in Dental Pulp Stem Cells with Emphasizing in the Expression and Function of TLR4

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Background:Inflammatory mediators along with stem cells highly regulate homeostasis of all tissues including dental pulp. In addition to the supportive activities of the human Dental Pulp Stem Cells (hDPSCs) in pulp regeneration/repair, cytokines and/or chemokineshave critical role in the orchestration of stimulated hDPSCs. It has been suggested that the inflammatory mediators gradient such as IL-6 & 8 are regulated by kind of innate immune receptor called Toll-Like Receptor (TLR). Among TLRs in human, TLR4 recognizes very divergent collection of exogenous and endogenous ligandssuch as lipopolysaccharide, heat-shock proteins and fibrinogen. It has been recently suggested that TLR4 has multi-functional activities in stem cell biology including stem cell proliferation & migration. This study investigates the expression of TLR4, IL-6 & IL-8 in hDPSCs. Materials and Methods:hDPSCs were enzymatically isolated from wisdom teeth (n=5) and characterized in terms of surface epitopes (mesenchymal stem cell (MSC)markers CD90/CD73/CD44/CD105 & hematopoietic/endothelial markers CD34/CD45/CD11b/CD31) as well as differentiation capacity into bone, cartilage & adipose cells. After the RNA extraction of hDPSCs, RT-PCR was used to show the expression of TLR4, IL-6 & IL-8 genes.Results:hDPSCsshowedMSC phenotype& differentiation capacity into three mesenchyme lineages. Immuno-phenotyping results confirmed the existence of MSC markers and the lack of hematopoietic/endothelial markers. Interestingly, the results of RT-PCR indicated the expression of TLR4, IL-6 & IL-8 genes in hDPSCs.Conclusion:Immunological evaluation of human adult stem cells is a challenging issue for the stem cell therapy & regenerative medicine. The expression of IL-6 & 8 followed by the expression of TLR4 in hDPSCs not only shows the importance of innate immunity in adult stem cell defense but also the immune potential capacity of these cells in pulp repair & regeneration.
Keywords:hDPSCs, Stem Cells, TLR4

837. Influence of DiD (Dioctadecyl-3,3,3,3-Tetramethylindodicarbocyanine) Labeling on Differentiation and Immunosuppressive Function of Mice Mesenchymal Stem Cells

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Background: Mesenchymal stem cells (MSCs) are multipotent cells with the ability of inhibition of various immune cells. Therefore, MSCs are efficient cells for immunotherapy of autoimmune diseases if they recruited efficiently to the site of inflammation. To track the MSCs fate after in vivo injection, their labeling should be done with agents that do not interfere with their function. In this regard, DiD is an appropriate lipophilicfluorescent dye for *in vivo* imaging. However, the effect of DiD on immunosuppressive properties of MSCs or their differentiation capability have not been investigated. Materials and Methods: MSCs were isolated from bone marrow of Balb/C mice and labeled with DiD for 20 min. Labeling was assessed with flowcytometry. For determination of suppressive capacity of MSCs, MLR (Mixed Lymphocyte Reaction) was done using Balb/C splenocytes as responder cells and C57BL6 mice splenocytes as stimulator cells in the presence or absence of MSCs that labeled with DiD or not. ^{3}H-thymidine incorporation was used to assess the proliferation. The effect of DiD on differentiation capacity of MSCs to adipocyte and osteocyte cells were also tested by culturing the MSCs in proper differentiation media. Results: More than 99% of MSCs were tagged with DiD. Suppression activity of MSCs were not altered after DiD labeling. In fact, both DiD labeled and un-labeled MSCs significantly suppressed (p= 0. 001) the ^{3}H-thymidine incorporation in the Balb/C splenocytes (248±60 and 282±50, respectively) compared to Balb/C splenocytes proliferation in the absence of MSCs (2260±53). Also DiD labeling did not affect the differentiation of MSCs to adipocyte and osteocyte cells. Conclusion: The results showed that suppressor activity of MSCs and their differentiation to adipocyte and osteocyte did not affect by DiD labeling. Therefore, DiD is an appropriate vital dye that can be used for tracking of MSCs without any affect on their functions.
Keywords: DiD, Differentiation, Immunosuppressive, Mesenchymal Stem Cells

838. Investigatingthe Apoptotic Activity of TLR4-Primed Mesenchymal Stem Cells toward the Activated Tcells

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Background:The therapeutic application of mesenchymal stem cells (MSCs) in treatment of autoimmune diseasesis largely attributed to their anti-inflammatory properties. Enhancement of anti-inflammatory potentials of MSCs is a pivote aim in therapeutic strategies toward autoimmune diseases. TLR4 describes as the early immune sensor, expressed by MSCs and effectively regulate their anti-inflammatory functions. Induction of apoptosis in activated immune cells is one of the important aspects of MSCs-exerted immunosuppression. The aim of this study was to investigate the apoptoticactivity of TLR4-primed MSCs toward the activated T cells. Materials and Methods:MSCs were isolated from bone-marrow of male BALB/c mice and cultured ex vivo. At the 3rd passage, MSCs were unprimed (control) or primed with a TLR4-agonist (LPS,10ng/ml) for different times (1h and 24h).Consequently,cells were co-cultured with PHA-activated splenic mononuclear cells (MNCs) at ratios of 1:10 (MSC:MNC)for 72h at 37 °C in a humidified 5%CO2 atmosphere. After 72h, levels of apoptosis in activated T cellswere assessed by anti-CD3PE,annexin-V/PI staining in flow cytometry.Results:Data showed that in contrast to the short term priming (1h), long term priming (24h) of TLR4 on MSCs significantly increased levels of apoptosis in activated T cells as compared to the control group ($p<0.01$).
Conclusion: Findings suggest that the different-terms of MSCs exposure to TLR4 agonist, differently affected apoptotic activity of MSCs. The results can be applied potently for more successful MSC-based therapy programs.
Keywords:Mesenchymal stem cell, TLR,Apoptosis, LPS, T cell.

839. Evaluationthe Expression of αv Integrin Family Receptors during Differentiation of Human Stem Cells to Oligodendrocytes

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Background:Repair by remyelinatingOligodendrocytes(OLG)is well recognized in the inflammatory demyelinating disease such as MS.Remyelinating cells are generated by oligodendrocyte precursor cells (OPCs) that migrate into the lesion, where they proliferate and differentiate into newly Oligodendrocytes.Extracellular matrix (ECM) ligands such as osteopontin are expressed at high levels in demyelination lesions.The principal receptors for ECM molecules are integrins, heterodimers of an alpha and a beta chain.FInteractions between αv integrin family receptors and their ECM ligands such as osteopontin will make the major contribution to integrin-mediated signalling during remyelination. Therefore, we have defined the pattern of αvintegrin family receptors expression during in vitro oligodendrocyte differentiation from human setm cells using real-time quantitative PCR analysis.Materials and Methods:human stem cells were cultivated in specific medium and differentiated into oligodendrocytesusing optimum protocol. After RNA isolation and cDNAsynthesis in three different stages of differentiation includinghuman stem cells, OPCs and oligodendrocytes, relative gene expression was performed for human αv-, β1-,β3-, β5-,and β8-integrin subunits with specific primers using SYBR Green quantitative PCR and normalized by beta actin(β actin)housekeeping gene.Results:Compare to low levels of **αv** -,β1- and β8-subunits mRNA inhuman stem cells,we observed that OPCs strongly expressed aforementioned subunits while their expression were markedly reducedin oligodendrocytes.TheExpression of β3-subunitin stem cells and OPCs waslower than predominantly expression level in oligodendrocytes. The similar exoressionpattern was seen for β5- subunit except that itsexpressinwas not detectable in human stem cells.Conclusion:This results show thatαv Integrin family receptors were differentially expressed during oligodendrocyte maturation.Despite of low expression level, in human stem cells , αvβ1 and αvβ8-integrinwere strongly expressed in the oligodendrocyte precursor phase Whereas αvβ5 and αvβ3-integrin expression increasedinmature oligodendrocytes.
Keywords:αv Integrin, OPCs, Stem Cells

840. Immunomodulatory Effects of Human Umbilical Cord Wharton's Jelly-Derived Mesenchymal Stem Cells on Differentiation, Maturation and Endocytosis of Monocyte-Derived Dendritic Cells

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Over the recent years, the Wharton's jelly of the umbilical cord is believed to be a source of mesenchymal stem cells which can be therapeutically applied in degenerative diseases. In this study, the fresh umbilical cords were prepared from newborns and transferred in sterile way to laboratory. Next, the umbilical cord mesenchymal stem cells were collected enzymatically and cultured in a suitable cell media. We investigated the immunomodulatory effect of UC-MSCs and BM-MSCs on differentiation, maturation, and endocytosis of monocyte-derived dendritic cells in a Transwell culture system under laboratory conditions. The initial induction of monocyte differentiation to immature dendritic cells in exposure to GM-CSF and IL-4 for 6 days followed by TNF-stimulation for 48 hours to finally generated mature dendritic cells. In every stage of differentiation, immature and mature dendritic cells were separately co-cultured with UC-MSCs and BM-MSCs. The findings showed that UC-MSCs and BM-MSCs inhibited strongly differentiation and maturation of dendritic cells at higher dilution ratios (1:1). The BM-MSCs and UC-MSCs showed more inhibitory effect in CD1a, CD83, CD86 expression, and dendritic cell endocytic activity, respectively .On the other hand , these cells up-regulated severely CD14 marker expression. We conclude that, UC-MSCs and BM-MSCs can inhibit differentiation, maturation and endocytosis in monocyte-derived dendritic cells through the secreted factors and free of any cell-cell contacts under laboratory conditions. As dendritic cells are believed to be the main antigen presenting cells for naïve T cells in triggering immune responses, it is logical that their inhibition to differentiate, maturate and function could affect in lessening or modulating of immune and inflammatory responses and even suppressing allograft rejection.
Keywords:Mesenchymal stem cells, Wharton's jelly, Umbilical cord, dendritic cell, Endocytosis

841. Investigation of miR-146 Family Expression, the Inhibitor of Mesenchymal-Amoeboid Transition in Cancer Stem Cell Rich MDA-M231 Cell Line

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Background: Breast cancer especially invasive ductal carcinoma is the most prevalent type of cancer and the second leading cause of cancer deaths in women worldwide. The major reasons for cancer deaths are complications arising frommetastasis.Although all factors governing tumor cell metastasis have not been fully elucidated, small non-coding microRNAs (miRNAs) are one of the most important contributers to cancer development and progression, and are differentially expressed in normal tissues and cancers. MetastamiR, a specialized family of miRNA, have been shown to have pro- and anti-metastatic effects.miR-146 family play an important role in suppression of metastasis by inhibiting the mesenchymal- amoeboid transition.It also inhibits invasion and migration of breast cancer cells by down-regulating NF-κB by targeting IRAK1 and TRAF6, so the reduction of this family could be an indicator of metastasis disease in all types of breast cancer. Materials and Methods: Metastatic MDA-MB231 cell line which is derived from plural effusion of patient with invasive ductal carcinoma as a test and non-metastatic MCF-7 cell line as a control have been cultured. The proportion of $CD^{44+}/CD^{24-/low}$ cells in these samples were evaluated by flowcytometery. Isolation of miRNA was performed by High Pure miRNA Isolation kit and expression of miR-146 family have been quantified by LNA enhanced RT microRNA PCR primer.Result: The result of our evaluation shows that MDA-MB231 has high proportion of $CD^{44+}/CD^{24-/low}$ cells in comparison to MCF-7. The expression of miR-146a and 146b in MDA-MB231 has been reduced to 100% and 17% respectively in comparison to MCF-7. Conclusion: MDA-MB231 cell line have high proportion of $CD^{44+}/CD^{24-/low}$ cells which is one of the important marker of breast cancer stem cells and the expression of mir-146 family, specially miR-146a have been reduced significantly, so the reduction of this family could be a marker of metastasis.
Keywords:miR-146 Family, Cancer Stem Cell, MDA-M231 Cell Line

842. Isolation and Characterization of Stem-Like Cells from Human Breast Cancer

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Background: The recently emerged concept of cancer stem cells has led to new hypotheses about tumor progression. Cancer stem cells can divide asymmetrically to self-renew and generate transient-amplifying tumor cells that cause tumor formation and subsequent metastasis. Thus, within the population of cancer cells, cancer stem cells are the ones which can form new tumors and their asymmetric division contributes to tumor heterogeneity. Breast cancer stem cells have been isolated from human breast tumors or breast cancer-derived pleural effusions using flow cytometry to find subpopulations of cells with a specific pattern of cell surface markers ($CD44^+$, $CD24^{-/low}$, ESA^+ (epithelial specific antigen)) but lacking expression of specific lineage markers (Lin2). These cells expressed epithelial-mesenchymal transition (EMT) markers and had higher tumorigenic potential than bulk tumor cells after transplantation in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. Most current treatments of cancer can remove the bulk of the tumors and not the cancer stem cells. Thus, these cells can be therapeutic targets for cancers. Antibodies for cancer stem cells surface antigens can help us to identify and isolate these cells. Materials and Methods: Tumor specimens were obtained from Milad hospital. After mechanical disaggregation and enzymatic digestion, single cells were produced. These cells and breast cancer cell lines were cultured at mammosphere condition. After two weeks mammospheres were collected and using a FACScan $CD44^+$ $CD24^-$ cells were isolated. Expression of survivin and oct-4 were identified using real-time PCR. Results: The $CD44^+$ $CD24^-$ cells could grow as mammospheres at non-adherent condition. There were the low percentage of the tumor bulk (1-5%). These cells had increased expression of survivin (an anti-apoptotic factor) and oct-4 (a stem cells marker). In metastatic cell lines the number of these cells was increased. Conclusion: The cells, $CD44^+$ $CD24^-$ can be breast cancer stem cells candidate. Their growth as mammosphere shows transformation and aggressive phenotype of these cells. As they were increased in metastatic cell lines, they may have an important role in metastasis. Isolation and identification of these cells can drive us to the new therapeutic approaches for the breast cancers.

Keywords: breast cancer stem cells, $CD44^+$ $CD24^-$, metastasis, mammosphere

843. Isolation and Characterization of Human Umbilical Cord Blood Mesenchymal Stem Cells

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Background: Umbilical cord blood is known as a rich source of hematopoietic stem cells,but there are few studies about mesenchymal stem cells in umbilical cord blood and still need to be established and evaluated.In this study,We investigated the isolation,expansion and characterization of human umbilical cord blood mesenchymal stem cells (UC-MSC). Materials and Methods: The mononuclear cells were isolated and cultued in appropriate growth medium.The adherent cells were expanded and maintained with periodic passages until a relatively homogeneous population was established.The isolation efficiency, colony-forming unit-fibroblast (CFU-F) frequency, phenotypic characteristics as well as multi-lineage differentiation capacity of UC-MSC were determined. Results: UC-MSC shared most of the characteristic of Bone Marrow Mesenchymal stem Cells (BM-MSC), including fibroblastic-like morphology, immunophenotype, adipogenic and osteogenic differentiation potentials. In Cell Surface analysis with flow cytometry,The following markers; CD29,CD44,CD73,CD90,CD105,CD106 and CD271 were positive and HLA-DR,CD45 and CD14 markers were negative.These results confirmed that isolated cells were mesenchymal stem cells.Conclusion: In summary, the present study describes the isolation of MSC from human full term umbilical cord blood. These cells share most of the characteristics with BM-MSC, including morphology, multi-lineage differentiation capacity. It seems that this source of cell with the distinct advantages , such as accessibility, painless procedures to donors, possible source for autologous cell therapy and lower risk of viral contamination, should be considered a promising alternative to Bone Marrow (BM) as a source of MSC.
Keyword: Umbilical Cord Blood, Mesenchymal stem cells, Isolation, Characterization

844. Isolation and phenotyping analysis of Mesenchymal stem cells from murine lung tissue

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Background: Regeneration and repair in the adult lung are mediated by endogenous lung stem and progenitor cells. Mesenchymal stem cells (also named multipotent stromal cells) a subset of adult stem cells present in virtually all tissues. The aim of this study was to isolate, differentiate and examine the expression of major surface markers on murine lung derived mesenchymal stem cells. Materials and Methods: Mesenchymal stem cells(MSC) were isolated from the collagenase digests of murine lung tissues and expanded through several passages. To investigate the mesenchymal nature, cells were differentiated into the osteogenic and adipogenic lineages after 2-4 passages. Flow cytometry analysis was performed on cells from passages 2-4 and the cultured cells stained by different fluorescent-labeled monoclonal antibodies against specific cell surface markers. Results: After several passages, adherent cells with fibroblastic morphology appeared in culture flasks. The cells from different passages were capable of differentiating into Adipocyte and osteocyte. The cell populations expressed CD90,CD73 and CD105.They did not express CD34, CD45 and CD11b. Conclusions: Mesenchymal stem cells could be successfully isolated and cultured from murine lung tissue.
Keywords:Mesenchymal stem cell, Isolation, Lung tissue, Cell differentiation, flowcytometry analysis

Poster Presentation

845. Vit-D3, Phorbol Ester and LPS Induce CD14 Expression in Promyelocytic Cell Lines

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Background: CD14 was described as the first differentiation marker on the surface of myeloid lineage cells. CD14 acts as a key role in activation of LPS-induced monocytes. LPS (lipopolysaccharide) binds to LPS-binding protein (LBP) in plasma and is delivered to the cell surface receptor CD14. LPS stimulates the human monocytes activation via several intracellular signaling pathways that involves the proinflamatory molecules. CD14 also reported to be a membrane receptor for a range of ligands .We investigated the effect of various factors that can affect the level of expression of CD14 in the human HL-60 and human U937 promonocytic cell lines. Materials and Methods: HL-60 and U937 cells were prepared. For each cell line,3 flask containing 10^6 cells were incubate for 18 days with vit-D3, 14 days with LPS and 72 hours with phorbol ester; then their responses to mentioned factors were observed by means of anti-CD14 antigen by FACS (flow cytometry analysis cell sorting). Responses of both cells to vit-D3, phorbol ester and LPS were compared with control sample, by FACS analysis. Results: FACS analysis demonstrated that HL-60 and U937 cells were induced by both vit-D3 and phorbol ester to obtained characteristics of adherent cells and expressed CD14 protein; moreover, LPS at a low dose increase CD14 on surface of this cells. Conclusion: According to the obtained results, it is speculated that CD14 gene expression may be induced in human HL-60 and U937 cell lines by different factors including vit-D3, phorbole ester and LPS.
Keywords: CD14, Vit-D3, LPS, phprbol ester, flow cytometry

846. Stem Cell from Human Exfoliated Deciduous Teeth (SHED) Inhibits theProliferation of Human T lymphocytes

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Background: Mesenchymal stem cells (MSC) are a specific type of adult tissue stem cell; in addition to stem cell attributes, they have the immunosuppressive effects that make them valuable targets for regenerative medicine and treatment of many human illnesses. So, MSC have been the subject of several basic and preclinical studies. The classical source of MSC is adult bone marrow (BM). Because of many shortcomings of harvesting MSC from BM, finding the alternative sources for MSC is an urgent. These sources should not endanger the donor healthy, be easily accessible and ethically uncontroversial. Stem cells from human exfoliated deciduous teeth (SHED) are relative new MSC populations that fulfill these criteria but their potential immunosuppressive effect has not been studied enough yet. Thus, in this work the effect of SHED on the proliferation of in vitro activated T lymphocytes by mitogen or alloantigen were explored.Materials and Methods: In this study, both mitogen and alloantigen activated T cells were cultured in the presence of different numbers of SHED. In some cocultures, activated T cells were in direct contact to MSCs and in other cocultures; they were separated from SHED by a permeable membrane. In all cocultures, the proliferation of T cells was measured by ELISA. BrdU proliferation assay. Result*:* In general, our results showed that SHED significantly suppressed the proliferation of activated T cells in a dose-dependent manner. Moreover, the suppression was stronger when MSCs were in physical contact to activated T cells. Discussion: This study showed that SHED likewise BMMSC and other MSC populations have immunosuppressive properties that may be exert by individual mechanisms. However, further studies are necessary to the exact mechanisms of their immunosuppression can be revealed. Generally, SHED is a proper MSC population that can be used instead of BMMSC in many basic, preclinical and clinical settings.
Keywords: Stem Cell, Exfoliated Deciduous Teeth (SHED), T lymphocytes

847. Culture and Characterization of Human Spermatogonia in vitro

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Background: Spermatogonia are the male germ line stem cells with life long expansion that is needed for permanent production of male germ cells. Spermatogonia are the only cells of the germ line, which proliferate in adulthood and cause fertility. Because of limitations on human SSCs studding there are little progress on culture and identification of these stem cells. Purpose is to establish in vitro culture of human spermatogonial stem cells from testicular tissue to obtain an adequate number of cells for 40 days and indicate presence of stem cells by stem cells marker GPR125 and PLZF. Materials and Methods: Study performed for 40 days using testis material donated by 8-10 parients. Testicular cells were isolated by collagenase IV (5mg/ml) and trypsin. Cells were cultured in supplemented SFM by factors as GDNF, bFGF, EGF and LIF to promote self-renewal divisions; germ line stem cell clusters that arose were subcultured on another dishes in the same medium by passaging every week. Immunofluorecent study was performed by anti-GPR125 antibody as specific marker of SSCs entire the culture. Skin and testis tissue were stained as positive control. Additionally, the expression of spermatogonial stem cells gene PLZF was studied in testis tissue and germ stem cells entire the culture. Results: Germ line stem cell clusters arose in the testicular cell cultures from patients and could be cultured and propagated up to 6 weeks. GPR125 is could be a specific marker for identification of Germ stem cells in testis tissue and during the culture. Expression of PLZF was applied on RNA level of Germ stem cells. Conclusion: Human germ stem cells could be culture and propagate for long-term in vitro conditions and maintenance of them could be prove by indication of SSCs specific marker on RNA or protein level.
Key words: Human spermatogonia, culture, GPR125, PLZF

848. Effect of Mesenchymal Stem Cell-Derived Exosomes on Rat Sperm Quality Parameters

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Background: Exosomes are described as microvesicles released from cells and serve as shuttles for bioactive component of cells in paracrine actions. Attachment or internalization of exosomes into target cells eventually coused to a wide variety of epigenetic and phenotypic changes in recipient cells. Recently, mesenchymal stem cell (MSC)-derived exosomes are considered as potent tools for paracrine induction of cell regeneration. Cell protective potentials of MSC-derived exosomes toward environmental stresses are demonstrated in several *in vitro* and *in vivo* studies. One of the major problems in the field of reproductive biotechnology is diminishing sperm quality after freezing (thawed frozen semen) which eventuates in low rate of *in vitro* fertilization of oocyte. The aim of this study was to investigate the possible protective effect of exosomes as an additive for cryoprotectant medium on sperm quality parameters.MaterialS and Methods: Exosomes were isolated from rat MSC-cultures'conditioned-medium by differential centrifugation. Sperms were obtained from cauda epididymis of 20 male wistar rats. Exosomes (20 µg/ml) were added to sperms suspended in cryoprotectant medium. After incubating for 6h at 37°C, sperms were frozen. Sperm parameters (viability, motility, acrosome integrity) and total antioxidant capacity were assessed both before freezing and after freezing in comparison to the control group (sperms suspended in cryoprotectant medium without the exosomes). Results: Results revealed that the addition of exosomesto cryoprotectant medium, significantly amplified sperm parameters and total antioxidant capacity, both before ($p<0.01$) and after the sperm freezing ($p<0.05$) as compared to the control group. Conclusion: Our data suggests that MSC-derived exosomes are the potent organelles for sperm quality enhancement over the time of maintenance in cryoprotectant medium and post-freezing, probably through reducing oxidative stress. Currently, the overall protective mechanism of exosomes is not yet understood.
Keywords: Mesenchymal Stem Cell, Exosome, Sperm, Viability, Motility, Acrosome integrity

849. Impact of chronic GVHD on survival of acute myeloid leukemia patients after non-T-cell depleted HLA-identical sibling peripheral blood stem cells transplantation

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Background: Peripheral blood stem cells (PBSC) are increasingly being used as the source of stem cells in allogeneic transplantations. Graft versus host disease (GVHD) is a major complication of allogeneic hematopoietic stem cells transplantation, and also is an important factor affecting the outcome of transplantation. An increased incidence of GVHD has been suggested following unmanipulated allogeneic PBSC transplantation (PBSCT), however, how this affects survival is not yetwell clear. In this study, our aim was to assess the impact of acute GVHD (aGVHD) and chronic GVHD (cGVHD) on overall survival (OS), disease-free survival (DFS) and relapse following non-T-cell depleted HLA-identical sibling peripheral blood stem cells transplantation (PBSCT). Materials and Methods: Data were analyzed from 78 patients, including 40 patients with acute myeloid leukemia (AML) and 38 patients with acute lymphoblastic leukemia (ALL), undergoing non-T-cell depleted HLA-identical sibling allogeneic PBSCT. All patients were received a uniform myeloablative conditioning regimen and prophylaxis for GVHD. We studied the incidence of aGVHD and cGVHD and theirs affect on survival and relapse in these patients. Results: The overall incidence of aGVHD and chronic GVHD was 82.5% and 42.5% in the AML patients and 84.2% and 26.3% in the ALL patients. The occurrence of aGVHD had no effect on OS, DFS and relapse in AML and ALL patients receiving transplants. Although incidence of 2-year OS and DFS were significantly higher in the AML Patients with cGVHD compared to patients without cGVHD (P=0.024 and P=0.033, respectively), this difference was not due to the low incidence of relapse. Conclusion: These data indicate that the occurrence cGVHD is an important predictor of outcome of non-T-cell depleted HLA-identical sibling allogeneic PBSCT, in that AML patients who develope cGVHD have a high chance of survival.
Keywords: GVHD, overall survival (OS), disease-free survival (DFS), peripheral blood stem cells transplantation (PBSCT)

850. Isolation and Immunophenotyping of Human Amniotic Epithelial Cells

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Background: Human Amniotic epithelial cells (hAECs) have been recently introduced as a new source of stem cells with therapeutic applications. These cells are heterogeneous population with differentiation capability in to all three germ layer cells. The aim of the present study was to establish an efficient and optimized isolation procedure of hAECs and characterization of purified cells with a panel of monoclonal antibodies against stem cell markers. Materials and Methods: Six human placentas were collected from healthy women undergoing cesarean delivery at term under sterile condition. Placentas were delivered immediately at cold chain to the laboratory and their amniotic membranes were carefully pilled off from the underlying chorion. Membrane fragments were enzymatically digested by trypsin and resulting single cell suspension were layered over Ficoll density gradient medium. Cells at the interface were collected and their epithelial origin was confirmed by assessment of cytokeratin expression. Cells were then evaluated for the expression of CD29, CD9, CD34, CD45, CD44, CD133, CD73, CD105, CD38, CD10 MHC-I, MHC-II, HLA-G, SSEA-4, STRO and OCT-4 by flow cytometry. Results: The overall yield ranged from 70-130 million purified cell/placenta. Microscopic examination revealed that hAECs are large and refractile cells with great capacity to adhere plastic surfaces. The cells exhibited substantial proliferative capacity as judged by XTT assay. The cells substantially expressed cytokeratin implying their epithelial origin. Flow cytometric analysis confirmed their stem cell-like nature by expression of CD9, CD10, CD29, CD73, CD105, HLA-I, HLA-G, OCT-4,SSEA-4 and STRO. These cells, however, failed to express CD34, CD38, CD44, CD45, HLA-DR and CD133. Conclusion: The procedure presented here is a simple and cost-effective protocol for isolation of a large numbers of purified hAECs. Based on their immunophenotype, it seems that this population share common markers of Embryonic stem cells. Regarding the fact that this cell type is readily accessible with potential

differentiation ability toward all germ layers, they can now be introduced as a novel source of stem cells for potential therapeutic or immunomodulatory purposes.
Keywords: Placenta, Stem cell, Amniotic membrane, Epithelial cells

851. Menstrual Blood Stromal Stem Cells inhibit Generation of Dendritic Cells from Peripheral Blood Monocytes

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Background: Menstrual blood stromal stem Cells (MBSCs) share some phenotypic and functional similarities with mesenchymal stem cells (MSCs). MSCs have been shown to inhibit either function or generation of different immune cells, including: T cell, B cells, Natural killer cells, and dendritic cells (DCs). However, data regarding MBSCs' potential effects on cells of the immune system are still elusive. Here, we examined whether MBSCs affect the generation of human monocyte-derived dendritic cells (DCs). Materials and Methods: Menstrual blood samples were collected from appearantly healthy women in the second day of menstrual cycle. Mononuclear cells were seprated using Ficol-Hypaque desity gradient medium and cultured. The adherent portion was subcultured to omit unwanted cells and obtain MBSCs. Magnetically-isolated peripheral blood monocytes were differentiated towards DCs through treatment with recombinant granulocyte monocyte colony-stimulating factor (GM-CSF) and interleukin (IL)-4 in the presence or absence of MBSCs. After five days, monocyte-derived DCs were analyzed for the expression of CD14, CD1a, CD40, CD86, and HLA-DR using flowcytometry. IL-6 level was also determined in co-culture supernatants by ELISA. Results: Co-culture with MBSCs, significantly downregulated the expression of DC markers (CD1a, CD40) and upregulated the expression of monocyte marker (CD14) on the monocyte-derived DCs compred with the control group. IL-6 levelwas shown to be significantly lower in the supernatant of monocyte-MBSC co-culture. Conclusion: Collectively, this is the first study to show the inhibitory impacts of MBSCs on the generation of DCs. IL-6 could be viewd as a potential factor mediating this effect. Regarding their so far known advantages over MSCs, MBSCs could be considered as a future promising candidate to be used for immunomodulatory purposes in the clinic.
Keywords: menstrual blood stem cell; dendritic cell; monocyte; maturation; surface marker

852. In Vitro Cardiac Differentiation of Bone Marrow Mesenchymal Stem Cells

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Background: Bone marrow mesenchymal stem cells are able to differentiate into cardiomyogenic cells. Selection the optimum conditions for directed differentiation of stem cells is one of the main topics in stem cells researches. In this study, we evaluated epigenetic processes that influence the differentiation of mesenchymal stem cells to cardiogenic cells. Materials and methods: MSCs were isolated and cultured from femoral bone marrow of rats and identified with morphological observations and differentiation tests. Then, Rat MSCs divided into two groups, in group 1, treated with 5-azacytidine for 24h and in group 2 first cells treated with trichostatin A for 3 days and then with 5-azacytidine for 24h. After differentiation, the expression of early cardiac transcription factor GATA-4 and specific cardiac genes such asB-MHC, Des and NPPA were determined with RT-PCR. Results: Morphological observations, differentiation results and RT-PCR tests revealed that our isolated cells were MSCs. Cardiomyogenic results show that, after 5-azacytidine and trichostatin A treatment, cell morphology changed to myotube-like structures and cells connected with adjoining cells. RT-PCR results show that, In both groups, cells expressed GATA4, GAPDH, B-MHC and Des.NPPA gene not expressed ingroup 1in 1 and 4 week but expressed in all times in group2. Conclusion: These data suggested that treatment with 5-aza induced cardiac specification of MSCs in vitro. However, addition of TSA as pretreatment enhanced the cardiomyocyte differentiation of MSCs with increased expression of cardiomyocyte-specific genes (through a mechanism beyond the inhibition of HDAC activity).
So in spite of these results, better understanding of MSCs behavior in vitro and in vivo is needed to develop strategies for therapeutic applications by these cells.
Keywords: Mesenchymal stem cells; cardiac differentiation; RT-PCR

853. Chemokine and Chemokine Receptor Expression in Mesenchymal Stem Cell like Cells Isolated from Brain Tumors

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Background:Mesenchymal stem cells (MSCs) astype of adult stem cell are very heterogeneous spindle shaped cells with high capacity for self-renewality. Recently significant evidence has been made in understanding the contribution of MSC in cancer by immune modulating of cancer, angiogenesis and metastasis by chemokines and chemokine receptors expression. The purpose of this study wasto investigate the expression of IP-10, RANTES and CCR5 in cells isolated fromdifferent types of brain tumorsincludingglioma and meningioma. Materials and Methods: We cultured cells isolated from brain tumor tissue (n=27) using collagenase type I, then cells were assessed for the expression of mesenchymal specific markers. The mRNA expression of RANTES/CCR5 and IP-10 was determinedby real-time quantitative RT-PCR.Results: Results of this study indicate that cells isolated from brain tumors were positive for the expression of CD166, CD105, and CD44 and negative for CD14, CD34 and CD45 by flowcytometry method. Expression of CCR5 in cells isolated from meningioma was 3.3-fold higher compared to those from glioma but this difference wasstatistically insignificant (p value >0.05). In addition, expression of RANTES and IP-10 in glioma was significantly higher compared with meningioma samples(p value <0.05).Conclusion:Morphological characteristics and expression of mesenchymal markers showed the similarity of isolated cells to the mesenchymalstem cells. Expression of chemokine and chemokine receptors particularly IP-10 and RANTES in mesenchymal stem cell like cells indicates that they may participate in recruitment of inflammatory cells to the brain tumor microenvironment and consequently exacerbating the antitumor immune responses.
Keywords: Mesenchymal stem cells, Chemokine, Brain Tumors

854. Human Mesenchymal Stem Cells Stimulated by Tumor Necrosis Factor-α Produce Growth Factor Receptors

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Background: Acute hepatic failure, despite recent therapeutic advances, remains associated with significant morbidity and mortality. Recent evidence indicated that insulin-like growth factor1 receptor (IGF-I R) is involved in liver regeneration upon activated by insulin-like growth factor1 (IGF-I). Aim: We hypothesized that inflammatory cytokines activate liver progenitor mesenchymal stem cell (MSCs) to induce IGF-I/ IGF-I R signaling pathway. Materials and Methods: Bone marrow of healthy donors was aspirated from the iliac crest. The adherent cells expanded rapidly and maintained with periodic passages until a relatively homogeneous population was established. The identification of the MSCs was carried out by differentiation potential into osteocyte and adipocyte. The MSCs were treated by 1 ng/ml, and 10 ng/ml of tumor necrosis factor-α (TNF-α) for 2, 10, 24, and 48 hours. Untreated MSCs were used as control. Real time polymerase chain reaction (RT-PCR) was used to evaluate the expression of IGF-I R in all cell-groups. Results: Flow cytometric analysis, and the differentiation potential into osteoblast and adipocytes showed that more than 90% of human MSCs which were isolated and expanded were positive by specific markers and functional tests. RT-PCR analysis indicated increase IGF-I R expression in MSCs treated by 1 ng/ml of TNF- α after 10 hours incubation. Conclusion: The data presented in this study, reflects increase expression pattern of IGF-I R in the human MSC upon *in vitro*-TNF- αstimulation, may be used for *in vivo*-clinical stem cell therapy and liver regeneration.

Keywords: MSCs, TNF-α, Growth Factor Receptors

855. Flow Cytometric Characteristics of Stem Cells in Medium with Different Sera and after Switching of Serum

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Background: Mesenchymal stem cells (MSC) are used for tissue engineering purposes. Fetal bovine serum (FBS) in culture media is essential for growth of MSCs. As FBS may induce an immunological reaction and may transfer pathogenic agents to MSC recipients, this study was designed to evaluate Adipose tissue stem cells (ASCs) in FBS, human serum by flow cytometry and histology. We also changed FBS of medium to human serum. Immunophenotypic characteristics and morphology of ASCs were compared in three culture condition. Results: ASCs isolated and expanded in medium containing FBS were flat shape and slowly growing versus to those grown in medium containing human serum. The forward scatter data significantly demonstrated ASCs in FBS had large size to others ($p<0.05$). ASCs appeared in the upper right quadrant of the dot plots, when the serum of medium were changed human serum. Morphology of ASCs were similar to flow cytomertic findings. There is no significantly differences in immunophenotypic markers of ASCs such as CD44, CD90 grown up in different media. However mean fluorescence intensity was higher for CD44 in human serum groups. Conclusion: These results indicate that medium enriched with human serum improved culture condition of ASCs in comparison with medium enriched with commercially available FBS. Switching of FBS to human may be useful method for stem cells characteristics that grown in medium containing FBS or freezed in FBS
Keywords: human serum, FBS, MSC, ASC

856. Adipose-Tissue Mesenchyme Stem Cell-Conditioned Medium Alleviate EAE by a Cell-Free Mechanism

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Multiple sclerosis is an autoimmune disease of the central nervous system in which body's own immune cells attack to insulating sheath around nerve cells and led to irreversible lesions in axons. In this regard Stem cell therapy, despite of broad application, has been associated with limitations such as etic problems, accessibility, cell harvesting, immune-incopmability, cost and contamination with other type of cells.in this study we analyze the effect of CM on alleviating courses of EAE. Due to Importance TH1 and TH17 cytokines in pathogenicity of EAE we indicated efficiency of AT-MSC-CM on IFNγ and IL17 as typical production from these cells. Here we investigated the immune regulatory properties of adipose-tissue CM by evaluation of their effects on splenic CD4+/CD25+/Foxp3+ T cell population and CNS histopathology by tracing leukocyte infiltration following Hematoxylin and Eosin staining. We also evaluated cell proliferation following specific and non-specific stimulation of splenic cells. CM from AT-MSC led to a significant reduction in severity of EAE by down regulating IL17 and IFNγ secretion.CM also inhibited splenic cell proliferation following stimulation by specific and non-specific antigens and reduced CNS leukocyte infiltration significantly.
Keywords: Mesenchymal stem cells; Experimental Autoimmune Encephalomyelitis; Immunomudulation; Regulatory T Cells.

857. Isolation and Propagation of Colon Cancer Stem Cells In vitro

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Background: Recent investigations in colon cancer biology suggest that cancer growth is driven by colon cancer stem cells (CSCs). CSCs are responsible for tumor initiation, maintenance, spreading and relapse. The aim of this study was to isolate and propagate tumorigenic colon CSCs in vitro. Materials and Methods: Tumor samples from colon cancers were subjected to mechanical and enzymatic disassociation. After passing of tumor cell suspensions through the cell strainers, tumor cells were counted and their viability was determined. Then tumor cells were plated in the specialstem cells medium. Tumor cells growth was evaluated by inverted microscope. Results: After about 3 weeks of plating in specific medium, colon CSCs were appeared (grown) as cell spheres whereas differentiated tumor cells and nonmalignant cells were unable to grow. Colon cancer spheres were able to propagated and passaged in the specific stem cells medium. Conclusion: Isolation and propagation of tumorigenic colon CSCs in vitro would help to devise novel diagnostic and therapeutic methods. Isolated colon CSCs can be applied for the studying of cell signaling and assessment of the effect of anti-cancer drugs.
Keywords: Isolation, Propagation, Colon Cancer, Stem Cells

858. *PEP* gene knock down using an expression vector producing specific designed shRNA in mouse embryonic stem cell during the process of cardiomyogenesis

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Background: Peroxisomal Protein (PEP) encodes one of the proxisomal matrix proteins, termed PeP, consists 209 amino acid resdiues. PeP contains a three peptide, SKL, at its C-terminus, acts as protein targeting signal (PTS1). Previous studies have shown that PEP is mainly expressed in heart, skeletal muscle, and brain tissues in adult mouse. Materials and methods: In order to clarify the PeP function in cardiomyocyte differentiation of mouse embryonic stem cells the present study was carried out. Thus, several siRNAs to silence PeP gene expression were designated using online softwares for siRNA designing. Results: The most appropriated siRNAs were 19nt in length along with a negative control siRNA (scramble) changed to shRNA and were ordered to synthesis. Synthesized oligonucleotides were annealed and inserted into an inducible eukaryotic expression vector at HindIII and XhoI sites. The recombinant vectors were transected in to the mouse embryonic stem cells. Stably transformed colony cells expressing PEP shRNA were selected after antibiotics treatment of the transfected cells. Doxycycline was used to induce the production of PEP shRNA and concomitant silencing of PEP gene. Conclusion: Real time quatitative RT-PCR revealed that one of the constructed PEP shRNA had a significant decrease in PEP expression. The PEP gene knock down using aforementioned approach during the stage of cardiomyogenesis is in progress.
Keywords: *PEP* gene, knock down, expression vector, cardiomyogenesis

859. To Evaluation of Differentiation Potential of Human Cord Blood CD133$^+$ Cells into Insulin Secreting Cells

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Background: The production of sufficient number of pancreatic endocrine cells similarly to primary islets is one of cell therapies to treat diabetes. CD133$^+$ cells, a type of adult stem cells, which express some embryo specific markers such as SSEA-4 and OCT4, are candidate for differentiation into other cells including insulin secreting cells. Therefore, here we attempt to determine their differentiation potential of UCB-CD133$^+$ cells into insulin secreting cells. Materials and Methods: CD133$^+$ cells were isolated from healthy human cord blood, by magnetic activating cell sorting (MACS). Subsequently, cells differentiated using activin A, retinoic acid, bFGF, nicotine amide, B27 and N2 during 9 Days.The expression of Insulin and c-peptide protein was detected by Immonocytochemistry. ELISA was performed to analysis the function of

differentiated cells in different glucose concentrations, and the expression of insulin, Nkx6.1, Pdx1, Glocagon genes was analyzed by RT-PCR. Results: Our results determined that UCB-CD133$^+$ cells were expressed oct4 and nanog in mRNA level and expressed insulin and c-peptide at protein level after 9 days of culture. However the insulin, Nkx6.1, Pdx1, Glocagon genes were not detected in differentiated cells and they could not respond to different concentrations of glucose. Conclusion: We suggested that CD133$^+$ cells ability into insulin secreting cells, however they are not functional and need to be more studies to find better microenvironment for production of functional differentiated cells.
Keywords: umbilical cord blood, CD133 cell, insulin-secreting cell, diabetic

860. Efficient and Expedited Derivation of Induced Pluripotent Stem Cells in Stirred Suspension Bioreactors

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Background: The therapeutic use of stem cells requires the availability of pluripotent cells that are not limited by technical, ethical, or immunological considerations. The recent derivation of induce pluripotent stem cells (iPSCs) is opening a new era in the field of tissue engineering and regenerative medicine. It will remove the existing ethical hurdles related to human ESCs and also can potentially eliminate the problem of immune rejection. The generation of induced pluripotent stem cells (iPSCs) is an inefficient process, which requires several weeks before a cell line is established. Here, we report that iPSCs can be generated more quickly and efficiently in stirred suspension bioreactors (SSBs). We have previously shown that SSBs provide a homogenous and dynamic environment, suitable for expanding mouse and human embryonic stem cells (ESCs). Our work suggests that SSBs favour the maintenance of pluripotency over differentiation. Materials and Methods: In this study, we examined whether SSB culture presents a selective advantage in iPSC derivation. We transduced mouse embryonic fibroblasts (MEFs) with retroviral vectors of the four reprogramming genes (Oct4, Sox2, klf4 and c-Myc). Two days after transferring to a 100mL SSB at 100RPM, the MEFs formed aggregates, which were morphologically similar to ESC aggregates. These bioreactor-derived iPSC (BiPSC) aggregates expressed alkaline phosphatase (ALP) only 5 days post-transfection, whereas ALP expression was totally absent in the control static culture condition. Results: We observed the *de novo* expression of major pluripotency markers such as Oct4, Nanog, SSEA1, Rex1, Dax1 and E-Ras on day 10. We were able to expand the culture to more than 50 million cells in less than 10 days, which is an outstanding difference, compared to conventional static culture methods. We observed about 2.5×10^5 ALP$^+$ aggregates (*i.e.* iPSC colony equivalents) from an input of 5×10^5 original fibroblasts, suggesting an efficiency of 50%. This number is substantiated by our observation that approximately 50% of the cells in the bioreactor were ALP$^+$ on day 12. BiPSCs from day 12 were karyotypically normal and could spontaneously differentiate into all three germ layers both *in vitro* and *in vivo*. We have recently generated several high percentage chimeras using BiPSCs and are awaiting the results of germline transmission. Conclusion: Our results suggest that liquid shear stress plays an important mechanistic role in bioreactor induced pluripotency. When we examined the nuclear translocation of β-catenin in ESC, using the TCF/LEF GFP reporter system, we observed that considerably more β-catenin resides in the nucleus of cells undergoing liquid shear stress. We suggest that suspension cultures provide a selective advantage in enhancing iPSC generation partly by inducing nuclear β-catenin activation. Our results show for the first time that fibroblasts can be efficiently reprogrammed in SSBs. Combined with new methods of reprogramming that only use epigenetic reprogramming factors, our BiPSC technology has the potential to accelerate and standardize iPSC research, bringing it to clinical application more quickly.
Keywords: Stem cells, Bioreactors, iPSCs, ESCs.

861. Autologous Transplantation of CD133+ Mesenchymal Stem Cells with Coronary Artery Bypass

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Background: Cell therapy with bone marrow-derived stem/progenitor cells is a novel option for improving neovascularization and cardiac function in ischemic heart disease. In this study we presented a series of patients with heart failure who had underwent CD133+ and mesenchymal stem cells transplantation. Materials and Methods: Nine patients with coronary artery disease and ischemic cardiomyopathy (EF<35%) who undergoes conventional CABG received an implantation of one ml from mixture of CD133+ and mesenchymal stem cells was injected for each patient into five points in peripheral regions of scars in myocardium. Results: The mean before-intervention ejection fraction (EF), end-diastolic, and end-systolic volumes were 35.5%, 6.3 cm, and 4.6 cm, respectively. The mean before-intervention EF, end-diastolic, and end-systolic volumes were 46.3%, 7.9 cm, and 6.2 cm, respectively. There was a statistically significant difference between before and after intervention EF, end-diastolic, and end-systolic volumes ($P < 0.0001$). None of the patients were experienced complications. Conclusions: According to obtained findings in this interventional study, the use of mesenchymal and CD133+stem cells is a safe and effective therapeutic option leading to increased functional cardiac capacity in patients with post ischemia heart failure.
Keywords: Transplantation, CD133+, Mesenchymal Stem Cells, Coronary Artery Bypass

TRANSPLANTATION IMMUNOLOGY

Oral Presentation

862. Study of the Relationships between IL-23R ،IL-17 and IL-21 Polymorphisms with Acute Graft Rejection in Kidney Transplant Recipients

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Background: Cytokines are one of the important factors in transplant outcome. IL-17, IL-21 and IL-23R are inflammatory markers that associated with episodes of acute rejection. Any variations in cytokine levels may directly influence the course of transplant. Since the importance of genetic polymorphisms for the immune response in graft is irrefutable so in the present study we investigated the effect of functional polymorphism of IL-17, IL-23R and IL-21 genes in acute rejection of Kidney Transplant Recipients. Materials and Methods: For IL-17, IL-23R and IL-21 genotyping, genomic DNA was extracted from Buffy coat of 250 kidney transplant patients using a DNP Kit according to the manufacturer's instruction. SNPs including: 197A/G polymorphism of IL-17 gene in chromosome 6, C/T polymorphism of IL-23R gene in chromosome 1, and C5250T and G1472T polymorphisms of IL-21 gene in chromosome 4 were evaluated by PCR based methods. IL-17 and IL-21 G1472T genotyping was performed by PCR-RFLP and IL-23R and IL-21 C5250T genotyping was performed by ARMS-PCR. Results: The study group composed of 250 patients , 70 of 250 (28%) studied kidney transplant patients, showed acute rejection and 180 of 250 (72%) them did not experience acute rejection. the frequencies of three genotypes in IL-17 (AA,AG,GG) in rejection were 8.58%,38.58% and 52.86% respectively while 15%, 46.11% and 38.89% in Non-Reject. there was a significant association between IL-17GG genotype (P= 0.045) and IL-17AG alleles (P=0.032) and acute rejection of Kidney Transplant Recipients. Conclusion: The mentioned results indicate that IL-17 polymorphisms and alleles play role in acute kidney rejection. Therefore we suggest IL-17G197A promoter polymorphism of IL-17 as a regulatory cytokine could be a genetic risk factor for the development of acute rejection.
Keywords: Gene Polymorphisms, Kidney Transplant, IL-17, IL-23R, IL-21

863. Association of the IL-17, IL-21, and IL-23R Genetic Polymorphisms with HBV Infection in Kidney Transplant Recipients

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Background: Hepatitis B virus infection is one of the most important chronic viral diseases all over the world. Several studies have reported cytokine genes are associated with HBV chronicity, persistence and/or disease severity. IL-17, IL-23R and IL-21 are cytokines that play a key role in modulating the inflammatory response such as acute rejection. The aim of this study is to investigate of the relationships between IL-17, IL-23R and IL-21 gene polymorphisms with HBV infection in recipients with acute kidney rejection. Materials and Methods: For IL-17, IL-23R and IL-21 genotyping, genomic DNA was extracted from plasma of 220 kidney transplant patients using a DNP Kit according to the manufacturer's instruction. SNPs including: 197A/G polymorphism of IL-17 gene in chromosome 6, C/T polymorphism of IL-23R gene in chromosome 1, and C5250T and G1472T polymorphisms of IL-21 gene in chromosome 4 were evaluated by PCR based methods. PCR-RFLP method was carried out for analyzing IL-21 C1472, IL-17 197GA SNPs and ARMS-PCR method was carried out for evaluating IL-23R and IL-21 C5250T gene polymorphisms. The prevalence of HBV infection was evaluated by a qualitative PCR test, using a HBV PCR detection kit according to the manufacturer's instruction in kidney transplant patients. Results: The 60 of 220 (27.28%) studied kidney transplant patients were shown acute rejection and 160 of 220 (72.73%) them were not experience acute rejection. The HBV genome was detected in 52 of 220 (23.64%) kidney transplant patients. Significant association was found between IL-23R alleles with the chronicity of HBV infections in patients with acute rejection (p=0.03). But other gene polymorphisms were not associated with acute rejection. Conclusion: Diagnosis of the relationships between IL-17, IL-23R and IL-21 gene polymorphisms with HBV infection and also association between IL-23R (A/C) alleles with the chronicity of HBV infections announced the importance of cytokine gene polymorphism in relation of kidney transplantation.
Keywords:HBV Infection, kidney Transplantation, IL-17 Gene, IL-23R Gene, IL-21 Gene

864. Donor-Derived DNA Is Associated with Stable Kidney Allograft Function

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Background: A large body of literature has documented an inconsistent relationship of peripheral donor cell chimerism with alloimmune tolerance following kidney transplantation. We revisit this association with assays capable of quantifying cellular microchimerism with 150–1500-fold greater sensitivity than previously utilized allo-antibody based flow cytometric approaches. Materials and Methods: Forty renal transplant patients, 20 with concurrent donor bone marrow infusion (DBMI) and 20 control participants without infusion were prospectively monitored for peripheral blood microchimerism using donor polymorphism-specific quantitative real-time PCR. Thirty-eight patients were evaluated for microchimerism, 19 in each group. Results: The frequency of testing positive for (95% vs. 58%, p = 0.02) and mean concentrations of microchimerism (115 ± 66 vs. 13 ± 3 donor genomes/million recipient genomes, p = 0.007), respectively, were higher in infused patients compared with controls. Thirty-one patients maintained stable graft function; 17 in the DBMI group vs. 14 in controls. Patients with stable graft function in the DBMI group compared with control patients harbored microchimerism more frequently (94 vs. 50%, p = 0.01) and at higher concentrations (123 ± 67 vs. 11 ± 4, p = 0.007), respectively. Significant correlation between dose of infused cells and microchimerism levels was found post-transplant (p = 0.01). Conclusion: Using very sensitive assays, our findings demonstrate associations between the presence and quantity of microchimerism with stable graft function in infused patients.
Keywords: Donor-Derived DNA, Kidney Allograft

865. Expression CD44 and CD74 in Liver Transplanted Cases

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Background:Hepatic dendritic cell, T cell co-stimulator molecule, cytokine and chemokine production has pivotal role in alloimmune response and allograft rejection. We aimed to demonstrate the feasibility of gene expression profiling for more than 91 human genes in formalin-fixed, paraffin-embedded tissue in liver transplant patient.Materials and Methods:We used PCR Array technology for assessment gene expression profile of cytokine, cytokine receptor,… in both rejected and non rejected groups.We studied biopsy sample from 12 hepatic patients (6 patient whit acute rejections and 6 patient non rejections). Between 2009 and 2011 from department liver transplant archive Namazi Hospital Shiraz-Tehran. Total RNA was extracted by Rneasy FFPEkit (Qiagene, Germany). Quality of RNA was evaluated spectrophotometric. CDNA and PCR Array were done according to SABA Science RT Profiler PCR Array Human Dendritic and APC-PAHS-406A kit.Result:We identified the gene expression pattern of CD74 and CD44 dramatically change in rejected transplant cases vs. non rejected according to relative quantification data the relative expression of CD44 were 4 and CD74 100 fold in rejected cases.Conclusion:Both CD44 and CD74 molecule are involved in B cell and macrophage migration and inflammatory reaction. In our studies both ligand dramatically increased in rejection cases. It seems this over gene expresses may involve in pathogenesis of acute rejection in liver transplant cases.
Keywords: CD44, CD74, Liver Transplanted Cases

866. Different Genotypes of HLA-E in Hematopoietic Stem Cell Transplantation

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Background: HLA-E is a member of MHCclass Ib moleculescrucially involved in the regulation of innate immunity. Considering the importance of HLA-E in NK cell function which affects hematopoietic stem cell transplantation (HSCT), we investigated the association of different genotypes of HLA-E withthe clinical outcomes after HSCT. Materials and Methods: In our study, patients with underlying malignant hematological disorders who received allogeneic HSCT were examined for acute and chronic graft-*versus*-host disease (GvHD), transplant-related mortality (TRM) and Disease Free Survival (DFS). Donor selection was performed using molecular typing for HLA-A, -B, -Cw, -DRB1, -DQ, -DP and -E. Results: We found a decreased incidence of acute GvHD (grade II or more; p=0.02) and chronic GvHD (extensive; p=0.04) as well as a trend towards a lower frequency of TRM (p=0.09) in the patients with HLA-E*0103/0103 genotype compared to HLA-E*0101/0101and HLA-E*0101/0103genotypes. Conclusion: We also showed an association between HLA-E*0103/0103 and a better DFS (p=0.001). In conclusion, our results suggest a protective role for HLA-E*0103/0103 genotype against acute and chronic GvHD, TRM as well as an association between this genotype and improvedDFS after allogeneic HSCT.
Keywords:DFS; GvHD; HLA-E polymorphisms; HSCT and TRM

Poster Discussion Presentation

867. Evaluation of the Relationships between Genotype Polymorphisms of PD1, CD28, and ICOS Genes with Outcome of HBV Infection in Bone Marrow Transplant Patients

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Background: Positive and negative effects of co-stimulatory molecules have role in outcome of bone marrow transplantation and affects withGenetic polymorphisms. Also single nucleotide polymorphisms of co-stimulatory moleculesmay interact with viral pathogenesis. Therefore

in this study the relationships which may occur between co-stimulatory molecule gene polymorphisms with HBV infection was evaluated in bone transplant recipients. Materials and Methods: In a cross sectional study EDTA-treated blood samples were collected from 72 Allogenic and 59 Autologus recipients of bone marrow between years 1383-1390. The genetic polymorphisms were evaluated in co-stimulatory genes including: of PD-1 gene including: PD-1-1.3G/A, PD-1-1.9C/T, CD28-17 C/T, ICOS-1720-T/C were analyzed by different in-house-PCR-RFLP protocols. Also the prevalence of HBV infection was evaluated by analyzing the HBsAg titer by a third generation ELISA method according to manufacturer instruction. Results: HBsAg was detected in 31 of 59 (52.5%) and 20 of 72 (27.8%) Autologus and Allogenic bone marrow transplant patients, respectively. HBV infection was found significantly in Allogenic bone marrow transplant recipients (P=0.003; OR=0.347; CI 95%=0.168-0.718). The GG genotype of PD-1-1.3 locus, the CC genotype of PD-1-1.9 locus, the TT genotype of CD28-17 locus, and the CC genotype of ICOS1720 locus have higher frequency in bone marrow transplant patients. These most frequent CD28-17 C/T gene polymorphisemis significantly found in HBV uninfected transplant recipients. Conclusions: Homozygote format of all studied co-stimulatory molecules was found with higher frequency in bone marrow transplant patients. Alsothe genetic polymorphisms of all co-stimulatory molecules were not correlated with HBV infection in both Autologus and Allogenic bone marrow transplant patients. But for better define the accurate role of co-stimulatory gene polymorphisms in clinical outcome of bone marrow transplantation and pathogenesis of HBV, the completed studies are needed.

Keywords**:** Polymorphisms, PD1**,** CD28**,** ICOS**,** HBV, Bone Marrow Transplant Patients

868. Influence of *GSTO2* (N142D) Polymorphism on Acute Renal Allograft Rejection in Iran Population

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Background: Despite last progresses and high potation of immunosuppressive agents, acute renal allograft rejection is remained one important threaten for graft survival during of post transplantation. Several immunological and non-immunological factors intervene in renal graft rejection. Glutathione S-transferase super family is one of the important enzymes for biotransformation of both exogenous and endogenous xenobiotic compounds such as immunosuppressive agents. The new class of this family is omega that includes two subunits GSTO1, GSTO2. They have unique features through existence of cysteine amino acid in their active site, cause to separate from others for substrate. Materials and methods: In this study 282 samples of buffy coat were calculated from renal recipients of Namazi hospital in Shiraz-Iran during 2007-2010 years. Also 300 healthy samples as control group were calculated from Shiraz population, included in our study. The primary outcome of this study was defined as biopsy-proven acute rejection (BPAR) during 1 year of renal transplantation. We applied PCR-RFLP method for determination of *GSTO2* N142D polymorphism. Results: According to this study, no significant association was observed between this genetic variant and acute rejection. Also *GSTO2* polymorphism has no significant effect with risk of ESRD. Cadaveric donor type for acute rejection significantly differed between acute rejection and non acute rejection patients (P=0.004). The combination effect of donor type and *GSTO2* polymorphism indicates DD genotype with cadaver donor type increase odd ratio of acute rejection (OR=3.82 CI95%=1.80-12.37 P=0.02). Conclusion: present finding indicating that *GSTO2* DD genotypes accompanied by cadaveric donor type increase risk of acute renal allograft rejection.

Keywords: *GSTO2*, Polymorphism, Acute Renal Allograft Rejection

869. Association of *GSTO2* (N142D) Polymorphism and Incidence of Acute GVHD Bone Marrow Transplantation

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Background: Hematopoietic cell transplantation (HCT) is frequently complicated by graft-versus-host disease (GVHD), GVHD is one of the main problem after bone marrow transplantation (BMT). The highly variable clinical manifestations of acute GVHD frequently involve the skin, liver, upper respiratory tract, and lower gastrointestinal tract. GSTs family is the most important genes in phase 2 detoxification that interfering in xenobiotic and drug metabolism. *GSTO2* is one of member of this family. *GSTO2* (N142D) Polymorphism may influence metabolism of immunosuppressor drug that used for BMT. Materials and Methods: the present case-cotrol study included 20 patient whit GVHD as a case and 68 patient whit out GVHD in bone marrow transplantation as a control to know whether this polymorphism plays a role in incidence of GVHD or not. Moreover we investigate 88 patient with BMT as a case and 100 healthy person as a control to know whether this polymorphism plays a role in creating conditions that lead to BMT or not.To determine variants of *GSTO2,* we use polymerase chain reaction-restriction fragment length polymorphism method. Three genotype were identified, NN as wild type, ND as a heterozygote and DD as mutant genotype. Results: our result suggest that , there is no significant association between the *GSTO2* genotype and GVHD(NN:OR=1(ref), ND:OR=1.259 ,%95CI =0.452-3.512, P-value=0.66 and DD:OR=0.944 %95CI=0.094-9.526 P-value=0.961) ,moreover we don't observe an association between *GSTO2* and BMT(NN:OR=1(ref), ND:OR=1.116 ,%95CI =0.612-2.036, P-value=0.72 and DD:OR=0.465 %95CI=0.152-1.428 P-value=0.14). Conclusion: this result show that may be this polymorphism has not role in metabolism of drug that use for BMT and also has not main role in creating problem that lead to BMT.

Keywords: *GSTO2*, Polymorphism, GVHD

870. Study of the Relationships between Genotype Polymorphisms of PD-1, CD28, and ICOS Genes with Acute Rejection in Kidney Transplant Patients

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Background: Co-stimulatory Molecules are important in regulating immune response and related polymorphisms are associated with change of the pattern of expression and function in kidney transplant patients with acute rejection. Accordingly, the relationships between the polymorphism of different loci of CD28, PD-1, and ICOS genes with the rate of graft surveillance in kidney transplant patients were studied. Materials and Methods: In this study, the buffy coats of EDTA-treated blood samples were collected from 172 kidney transplant patients admitted to Shiraz Transplant Center, Namazee Hospital, Shiraz, Iran, between years: 2006-2010. The polymorphism of co-stimulatory genes including: CD28-IVS3TC locus, PD-1(PD-1.9-7625TC, PD-1.3 -7146AG loci), and ICOS 1720CT locus were analyzed by in-house-PCR RFLP protocols. Results: The higher frequency of TT genotype of CD28 -17 C/T locus was found in 44.4% of acute Rejected kidney transplant patients (p=0.67, OR 0.87, %95Cl 0.63-1.82). The higher frequency of GG genotype of PD-1.3 -7146 locus was found in 77.7% of acute Rejected patients (p=0.32, OR 1.49, %95Cl 0.63-3.60). Also the higher prevalence of CC genotype of PD-1.9-7625 locus was found in 73.3% of acute Rejected transplant recipients (p=0.84, OR 0.93, %95Cl 0.40-2.16), and the higher frequency of CC genotype of ICOS1720 locus was found in 60% of acute Rejected transplant patients (p=0.94, OR 0.97, %95Cl 0.46-2.07). Conclusion: Interestingly, the homozygote format of all studied co-stimulatory molecules was found with higher frequency (but not significantly) in kidney transplant patients with acute rejection. Therefore for better define the accurate role of co-stimulatory gene polymorphisms in clinical outcome of kidney transplantation, the completed studies are needed.

Keywords: PD-1, CD28, ICOS, Acute Rejection, Kidney Transplant Patients

871. Evaluation of the CTLA-4 Gene Polymorphism in Graft Survival of Kidney Transplant Patients

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Background: One challenging aspect of the immunological role of co-stimulatory gene polymorphism is in a kidney transplant.CTLA-4 has role in the regulation of T cells and it is a member of the same family of cell surface molecules including CD28. CTLA-4 antigen is only expressed on activated T cells, which binds to B7 molecules on antigen-presenting cells. CTLA-4-B7 complex delivers negative signals to T cells and affecting T cell proliferation, cytokine production, and immune response regulation. Breakdown in the B7-CD28/CTLA-4 pathway could alter T-cell response and lead to autoimmune disease. Materials and Methods: 172 EDTA-treated blood samples were collected from kidney transplant recipients, between years: 2006-2011 form Shiraz Transplant Research Center, Namazee Hospital, Shiraz, Iran. The genetic polymorphisms of CTLA-4 gene including: -318 C/T, 1722 T/C, 1661 A/G and 49 A/G, polymorphisms. The genotyping of CTLA-4 polymorphisms were analyzed by in-house-PCR RFLP protocols including MseI and BbvI restriction enzymes. Results: The AA genotype of CTLA-4 1661 locus, the TT genotype of CTLA-4 1722 locus, the CC genotype of CTLA-4 -318 locus, and the AA genotype of CTLA-4 49A/G locus have higher frequency in kidney transplant recipients. None of studied CTLA-4 gene polymorphisms are significantly correlated with kidney graft surveillance and HCV infection in transplantation patients. Conclusion: Interestingly, the homozygote format of all studied CTLA-4 molecules was found with higher frequency (but not significantly) in kidney transplant patients with acute rejection. Therefore for better define the accurate role of co-stimulatory gene polymorphisms in clinical outcome of kidney transplantation, the completed studies are needed.
Keywords: CTLA-4, Polymorphism, Graft Survival, Kidney Transplant Patients

872. Controversies Regarding the Hepatitis B Immune Globulin Prophylaxis (HBIG) for Liver Transplantation Recipients

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Liver transplantation is the best option for the end-stage liver disease following hepatitis B (HBV) infection. However, the success of such option has been hampered by a high rate recurrence of HBV infection following transplantation. Over the past two decades, prophylactic treatment for the prevention of HBV re-infection following liver transplantation has been consisted of passive immunity administered by low- to high-dose Hepatitis B Immune globulin (HBIG) plus an antiviral agent as a gold standard regimen.However, the effectiveness of HBIG in preventing recurrence of HBV depends upon the dosage, route of administration, duration of HBIG treatment and the viremic status at the time of transplantation.Moreover, there is currently no consensus standardized recommendation over those therapeutic options that include HBIG applications. Despite reported success of this method for the prevention of HBV recurrence following transplantation, high costs and patient's incompliance have limited the acceptance of this approach. Further, despite monitoring, patients with active replication still had 16% rate of HBV recurrence. Therefore, in an attempt to determine an optimal regimen for HBIG administration that can be more cost-effective, convenient, and widely-accepted alternative approaches have been studied including: lower HBIG doses, the use of intramuscular HBIG rather than the intravenous route, non-HBIG forms of anti-HBs, and low-dose or short-term HBIG in combination with antivirals and tailoring HBIG administration guided by plasma concentrations rather according to a fixed time schedule. The choice of oprtions depend on the availability of antivirals and cost of HBIG regimen.
Keywords: Liver transplantation prophylaxis, HBIG and nucleot(s)ide analogues,HBIG and HBV recurrence

873. The Comparison of Percentage of the Th1 Cells between Patient with Stable Grafts and Acute Rejection Cases

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Background: In spite of dramatic improvements in the allograft maintenance and survival, there are lots of problematic issues which affect long-term survival of a graft. The molecular mechanisms of rejection and major player cells are still unidentified. In the immune system, Th1 subset supply first line defense against intracellular bacteria and viruses. It has been shown that Th1 cells can take part in some dysregulated responses which may lead to autoimmune disease as well. Many papers have reported the important role of Th1 cells in rejection but there are some questions still waiting for an answer. Do they exactly play any role in rejection? Or does Th1 population undergo any changes during graft rejection? To answer these questions we did this study. Our aim was to comparison the percentage of Th1 cells in PBMCs of patients with or without stable graft function. Materials and Methods: We studied 30 patients who received renal transplants from living donor. We divided our patients in two groups; first group included 20 patients who have shown stable graft function and the latter one included 10 patients who have shown biopsy approved acute rejection. Then, we evaluated the percentage of Th1 cells in two groups. PBMCs of patients were extracted from patient blood samples by ficoll protocol. The PBMCs were stimulated by PMA/Ionomycin in the presence of BD GolgiStop™ then cells were stained with fluorescent monoclonal antibodies-specific for human CD4, IFN-γ. Subsequently, the amount of released fluorescent was analyzed. All statistical analyses were performed by SPSS 14.0. Results: Patients with acute rejection episodes have been indicated an increment in the percentage of Th1 cells. Differences between the percentage of CD4+ IFN-g+ cells have been shown among these two groups. Statistical analyses demonstrated that these differences were significant (P value: 0.04). Conclusion: As many papers and references pointed out Th1 cells can play a major role in the immune responses. Our data are in support of the studies, which explain higher level of Th1 cells can result in rejection. We proved that higher percentage of Th1 subset was observed in the rejection group. Finally, we suggested that Potentially Th1 cells can use as an immunologic marker to estimate the possibility of rejection.
Keywords: Th1 Cells, Stable Grafts, Acute Rejection

Poster Presentation

874. Evaluation of Circulating Concentrations of CXCL1 (Gro-α), CXCL10 (IP-10) and CXCL12 (SDF-1) in All Patients Prior and Post Bone Marrow Transplantation

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Background: Immune system is important in the development of leukemia. CXC chemokines as the molecular members of immune system that are involved in the immune responses. Therefore, this study was designed to compare the levels of CXCL1 (Gro-α), CXCL10 (IP-10) and CXCL12 (SDF-1) in ALL patients before and after bone marrow transplantation. Materials and Methods: In this experimental study, samples were undertaken from ALL patients and controls and subjected to ELISA for detection of chemokines. Demographic data were also collected by a questionnaire. Data were analyzed using SPSS software. Results: Our results showed that serum level of CXCL1 (Gro-α), CXCL10 (IP-10) and CXCL12 (SDF-1) significantly increased in ALL patients in compare to control. We also showed that the level of CXCL10 (IP-10) was increased after bone marrow transplantation in ALL patients while CXCL1 (Gro-α) and CXCL12 (SDF-1) were decreased. Conclusion: According to our results, it can probably be concluded that the CXCL1 (Gro-α), CXCL10 (IP-10) and CXCL12 (SDF-1) play important roles in pathogenesis of ALL. Noteably these chemokines could probably be used as pivotal biological markers in diagnosis of leukemia and recombinant CXCL1 (Gro-α), CXCL10 (IP-10) and CXCL12 (SDF-1) could be applied as a therapeutic reagent for treatment of leukemia patients.
Keywords:ALL, leukemia, chemokine, CXCL1 (Gro-α), CXCL10 (IP-10)andCXCL12 (SDF-1), BMT

875. Role of CD4 Gene Expression in Liver Transplant Rejection

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Background:Liver transplantation is critical therapy in end stage liver diseases. Since the liver is a part of immunologic organ, thus immunologic reactions especially rejection is more prominent in liver transplantation. Rejection processes are more complex and many cells and mediators involve in it. Materials and Methods: In this study, we compared gene expression profile of cytokine and co-stimulator molecules in both rejected and non rejected patients. Result:According to data obtained in PCR–array of relative quantification of expressed gene profile an increase in CD4 RNA level in rejected patient vs. non rejected(16 fold)was detected.Conclusion:CD4 molecule has central role in T cell based immunological reaction. It seem's increased expression of CD4 molecule in parts involves in pathogenesis of acute graft rejection in liver transplanted patients.
Keywords: CD4, Liver Transplant Rejection

876. Association of *GSTO2* (N142D) Polymorphism and Liver Acute Rejection in Iranian Population

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Background: Liver acute rejection is the main problem in liver transplantation that occurs in the first days or first months of transplantation that included histological and cellular rejection. Histological acute rejection determind with biopsy. Polymorphism in immune system genes may influence acute rejection. GSTs family is the most important genes in phase II detoxification that interfering in xenobiotic and drug metabolism. *GSTO2* is one of member of this family. *GSTO2* (N142D) Polymorphism may influence metabolism of immunosuppressor drug that used for inhibiting acute rejection. Materials and Methods: The present case-cotrol study included 120 patients with histological liver acute rejection as a case and 182 patients without liver acute rejection as a control. Case and control matched by sex and age. To determine variants of *GSTO2,* we use polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Results: Our results suggest that, there is a significant association between the *GSTO2* genotype and liver acute rejection. N allele increase risk of liver acute rejection. (NN: OR=3.642, %95CI =1.179-5.444, P-value=0.007) and (ND: OR=2.533, %95CI =1.672-8.149, P-value=0.002). So patient with NN and ND genotype show more liver acute rejection than DD genotype. Conclusion: In this polymorphism, (142D) allozyme were expressed at approximately 80% of the level of WT (N142) allozyme. Genotype with no or lower GST activity may increase the effectiveness of drug such as immunosuppressor drug so clearness these drug occur in lower rate than higher GST activity and given the reduced detoxification capacity of enzyme. So immunosuppressor drug can remain in long time in body but may also render patient more prone to drug side effects.
Keywords:*GSTO2,* Polymorphism, Liver Acute Rejection

URBANISM & IMMUNE SYSTEM

Oral Presentation

877. Modulatory Effect of Humic Acid on Phagocytosis

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Background: Humic acids are found wherever organic matter is being decomposed, or transposed as sediments via humification. As these substances have wide therapeutic usage in alternative medicine, the possible effects of humic acid (HA) on phagocytosis were studied. Materials and Methods: Thirty two male wistar rats were divided randomly into four groups. Groups includes: 1) Control (normal diet). 2) Normal diet + 0.25% HA per kg of diet, 3) Normal diet + 0.5% HA per kg of diet, 4) 1% HA per kg of diet. After eight weeks, blood samples were collected via cardiopuncture with heparinised syringe under anaesthesia from each individual rat. Peripheral blood mononuclear cells (PBMCs) separated by density gradient sedimentation on ficoll. The monocytes enriched by adherence to a slide surface by two hours incubation of PBMCs in 37 °C and 5% CO_2. The non-adherent cell (almost lymphocytes) washed away. Opsonised yeasts were added on slides and incubated again. Cells were stained with gimsa solution and observed with light microscope. Results: The comparison of mean percentage phagocytosis of groups show significant differences (P=0.008). The results show that the highest levels of phagocytosis were observed in groups 3 and 4 (56 and 58% respectively). Conclusion: Present results suggest that humic acids are biologically active immunodulators affecting phagocytosis branch of immune reactions.
Keywords: Humic Acid, Phagocytosis

878. The Modulatory Effect of TCDD on Menstrual Blood Stromal Stem Cells

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Background: Endometriosis is characterized by the growth of endometrial tissue outside the uterine cavity. Retrograde menstruation is shown to be involved in the pathogenesis of endometriosis through which endometrial stromal stem cells are implanted in the peritoneal cavity. Although, etiology of this disease remains poorly understood, environmental pollutants such as 2,3,7,8- tetrachlorodibenzo p-dioxin (TCDD) have been suggested as a possible etiologic factor. Here, we examined the immunomodulatory effect of TCDD on menstrual blood stromal stem cells (MBSCs) in an allogenic MLR co-culture system. Materials and methods: Menstrual blood was collected from 6 apparently healthy donors and 6 endometriosis patients. Mononuclear cell fraction was cultured and adherent cells were used as MBSCs. Purified MBSCs were co-cultured in the allogenic MLR system and treated with 10 nm TCDD. Expression of indoleamine 2,3-dioxygenase (IDO), production of cytokines and levels of regulatory T cells (Treg) were assessed thereafter by Real-time PCR, ELISA and flow cytometry, respectively. Results: TCDD treatment resulted in an up-regulated IDO expression in both normal and endometriosis MBSCs, being more pronounced in the latter group. In both groups, TCDD treatment significantly decreased IFN-γ, TNF-α and IL-18 levels in MBSCs/PBMCs co-culture and increased IL-10/IFN-γ ratio. Moreover, the ratio of IL-10/IFN-γ and the percentage of Tregs were significantly increased in endometriosis co-cultures. Conclusion: In line with the previous studies, our results corroborated that endometriosis is a Th2 phenomenon. TDCC treatment, on the other hand, induced IDO expression and declined pro-inflammatory cytokines in favor of Th2 profile. More importantly, differential effect of TCDD on IL-10/IFN-γ and the percentage of Tregs in endometriosis MBSCs may imply inhered susceptibility of these cells to the microenvironmental signals. This might, therefore, explain the role of TCDD as an environmental pollutant in the pathogenesis of endometriosis.
Key words:Menstrual blood stromal stem cells, TCDD, IDO, Cytokine, Regulatory T cell

879. Blood Cells in Non Sensitized and Sensitized Guinea Pigs

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Background: To examine the effect of lead acetate on serum hitamine, total and differential white blood cells (WBC) in blood of non sensitized and sensitized guinea pigs. Materials and Methods: Guinea pigs were randomly allocated in to control (group C), sensitized (group S), three non sensitized Pb-exposed groups: (0.1M Pb, 0.2M Pb and 0.4M Pb), three sensitized groups exposed during sensitization (DS+0.1M Pb, DS+0.2M Pb and DS+0.4M Pb) and three sensitized groups exposed after sensitization (PS+0.1M Pb, PS+0.2M Pb and PS+0.4M Pb), (n=6 for each group). Total and differential WBC count as well as histamine level was evaluated in blood of all animal groups. Results: Serum histamine level and total WBC number in sensitized and all exposed groups to lead were significantly higher compared to control group ($p<0.05$ to $p<0.001$). Compared to control group, the percentages of eosinophil, nuetrophil and basophil were also increased in sensitized guinea pigs and sensitized animals exposed to lead ($p<0.01$ to $p<0.001$). In addition, the percentages of eosinophil in non sensitized animals exposed to two higher concentrations of lead and that of basophil in animals exposed to highest lead concentration were also increased ($p<0.05$ t0 $p<0.001$). Monocyte percentage in animals exposed to two higher lead concentrations after sensitization was also increased ($p<0.05$ for both cases). However, the percentage of lymphocyte in all sensitized groups was decreased compared to control group ($p<0.001$ for all cases). Serum histamine level and total WBC number in all sensitized animals exposed to lead were significantly higher compared to non exposed sensitized group ($p<0.05$ $p<0.001$). In addition the percentage of eosinophil, exposed to last lead concentration were higher but percentage of lymphocyte exposed to two higher lead concentrations was lower than non exposed sensitized group ($p<0.05$ to $p<0.01$). Conclusions: These results showed that lead acetate can lead to increased in serum histamine level, total WBC percentage of eosinophil, neutrophyl and basophil but reduction in percentage of lymphocyte. In addition the results showed that all the observed changes further enhanced in sensitized animal exposed to lead.
Key words: Lead exposure, Sensitized animals, Total WBC, Differential WBC, Histamine

880. Living in High Background Radiation Areas: Impacts on Immune System

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Background: Urban living today involves several biological challenges, of which one is pollution (air pollution, lead, noise, etc.). Urbanism has continuously presented novel adaptive challenges to its residents. Although radiation is around us all the time in both natural and artificially forms, however, living in high background radiation areas and chronic exposure to low dose radiation may have impacts on health.The purpose of this study was to determine whether the chronic exposure to high level natural radiation affects the immune responses of the inhabitants. Materials and Methods: Lymphocyte subpopulations, lymphocyte activation factor and cytokines (by flowcytometry), serum concentrations of Immunoglobulins and components of the complement system (by SRID and ELISA methods) and serum concentrations of DHEA and Cortisol (by RIA) and chromosomal damage were measured among 50 inhabitants of high background radiation areas in Ramsar city who have lived there for several generations. The control group covered 40 inhabitants from the nearby city of normal background, matched by sex and age. Results: Results showed no significant difference between the exposed and control group in terms of number of WBC and lymphocytes, $CD4^+$ Tcells, $CD8^+$ Tcells, $CD19^+$ and $CD16^+56^+$ cells. However, significant expression of activation marker on $CD4^+$ Tcells was found in the exposed group. The results of cytokine production in response to PMA/I showed that IL-5 up-regulated, IL-2 and IL-12 down-regulated and IFN- γ did not change in the exposed individuals to the controls. Low levels of DHEA in the exposed group were correlated to their increased IgE levels. Cytogenetic results showed higher chromosomal damage in the exposed group.Conclusion: these data suggest that continuous exposure to high level natural radiation throughout life may influence the cascade of activities determining the pattern of immune response to this challenge by stimulating some cellular responses and modulating cytokine productions. While cytogenetic results show higher chromosomal damage, some immune responses are stimulated or modulated immunologically in the inhabitants.
Keywords: Living, High Background Radiation, Immune System

881. Effect of Benzo(α)Pyrene on the Pollen Allergenicity in Sunflower (*Helianthus annuus* L.)

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Background: Allergy occurrence was increased in recent years dramatically. The reason of this phenomenon was not clear but there is a scientific attention to air pollution. Diesel exhaust particles (DEP) are a major part of air pollutants that express both adjuvant activity for sensitization against common allergens and enhancing effects on allergic symptoms. Benzo(α)Pyrene (BaP) is considered as the most important part of DEP. The aim of this research was study of the effect of BaP on pollen allergenicity in *Helianthus annuus* L. Materials and Methods: *Helianthus annuus* L. var Record plants were grown from seed in greenhouse controlled condition and shoots treated by different concentration of BaP solutions in phosphate buffer saline (PBS) (0.002, 0.02, 0.04 gL^{-1}) daily. Allergenicity of collected pollens was studied by means of skin prick test, determination of blood cells and evaluation of total IgE in different studied groups using of sensitized guinea pigs as an experimental model. Pollen proteins were also studied by SDS-PAGE and Immuno-blotting method for BaP-induced changes in protein profile and detection of allergen bands. Result: Comparing of allergy potency showed that allergic skin reactions in animals treated by polluted pollen was 2-3 times more than the groups treated by normal pollen extract and 5-6 times more than BaP treated group. Eosinophil number and IgE level, as allergy indicators, were also increased considerably in the blood of the groups treated by BaP exposed pollen than control ones. Results of SDS-PAGE showed that there are additional two bands in the BaP treated pollens. Immuno-blotting study of pollen proteins showed that new bands act as allergens that are reacted with IgE strongly. Conclusion: Results of this research work concluded that BaP could increase the allergy potency of *H. annuus* pollen and also cause to formation of new protein bands that act as new allergens.
Key words: Air pollution, Diesel exhaust particles, Benzo (α) pyrene, Pollen allergy, Helianthus annuus

Poster Discussion Presentation

882. The Effects of Electromagnetic Field on Humoral Immunity in BALB/C Mice

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Background: different studies have been performed for investigation of magnetic field effects on the immune system. There are many controversies about the effects of different frequencies and amplitudes on the immune system. Materials and methods: In this experimental study, the effects of different frequencies of magnetic field (0, 5, 50, 500 and 5000 Hz) have been determined on the Humeral Immune system. Antigen such as SRBC was injected (10^8/0.1 ml) to different test and control groups (10 mice in each group).Each test group exposed to a defined frequency (0, 5, 50, 500 and 5000Hz) of magnetic field with constant amplitude (0.5 mT) 10 hours daily for seven days. Control groups were not exposed to any magnetic field while all other conditions were similar to the test groups. Humoral immunity was evaluated by Microhemagglutination test and HPFC respectively. Results: The Microhemagglutination results showed that in all except zero frequencies of magnetic field, the Antibody Titer were significantly reduced compared to the control group ($P<0.002$). The HPFC results were also decreased

significantly in 500 Hz (P<0.03) and 5000 Hz (P<0.002) measured in 24 hours with no changes in zero, 5 and 50Hz frequencies. Conclusion: It can be concluded that frequencies of 5, 50, 500 and 5000 HZ reduce the Antibody Titer and frequencies of 500 and 5000 HZ reduce the HPFC in BALBL/c mice.

Keyword: Electromagnetic Field, Humoral Immunity, BALB/C Mice

Poster Presentation

883. Bio Positive Effects of Low Dose Radiation on the Immune System

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Ionization radiation effects on biological and immunological systems are well established. Epidemiological studies from atomic bomb survivors and patients who undergo radiotherapy that received high level of radiation dose and dose rates are the best evident of lethal cluster damages of ionization radiation on immune system. In low dose radiation (below 0.5 Gy) there is not enough data to interpret the real effects of radiation. However there are challengeable events in low dose radiation region that not only reject the lethal and destructive effects of radiation, but consider radiation as a beneficial agent. Stimulatory effect of low level of radiation (called as hormesis) and radioadaptive response (adaptation to lethal doses by exposing to low radiation) are the two interesting phenomena that during those, radiation causes positive effects on immune system. Radiation induced signal transduction, cytokine releases, new proteins and genes expression in related to immune system are as double-edge sword that control the positive and negative effects of radiation. Anti-inflammatory, anti-aging, activation and amplification of immune system by low dose radiation against viral and bacterial infections, lower chromosomal and proteins damages due to exposure to low level of radiation are scientifically proved events in term of bio positive effects of radiation on immune system.

884. Acute Effect of Pulsed Electromagnetic Fields Resulting fromSignificant Frequencies of High and Low Triangular Waves on White Blood Cells

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The study of the effects of electromagnetic fields on biological systems have started in recent years and have had significant impacts and outputs which requires ample paying and further research, especially in therapy field. In this study, the effects of electromagnetic fields caused by low-frequency triangular wave (10 Hz) and high frequency (110 Khz) with 700 milli Gauss intensity on white blood cells of rats was comparatively studied. For this purpose 30 male Wistar rats were selected and divided into three groups of ten. The first group was under high frequency field (110 Khz) for two days and each time for two hours and the second group were under similar low frequency (10 Hz) but the third group (control) weren't exposed to any field. Then each of those group members was bled from their hearts and their blood samples were analyzed based on their approximate white cell numbers. The results of the study were compared using t-test and it showed that the total white blood cell counts in both groups were not statistically different. Differentiation in counting the number of neutrophils were not also statistically different but reduction in the number of lymphocytes was observed in high frequencies and also increase in the number of Eosinophils in high frequencies and decrease in low frequencies as well as an increase in monocytes in lower frequencies was meaningful. According to the result of this research and activities conducted by others, the effects of electromagnetic fields on blood factors including white blood cells were significant and provide a suitable ground for research and extensive field of therapeutic applications in this sector.

Key words:Pulsed electromagnetic fields, Triangular waves, White blood cells

885. Immunological Parameters of Common Carp (*Cyprinus Carpio)* From Polluted and Non-Polluted Sites in the Karoon River

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Biomonitoring means an environmental assessment through conducted observations in relation to effects of pollutants on the animal and plant life**.** This study evaluated the immunological response of common carp captured in two environments with different levels of pollution in the karoon river, khozestan state, Iran. One of them, hereby named reference site, is a non pollution site which there is no sewage discharge. The other, denominated polluted site, is characterized by discharge of domestic sewage. After water quality analysis, 62 fish were captured, transported to the laboratory and anesthetized for hematological exam. The immune factors that were evaluated in this study, be included lysozyme, total protein, albumin and the serum bactericidal. A significant increase was observed in total protein in ahvaz than the shooshtar ($p<0.01$). However a significant decrease was observed in lysozyme and albumin of ahvaz than the shooshtar. This study demonstrated that water pollution might affect the immunological parameters in *Cyprinus carpio*. The employment of immunological techniques has provided valuable knowledge for fishery biologists in the assessment of fish health and in monitoring stress responses. Thus assessment and identify of aquatic ecosystems is necessary for its management.

Key words: Pollution, Biomonitoring, karoonriver,Immunological parameters, *Cyprinus carpio*

VACCINE & VACCINE DEVELOPMENT

Oral Presentation

886. An Overview of Leishmaniasis Vaccine Development: Why don't we have a vaccine?

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Leishmaniasis is a protozoan parasitic disease endemic in many countries including Iran. There are three major clinical forms, cutaneous, mucocutaneous and visceral leishmaniasis. According to the great variety of epidemiological situations and the incomplete understanding of the *Leishmania*biology, disease control would be complicated. There are no vaccines available at present.*Leishmania* vaccine development has proven to be difficult and challenging task. Attempts to develop vaccines such as heat killed, subunit, or DNA vaccines have not resulted in a successful vaccine candidate that could be applicable to human. Current curative therapies are relatively ineffectual, expensive and often not tolerable by patients. Certainly, the profile of an anti-leishmanial vaccine would need to incorporate several important features, such as safety,

ease of production at a low cost in endemic countries, the induction of robust and long term T cell responses. Hopefully, by rapid progress in the fields of parasite immunology and genomics, a successful anti-*Leishmania* vaccine should be achieved in near future.
Keywords: Leishmaniasis, Vaccine

887. Basic HIV Vaccine Development; Obstacles and Where We Are

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Development of a safe, potent and globally affordable HIV vaccine is the best hope for the future control of the HIV-1 pandemic. Since 1987, scores of candidate HIV-1 vaccines have been developed, which elicited varying degrees of protective responses in nonhuman primate models, including subunit vaccines, DNA vaccines, live vectored recombinant vaccines and various prime-boost combinations. Several clinical trials of HIV preventive vaccines have been performed worldwide, however, to the exception ofthe recent RV144 Phase III trial in Thailand, which elicited a modest but statistically significant level ofprotection against infection, none has shown efficacy in preventing HIV-1 infection or in controlling virusreplication.It sounds that any success in this area needs to answer several specific questions about; the correlation of a protective immune response with the virus, control of the enormous sequence diversity of the virus, and the differential features of effective response in human and monkeys. Results of ongoing clinical studies definitely show that in order to develop an efficient vaccine against HIV global research efforts should refocus on basic discovery.Herein, outcomes of the latest clinical studies with the focus on the problems facing the development of an HIV vaccine will be discussed.
Keywords: HIV, Vaccine Development

888. Synergistic Anti-tumor Effect of IP-10 and PEI600-Tat in DNA Vaccination against E7-Expressing Tumors

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Background: Immunotherapy targeting human papillomavirus (HPV)represents a valid noninvasive treatment for cervical cancer. Regarding to previous studies, vaccination with HSP/antigen complexes efficiently elicit antigen-specific immune responses. The N-terminal of glycoprotein 96 (NT-gp96), a member of HSP90 family, has adjuvant effect and can induce effective immune response against several tumors and viral infections. Many studies demonstrated that IP-10, a Th1 directing chemokine, involves in the anti-tumor immune responses by T cells recruitment to the malignancies. RANTES,a CC chemokine, is able to polarize the specific immunity towards a dominant Th1 profile. In this study, a combined strategy for enhancing HPV-vaccine potencyincluding linkage of immunostimulatory HSP (NT-gp96) to E7, a HPV transforming protein representing perfect antigen for vaccines, as well as co-administration of chemokine (IP-10 or RANTES) and non-viral gene delivery system (PEI600-Tat) were evaluated.Materials and Methods: Fusedrecombinant DNA plasmid consisting of E7 and NT-gp96(E7-NT-gp96) was constructed. In addition, the effect of PEI600-Tat in combination with IP-10 and RANTES on DNA vaccine immunogenicity was evaluated. The preventive andalso therapeutic efficacy of different applied strategy was assessed against E7-expressing cell line (TC-1) in C57BL/6mice model.Results: Our data showed that co-administration of IP-10 along with E7-NT-gp96 delivered by PEI600-Tat was able to enhance the vaccine immunogenicity and consequently to convey protection against TC-1.Conversely, RANTES co-delivery resulted in tumor growth and associated with detrimental effects. In therapeutic setting, co-immunization of IP-10 at the same inoculation site of TC-1 along with E7-NT-gp96 delivered by PEI600-Tat is able to significantly suppress tumor growth. The lymph nodes of successfully-treated mice secreted elevated levels of IFN-γ and IL-2.Conclusion: In C57BL/6 mice, the fusedE7-NT-gp96 in combination with IP-10 and PEI600-Tat could synergistically enhance the potency of HPV vaccine, all of which induced a strong preventive and therapeutic response against TC-1.
Keywords: IP-10, PEI600-Tat, DNA Vaccination, E7-Expressing Tumors

889. Advances in Development of Hepatitis C Virus Vaccines: Turning the Immune System on a Moving Target

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Around 3% of the world population is infected with Hepatitis C virus (HCV) and 3-4 million newly infected subjects are added to this reservoir each year. At least 10% of infected people will develop liver cirrhosis or cancer while no specific therapy or approved vaccine against HCV infection is available to date.Currently, HCV is the greatest human health challenge after that of HIV and development of an effective vaccine against HCV infection is among globally important medical priorities. High sequence divergence level of HCV genotypes, induction of antibody and CTL escape mutants together with emergence of quasispecies in the course of infection and limitation of knowledge around correlates of protective immunity and HCV pathogenesis until very recently were among obstacles for HCV vaccine development. However, this disappointing scenario is rapidly changing mainly because of: *i)* invention of efficient HCV/JFH1-based culturing systems and HCV infection transgenic mice models which enhanced advancement of the basic knowledge on HCV pathogenesis and immunology and *ii)* advancements in novel vaccine strategies and formulations. In fact, despite exceptional problems associated with development of an effective vaccine against this viral infection, presence of several HCV vaccine candidates in the phase I/II of clinical trials indicate that the future for HCV vaccination and/or immune therapies will be bright. Herein, the latest knowledge on HCV molecular biology, pathogenesis and immunology in relation to HCV vaccine design as well as the cutting edge technologies and approaches currently employed in developing vaccines for this viral infection will be reviewed.
Keywords: Development, Hepatitis C Virus, Vaccines, Immune System

890. Formulation of Selected *Leishmania* DNA Vaccine Candidates in a Nanoparticulate Delivery System: Characterization and *In vitro/In vivo* Immunological Evaluations

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Background: Leishmaniasis with 14 million affected in 88 countries and 2 million annual incidence rates is still a major health problem in endemic areas mostly developing ones. Earlier generations of *Leishmania* vaccines have reached the third-phase of clinical trials, however none of them have shown adequate efficacy due to lack of an appropriate delivery system. Materials and Methods: In this study, cationic solid lipid nanoparticles (cSLNs) were used to formulate three pDNAs encoding *L. major* cysteine proteinase type I (*cpa*), II (*cpb*) and III (*cpc*). The formulations were characterized according to their size and zeta potential as well as pDNA integrity and stability against *DNase I* treatment. Lipoplexes' cytotoxicity was investigated on COS-7 cells by MTT test. BALB/c mice were immunized twice with a 3-week interval, with SLN-pcDNA-cpa/b/c, pcDNAcpa/b/c, SLN, SLN-pcDNA and PBS. Footpad assessments, parasite burden, cytokine and antibody responses were evaluated. Results: cSLN-pDNAs complexes revealed suitable size and zeta potential. Efficiency/cytotoxicity ratio of these formulations was comparable to related linear PEI-25KD- polyplexes while exhibiting significantly lower cytotoxicity. Mice vaccinated with SLN-pcDNA-cpa/b/c significantly ($p<0.05$) showed higher protection levels with specific Th1 immune response development compared to other groups. Conclusion: This is the first report demonstrating cSLNs as a nanoscale vehicle boosting immune response quality and quantity; in a designable trend. The nanomedical feature of this novel formulation can be applied for wide-spread use in genetic vaccination against leishmaniasis, which is currently managed only through relatively ineffectual therapeutic regimens.

Keywords: *Leishmania* DNA Vaccine, Nanoparticulate Delivery System, cSLNs

891. Vaccine Production Vision in Production Complexof Pasteur Institute of Iran up to the year 2025

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Production and Research Complex was inaugurated formally in the year of 1988 in order to satisfy hygienically domestic demands.
At present, Production and Research Complex (as a part of National Health System) makes every effort to supply some of the required vaccines (such as B.C.G & HB Vaccine) and to achieve the most prestigious position in vaccine production according to IRAN'S 2025 vision policy.In the past, Complex activities in the vaccine field concentrated on vaccine production against pox, tuberculosis, and typhoid fever. Following the eradication of some diseases and the emergence of recombinant vaccines, complex priorities were reprogramized. Based on modern and advanced required facilities and equipment, we are planning to produce new generation vaccines such as Hib sub-unite vaccine Against *Haemophylus Influenza*, tetravalent (DPT + HB vaccine), pentavalent (DPT + Hib + HB vaccine) and Human rabies vaccine in future.
Keywords: Vaccine Production, Pasteur Institute, Iran

892. Application of Efficient Immune Adjuvants and Combinatorial Therapies Remain Viable Options to Treat HPV-inducedLesions

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Efforts to test novel strategies targeted toward HPV should help to develop the field of immunotherapy significantly. While prophylactic vaccines aim to prevent infection with HPV by inducing a neutralizing antibody response, therapeutic vaccines aim to develop a strong cellular immune response to HPV antigens that are expressed in transformed cells. In recent years, considerable progress has been made in the development of prophylactic vaccines against HPV, while, advances in the field of therapeutic vaccines seem to have progressed more slowly.Due to high cost and some side effects of prophylactic HPV vaccines in developing countries where screening programs are minimal,thus, the need for developing therapeutic strategies to treat HPV induced lesions is emerged. Combining novel and improved immunotherapeutic strategies with current standard of care treatments may potentially lead to a decline in recurrence rates and also leave the patient with long-term immunity. In addition to improving the design of efficient vaccines and clinical trials, future studies should concentrate on addressing local immune suppressive mechanisms and immune evasion strategies used by HPV. However, the application of proficient immune adjuvants and combinatorial therapies remain viable options for enhancing immune responses in clinical trials.
Keywords: Adjuvants, Combinatorial Therapies, Viable Options, HPV-inducedLesions

893. Protection against Rabies after Exposure Past History-Present State and Future Outlook

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Until the findings of L. Pasteur for thousands of years, the protection methods of exposed individuals against rabiesvaried in different ethnic groups and territories. These methods were disastrous and useless. The long dark of human knowledge about the protection of man against rabies came to end whenin 1885 the first human being severely woundedby a suspectedrabiddog(Joseph Meister) received the Pasteur's rabies vaccine and was protected. Pasteur's Rabies Vaccine and other nervous tissue vaccines prepared after L.Pasteur were abandoned due to post vicinal reactions. At present cell culture rabies vaccines are used in all over the world. Today as a result of four decades researches and field trials in Pasteur Institute of Iran and other research centers,the exposed persons can benefit from a simple, safe and protective prophylactic treatment. The protection of exposed persons is expensive and this causes a great deal of strain on budget of health organizations. Therefore in future it is necessary to provide a cheaper cell culture rabies vaccine and rabies immunoglobine.
Keywords:Rabies, Past History-Present, State, Future Outlook

894. Development of a Multi-species Malaria Vaccine against *Plasmodium falciparum and Plasmodium vivax* Species

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Background: Vaccines could be a crucial component of efforts to eradicate malaria. Current attempts to develop malaria vaccines are primarily focused on *Plasmodium falciparum*. However, because of increasing drug resistance, recent observations of severe and lethal *P. vivax* cases and coexistence of *P. falciparum/P. vivax* in many malaria endemic regions, a multi-species vaccine should be considered. Different studies have been shown that the carboxy-terminus of the merozoite surface protein1 (MSP-1_{19}) is one of the main malaria vaccine candidate antigens. Therefore, in the present investigation to develop a multi-species malaria vaccine, the immune responses elicited by co-immunization of PvMSP-1_{19} and PfMSP-1_{19} antigens were evaluated. Materials and Methods:The recombinant PvMSP-1_{19} and PfMSP-1_{19} proteins were expressed in prokaryotic system using pQE30 and pGEX-KG plasmids, respectively. To produce DNA constructs, the corresponding genes were cloned in pcDNA3.1hygro(+) vector. Balb/c mice were immunized subcutaneously 3 times with 3 weeks intervals with PvMSP-1_{19} and PfMSP-1_{19} antigens (protein antigens were emulsified in IFA) either alone or in combination using DNA/protein regimen. Immunized mice sera were evaluated for anti-PvMSP-1_{19}- and -PfMSP-1_{19}-specific antibodies at weeks 8 and 40 by ELISA. IFN-γ production was analyzed in supernatants of stimulated splenocytes of immunized mice with antigens using murine cytokine immunoassay kits at week 8. Results: High level of specific anti-PvMSP-1_{19} and -PfMSP-1_{19} IgG1, IgG2a and IgG2b antibodies and IFN-γ response were induced in mice immunized with either of antigens alone or in combination. In addition, anti-PvMSP-1_{19}- and -PfMSP-1_{19}-specific antibodies persist near a year. Conclusion: The inclusion of both antigens in combination in a vaccine mixture could not inhibit induction of antibodies or cytokines to the other antigen. Therefore, for the first time, this study is encouraging to develop a multi-species malaria vaccine based on MSP-1_{19} antigen using DNA/protein regimen.
Keywords:Multi-species Malaria Vaccine, *Plasmodium falciparum, Plasmodium vivax*

895. Challenges in Malaria Vaccine Research

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Despite major efforts over the past 50 years to develop a malaria vaccine, there is no approved vaccine against this disease. Expectation that a malaria vaccine can be developed comes from the studies that malaria immunity can be acquired through natural and experimental infection. However, efforts to develop a vaccine have been blocked by the complexity of the parasite's life cycle and the ability of the parasite to suppress and evade the immune response. Although clinical immunity to malaria has been well documented in adults living in malaria endemic areas, our understanding of the host-immune responses operating in such malaria immune persons remains poor, and limits the development of immune control of the disease. Current malaria vaccine candidates are directed against human and mosquito stages of the parasite life cycle that focused on reducing infection rates, blocking replication of the parasite in the bloodstream, and the pathologic effects of the parasite in individuals. As one of the WHO goals is to eliminate and eradicate malaria from the world by 2050, it is important to have vaccines for their potential contribution to reduction of disease transmission. Therefore, in this presentationthe state of malaria vaccine development in the world and challenges facing for such a development are discussed. Finally, our malaria vaccine development activities, based on locally generated evidence

will be presented, mainly transmission blocking vaccine and regional pre- and-erythrocyte stages vaccine against both *P. vivax* and *P. falciparum* using different prime-boost strategies.
Keywords:Challenges, Malaria, Vaccine

896. A Novel Adjuvant, Mixture of Alum and Propranolol, Elicits both Humoral and Cellular Immune Responses for Heat-killed *Salmonella typhimurium*Vaccine

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Background: To determine the efficacy of the mixture of propranolol (PRP), a β-adrenergic receptor antagonist, and alum, as a new adjuvant, in the induction of humoral and cellular immunity in response to heat killed *Salmonella typhimurium (S. typhimurium)* (HKST) as a model vaccine. Materials and Methods: Methods: BALB/c mice were divided into five groups. Mice in the experimental groups received either the HKST vaccine alone or in combination with the adjuvant alum, PRP or the alum-PRP mixture. Mice in the negative control group received phosphate-buffered saline. All mice were immunized two times on days 0 and 14. Two weeks after the last immunization, immune responses to *S. typhimurium* were assessed. Results: Administration of the alum-PRP mixture as an adjuvant increased the ability of the HKST vaccine to enhance lymphocyte proliferation, shifted the immune response towards a T-helper (Th) 1 pattern and increased *S. typhimurium* specific IgG, IgG2a and IgG1. This resulted in improved protective immunity against *S. typhimurium*. Conclusion: Administration of the alum-PRP mixture as an adjuvant in combination with the HKST vaccine, can enhance both humoral and cellular immunity and shift the immune responses to a Th1 pattern. To our knowledge, this study is the first one in the literature to evaluate the adjuvant activity of a β-adrenergic antagonist alone or as a mixture with alum for use in combination with a vaccine.
Keywords: Adjuvant, Alum, Propranolol, *Salmonella typhimurium*

897. Invariant Natural Killer T Cells: Benchtop Research & Bedside Applications

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Invariant natural killer T (*i*NKT) cells constitute a tiny but potent subpopulation of lymphocytes with unique characteristics. They co-express NK cell markers and a canonical or invariant T cell receptor (*i*TCR) that recognizes glycolipid antigens within the deep hydrophobic pocket of CD1d. Once activated, *i*NKT cells rapidly secrete copious amounts of pro- and/or anti-inflammatory cytokines. This is owed to the presence of pre-formed mRNA for these cytokines in *i*NKT cells. Cytokines released by *i*NKT cells in turn act on various downstream effector cell types and modulate a wide spectrum of immune responses in health and disease. Recent years have witnessed increasing interest in *i*NKT cells as attractive targets for immunotherapy. Importantly, findings from mouse models are likely to be translatable to human conditions. This is due, at least in large part, to the fact that mouse *i*TCR can recognize human CD1d and vice versa. Furthermore, the same glycolipid antigens (*e.g.*, α-galactosylceramide) that are used as experimental tools in animal models have been tested in clinical trials for cancer and infectious viral diseases. I will summarize our recent data on costimulatory and signaling requirements of *i*NKT cells and their unconventional activation by bacterial superantigens. I will also highlight some of our (and others') recent findings in *in vivo* models, including in humanized mice, to discuss the therapeutic promise of *i*NKT cells in the context of autoimmune arthritis, toxic shock, type 1 diabetes and transplantation.
Keywords: Natural killer T cells, CD1d, glycolipid antigens, immune regulation, cancer, autoimmunity, transplant rejection, toxic shock syndrome, bacterial superantigens

898. Influenza Virus Vaccine

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Influenza virus is a highly infectious respiratory pathogen causingdifferent level of morbidity and mortality world-wide.The current licensed vaccine is efficacious when the vaccine and circulating strainsare well matched. Therefore, we need to develope an effective Influenza vaccine that needs no changes every year. The Influenza Research Lab of Pasteur Institute of Iran organized and performed different research projects in this approach. Having conserved regions in some internal proteins of the virus makes themsuitable for a broader vaccine to match more strains of the virus. The nucleoprotein of the virus as a conserve proteinof the virus inducesprotectiveimmunity against a variety ofinfluenza virusesarising both humoral and cellular immunity.Influenza virosome is one of the commercially available vaccines that have been used for a number of years. An advantage of virosome over other influenza vaccine platforms is intrinsic adjuvanticity and potential carrier capability which have been exploited in this study to broaden vaccine protectivity by incorporating internal highly conserved viral nucleoprotein (NP) and matrix (M1/M2) proteins to provide heterosubtypic immunity. We have developed the influenza virsome with the capacity to be loaded with DNA as well as proteins. Antigenic as well as conserved proteins of the virus are expressed in different expression system to be incorporated in the virosome.
Keywords: Influenza, Virus, Vaccine

899. Design and Study of Laboratory Production of Intranasal Nanovaccine against of *Esherichiacoli* O157:H7

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A prototypical prevalent serotype of enterohemorrhagic Escherichia coli(EHEC),*E.coli* O157:H7 is an attaching and affacing(A/E) diarrheal pathogen that can cause wide ranging diseases in human from watery or bloody diarrhea to life-threating systemic complication including hemolytic uremic syndrome(HUS) which specified by hemolytic anemia,thrombocytopenia and renal failure. Most outbreaks of *E.coli* O157:H7 occurs via contaminated food or water.Cattles are frequently recognized as the primary and sighinificiant animal reservoir for *E.coli* O157:H7,thus this common sours most considered as the infection sours for EHEC. EspA,Tir and Intimin are key virulence protein factors that cause to (A/E) lesions in mammalian host cells. EspA induces a filumentous structure that form a bridge for translucation of Intimin receptor(Tir) into the host cell. Intimin defined as the first adhesion of EHEC that is essential for induce A/E lesions.In previous work chimeric recombinant EIT protein include of EspA, Intimin and Tir has been constructed and used as edible and injected EHEC vaccine. In this research we employed the recombinant EIT to design a protective EHEC intranasal nanovaccine.Chitosan known as a mucoadhesive biopolymer with interest characteristics for mucosal vaccine researchs such as able to opening tight junctions between epithelial cells. We employed the electrospinningtechnique as a novel method to obtain particles of recombinant EIT loaded chitosan, on a nanometer scale. Mice were immunized with intranasal administration or intraparenterally by recombinant EIT. After mice immunization, recombinant EIT specific immune responses (IgG and IgA) were measured by indirect ELISA. Nasally administration only elicited detectable and effective secretory IgA levels.Mice challenged with E.coli O157:H7demonstrated that this intranasal nanovaccine capable to reduction duration of bacterial fecal sedding and thus can develop an effective and protective strategy of *E.coli* O157:H7 vaccine.
Keywords:EHEC-Intranasal vaccine-Chitosan- Electrospinning technique –Secretory IgA

Poster Discussion Presentation

900. Evaluation of the Correlation between HBsAgSpecific Activity and the rDNA Hepatitis B Vaccine Potency

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Background: Quality control of recombinant Hepatitis B vaccines performed by the manufacturer and the National Control Laboratories (NCL) prior to registration and marketing vaccine batches, requires *in vivo* and/or a well validated *in vitro* potency assays as recommended by WHO technical series. Such an *in vitro* testmust demonstrate its suitability for monitoring the consistency of the vaccine production. The aim of this study was to establish the relationship between specific activity of the Activepharmaceutical ingredient and *in vitro* potency of rDNA hepatitis B vaccine. Specific activity is defined as Hbs Ag content by ELISA method divided to the total protein by Lowry methodmultiplied to 100.Materials andMethods: rDNAHepatitis B vaccine produced by Pasteur Institute of Iran (*PastoHep*) was used for this study. Both *in vitro* potency and Hbs Ag content were measured by ELISA-based methods.Lowry method was used for measuring the concentration of total protein in rDNA Hepatitis B vaccine, as well.Results:A significant relationship ($p<0.05$) was found between concentration of HbsAg and the final product *in vitro* potency. When Hbs Ag concentration determined by ELISA method was low in a vaccine batch, the specific activity of the API was shown to be decreased. Consequently, the vaccine batches manufactured fromthe API with the lower specific activityrevealed the lower potency determinedby ELISA method, as well.Conclusion: To bear the increasing costs for the vaccine development, techniques and parameters to reduce the number and the size of the invivo and clinical studies must be developed. Specific activity is one of the characteristics of hepatitis b vaccine which is not considered as a pharmacopealcharacteristics but it is very important parameter to confirm the quality of HbsAgintended to use in thevaccine formulation with an acceptable potencyresultwhich in turn will stimulate the immune system sufficiently.
Keywords: Correlation,HBsAg,rDNA Hepatitis B Vaccine Potency

901. Immuno-stimulatoryEffects of anElectroporation-delivered DNA VaccineEncoding HPV 16 E7Fused to the C-terminal of gp96 in a Mouse Model

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Background: Human papillomavirus (HPV) is present in more than 99% of cervical cancers. The association between HPV infection and cervical cancer indicates that HPV serve as an ideal target for development of prophylactic and therapeutic vaccines.DNA vaccines are an attractive approach to elicit antigen-specific immunity. Several strategies have been used to increase the potency of DNA vaccine including heat shock proteins (HSP) as an adjuvant and electroporation as a physical delivery system. In current study, we prepared DNA construct encoding HPV 16 E7 linked to C-terminal fragment of gp96and evaluated immune responses after delivering with electroporation in C57BL/6 tumor mice model.Materials and Methods: Directional cloning of E7-CT (gp96) was performed into pEGFP and pQE30 expression vectors.Large-scale purification of pEGFP-E7 and pEGFP-E7-CT (gp96) was prepared using an Endo-free plasmid Giga kit. Recombinant E7-CT (gp96) protein expressed in *E.coli* wasconfirmed by SDS-PAGE and western blotting and thenpurified by FPLC. The E7 and E7-CT (gp96) DNA delivery into COS-7 eukaryotic cells was done using the GFP reporter construct and PEI, *in vitro*. Finally, we evaluated the ability of fusion E7-CT (gp96) DNA vaccine to elicit E7-specific immune responses by ELISA. The tumor growth was monitored for two months.Results: DNA constructs encoding HPV16 E7 and E7-CT (gp96) were prepared in large scale with high purity. The COS-7 cells transfected with DNA constructs exhibited increasing levels of GFP fluorescence, indicating *in vitro* protein expression. The recombinant E7-CT(gp96) protein migrated as a 57 kDa protein in SDS-PAGE.Finally, the enhancement of immune responses and preventive efficacy of E7-CT (gp96) DNA vaccine delivered by electroporation weredetected in C57BL/6 tumor mice model.Conclusion: The utilization of electroporation along with HSPwould possibly improve the efficacy of DNA based cancer vaccines.It may represent a promising approach for therapeutic HPV vaccines in future studies.
Keywords:Immuno-stimulatory Effects, HPV 16 E7, gp96, Mouse Model

902. Generation and Using Stably*gfp-luc*Transgenic *Leishmania major* for*invitro* and *in vivo* Drug Screening

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Background: Three main forms of leishmaniasis are caused by three species of *Leishmania* (*major*, *infantum* and *brasiliensis*). *Leishmania*is a protozoa parasite that transmits from animals (dog and rodent) to human by sand fly. Still, there is no any perfect anti-leishmanial drug or vaccine against that. The important issue is in order to develop new drugs and/or precise disease follow up study in animal model is lack of a good assay for fast and efficient screening. Reporter genes such as green fluorescent protein (GFP) and luciferase (LUC) could be use as an excellent tool to drug screening, disease progressionstudying and parasite burden estimation *in vitro* and *in vivo*. Previously, we showed that fluorescent intensity measurement in both forms of GFP-transgenic *Leishmania*(amastigote and promastigote) and monitoring of these parasites in mice and macrophages is possible as well. Because each reporter gene has some advantages and limitation, fusion between two or more genes make a strong (or polyvalent) reporter gene that enable it to detect by more than one specific detector. Materials and Methods: In present study, to solve some obstacles of GFP such as background and increasing the sensitivity and potential of detecting parasites in screening assay, we generated a recombinant parasite line that express stably *gfp-luc* dual reporter gene. To do this, fused *gfp-luc* gene was cloned in a specific vector including two neighboring sequence of SSU 18S RNA locus. After transfection of fused gene integrated downstream of promoter of 18S rRNA gene through homologous recombination and transcribed by RNApolI. Results: Presence and correct integration site of gene was confirmed at DNA level (by PCR), expression of GFP and its fluorescence intensity (by western blot, Florescence microscopy and Flow Cytometry) in promastigotes. Also, expression of LUC in promastigotes (extracellular) and amastigotes (intracellular) forms was measured by luminometer. Conclusion: The obtained result revealed a clear relation between parasitemia and luminosence or GFP intensity.
Keywords:

903. Enhanced Immune Responses Multigene Regiment and GM-CSF Injection Combined HIV-1 tat, rev and rt Genes

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Background: Despite all efforts to control HIV infection, it is still one of the major killers all over the world. A powerful preventive vaccine is one of the most important concerns for HIV researchers. Despite intensive research, a suitable vaccine with enough stimulation of cellular, humoral and mucosal immune systems is not yet developed.Adenovectors are valuable tools for developing vaccines against various pathogens.In this study we evaluated multigene strategy with combinations of Ad5[E1-E3] vector expressing HIV-1 clade A gag, tat, rt, env with or without GM-CSF to induce immune response in mice.Materials and Methods: We used recombinant Adenovector-5 separately. The HIV-1 genes, sequence were amplified from HIV-1RNA (clade A) using nested polymerase chain strategy. The cDNA of each gene was cloned into a transfer vector. Transfer vector then co-transformed into *E.coli* strain BJ5183 together with pAdenovector ΔE1/E3. Recombinant viruses were purified using CsCl gradient centrifugation and titrated on 293 cell plates and then injected at 10^{12} viral particles into 6 groups comprised of 5 mice as a single injection. After 2 weeks, the Humoral responses were evaluated using ELISA and cellular immune responses checked by cell proliferation and ELISpot assay (IL-2, IL-4 and IFN-γ).Results:multiple strategies did not change single protein humoral and cellular immunity significantly

however it obviously increased total antibody titer.Moreover, rGM-CSF as an adjuvant enhanced immune response along with an increase in cytokine expression.Conclusion: it can be concluded that simultaneous use of multiple antigen and adjuvant can enhance both humoral and cellular immune systems. Choiceof antigenand the pattern of combination are very important in vaccine design.
Keywords: HIV vaccine, Adenovector, Multiple Antigen, rGM-CSF

904. Studying the Immunogenicity of PolytopicPeptide and Recombinant Core Protein as HCV Vaccine Candidates

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Background:There is not available vaccine against Hepatitis C virus (HCV); however, cell mediated immunity (CMI) seems to play a critical role in natural clearance of infection and prevents from developing chronic liver disease. In spite of thatpolytopic peptide(PP)immunization is anew promising strategy inducing CMI;there is not comparative study of PP and protein context in epitope-specific responses.Herein, we evaluated the level of induced epitope-specific response against a HCV-core derived epitope within the contexts of designed PP and recombinant core protein (HCV cp). Furthermore,the adjuvant affects of pluronic acid (F127) and BCG compared.Materials and Methods:PP containing two HCV derivedHLA-A2 and three H-2d –restricted epitopes of core 132-142(c) , E2405-414 (E), NS31405-1414(N), E2614-622(E) and core 39-48(C39) was synthetized in tandem of CENEC39. HCV cpoverexpressed in E. coli BL21-AI cells and was purified by affinity chromatography. BALB/c mice subcutaneously received 2 x 50 µg of PP orHCVcp that were mixedeither with F127 orBCG (Pasteur 1173P2 strain). CMI was analyzed against H-2d-restricted epitope of C39 using IFNγ/IL4 ELISpot and CTL assays.Results:Vaccinated mice promoted different levels of cellular response against c39 epitopic peptide that should be discussed based on complementary experiments in favor of epitope processing. Moreover, mice immunized with BCG-mixed immunogens obviously provoked stronger immunity.Conclusion:This study pointed towards the importance of epitope engineering within the context of polytopic peptides to enhance their immunogenicity and further confirmed the immunopotential effects of BCG as a valuable candidate adjuvant.
Keywords:HCV core, BCG, Polytopic peptide, Pluronic acid

905. Development of an Effective Delivery System for Nasal Immunization against Tuberculosis Antigens using Chitosan Nanoparticles: Immunological Studies in Mice

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Background: This study was designed for development of an efficient delivery system for novel fusion protein of *M. tuberculosis* based on ESAT-6 and HSP70 (E6H70). We started out using trimethyl chitosan (TMC) nanoparticles, which has been successfully used as a delivery system for mucosal vaccines and drugs. Materials and Methods:The recombinant E6H70 fusion protein was expressed in prokaryotic system and purified by Ni–NTA affinity chromatography. This protein was encapsulated in TMC solution (1 mg/ml) by ionic gelation method. E6H70 loaded nanoparticles were characterized for their morphology, size, zeta potential, loading efficiency, and in vitro release of protein. The immunogenicity of encapsulated protein was investigated in BALB/c mice after intranasal administration. For comparison, groups of mice were immunized intranasally with soluble free protein. Three weeks after last immunization cytokine production and cytotoxic responses were examined. Results: The mean particle size, distribution and zeta potential of nanoparticles were measured and determined as 308 nanometer and +21 mV. We found an elevated T cell proliferative response and IFN-γ, IL-4 and IL-12 production in animals who was immunized with E6H70 loaded nanoparticles. However, free E6H70 recombinant protein induced a significantly moderate level IL-12. Moreover, when the fusion protein was administered in the TMC nanoparticles, a strong level of cytotoxic T cell activity was induced in compare to other groups. Conclusion: Taken together, the role of chitosan nanoparticles as a suitable mucosal delivery system for induction of appropriate immune response was supported in our study. However, these data offers the potential of E6H70 fusion protein as a novel candidate for subunit vaccine against tuberculosis.
Keywords:*Mycobacterium Tuberculosis*, ESAT-6, HSP70, Chitosan, CTL, Cytokines

906. Comparison of Neutralizing Antibody Response using DNA Vaccine Encoding FMDV/O/IRN/2007 VP1 Gene and Conventional Inactivated Vaccine in Animal Model

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Background:Foot-and-mouth disease virus is highly contagious and responsible for huge outbreaks among cloven hoofed animals. The aim of the present study is evaluation of a plasmid DNA immunization system to express *FMDV/O/IRN/2007* VP1 gene and comparison with conventional inactivated vaccine in animal model. Materials andMethods:The VP1 gene was sub-cloned into the unique *KpnI* and *BamHI* cloning sites of the pcDNA3.1+ and pEGFP-N1 vectors to construct the VP_1 gene cassettes. The transfected *BHKT7* cells with sub-cloned pEGFP-N1-VP1 vector expressed EGFP-VP1 fugene protein and displayed green fluorescence spots more than the transfected BHKT7 cells with sub-cloned pEGFP-N1 vector. Results:The significant neutralizing antibody response was induced in the mice which immunized using plasmid vectors expressing VP1 and GMCSF genes (co-administrate), VP1 gene lonely and the conventional inactivated vaccine ($P<0.01$).Conclusion: The DNA vaccine encoding FMDV/O/IRN/2007 VP1 gene with GMCSF gene as molecular adjuvant induced humeral immunity less than conventional vaccine.
Keywords: Foot-and-mouth disease virus, Neutralizing antibody, DNA vaccine, VP1 gene

907. Evaluation of Tumor Necrosis Factor Alpha (TNF-α) gene expression in Immunized Cattle with two types of Bovine Theileriosis Vaccine preparation

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Background: Bovine tropical theileriosis is a tick-borne disease, caused by *Theileria annulata*. The vector ticks are of the genus *Hyalomma*. In Iran live attenuated *T. annulata* vaccine has been prepared from cultured schizont-infected lymphoid cells to protect cattle from tropical theileriosis for near four decades. The vaccine has been produced in tissue culture flasks; therefore for scaling up the vaccine, it is necessary to handle a large amount of glass tissue culture flasks. We proposed the use of aerobic bioreactor instead of using a lot of tissue culture flasks to decrease the risk of contamination due to minimizing the handling the flasks, reducing the needed operators, and increasing the overall volume of the final product. Materials and Methods: The schizont-infected lymphoid cell culture was optimized in aerobic bioreactor. Therefore, the produced vaccine in new system has passed the quality control tests. Finally, the product was used in parallel to conventional flask produced vaccine in susceptible calves and cattle for immunological assay. The mechanism of protective immunity against bovine theileriosis has been shown to be related to establishment of a specific cell mediated response. Delayed type hypersensitivity test "Theilerin test", and *in vitro* evaluation of TNF-alpha gene expression were used to examine the immune response of immunized calves and cattle with two produced vaccine

preparation. Results:The results of both two tests revealed the close findings in two examined groups. The relative TNF-alpha gene expression and DTH response were comparable in both two immunized groups. Conclusion: These findings as well as vaccine inoculation to exotic cow breeds in endemic regions with potentially the natural challenge test encourage us to shift the vaccine production in aerobic bioreactor system.
Keywords: Tumor Necrosis Factor Alpha, gene expression, Cattle, Vaccine

908. Cloning and Expression of *Leishmania infantum* LPG3G by the Iranian Lizard *Leishmania* System

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Background: A variety of recombinant protein expression systems have been developed for heterologous gene expression in prokaryotic and eukaryotic hosts. In this study, we used a new protein expression system based on the Iranian Lizard *Leishmania*, a trypanosomatid protozoan as a host for expression of LPG3 gene from *Leishmania infantum* strain MCAN/IR/96/LON-49. Materials and Methods: The *L.infantum* LPG3 gene was amplified and cloned into pLEXSY- hyg2 expression vector for integration into the small subunit of ribosomal RNA gene of Iranian Lizard *Leishmania* genome by electroporationin. Expression of rLPG3 was confirmed by western blot and immunofluorescence assay. Results: Diagnostic PCR showed integration of recombinant cassette into the parasite genome. Western blot confirmed expression and production of rLPG3 protein respectively. In addition, immunofluoresence analysis revealed staining of throughout the cytoplasm of transfected parasites, indicating that the protein was expressed. Conclusion: This study showed that *Leishmnia* cells can be used as a useful biotechnological protozoan for expression of recombinant proteins such as LPG3 to further research in vaccine designing against Leishmaniasis.
Keywords: LPG3, *Leishmania infantum*, Iranian Lizard *Leishmnia*, Eukaryotic expression vector, Immunofluorscence assay

909. Evaluation of Specific Immune Response of Mice after Intranasal Immunization with *Mycobacterium tuberculosis* ESAT-6 Antigen

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Background: The majority of pathogens, such as *Mycobacterium tuberculosis*, initiate infection by interacting with host mucosa. Thus, delivery of vaccines through the mucosal route is considered as a non-invasive and efficacious strategy for the induction of immune responses. In this study, we demonstrate the proof of concept to develop a needle-free mucosal immunization protocol using a chitosan nanoparticles consisting of *M. tuberculosis* ESAT-6 antigen. Materials and Methods: Chitosan nanoparticles Consisting of ESAT-6 antigen prepared by ionic gelation method and it`s charactriztion were investigated by TEM microscope and zeta sizer. Protein encapsulation and release measured by micro BCA protein assay. Flowing three times of nasal immunization of Balb/C mice with ESAT-6 antigen in nanoparticles or native forms, serum levels of specific antibody was measured by indirect Elisa. Two weeks after last immunization The levels of IFN-γ and IL-10 released from cultured lymphocyte were investigated. Results: It was shown that ESAT-6 loaded chitosan nanoparticles were formed efficiently with the average size of 242 ±10 nm and zeta potential of 29±3 mV. After three times of nasal immunization, strong response of IFN-gamma and IL-4 were detected in ESAT-6 loaded in chitosan nanoparticles. However, this group shows a greater titer of specific IgG antibody in compare to who received ESAT-6 without chitosan. Conclusion: These results demonstrated that chitosan nanoparticles could be considered as an effective vaccine delivery system with adjuanicity and could elicit great humoral and cell-mediated immune responses.
Keywords:Immune Response, Intranasal Immunization, *Mycobacterium tuberculosis*, ESAT-6 Antigen

Poster Presentation

910. Immunogenicity of a Local MMR Vaccine in 1-1.5 Year Old Iranian Children, a Prospective Study

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Background: The prevalence of measles, mumps and rubella(MMR) diseases has declined dramatically over recent years as a result of universal immunization programs. The present study reports on the immunogenicity of a local MMR vaccine in Iranian children vaccinated at 12 and 18 months of age.Materials and Methods: In this prospective study, 104 children aged 12 months were administered with two doses of MMR vaccine manufactured by Razi Institute of Iran at 6 months time interval. Blood samples were collected 2 and 6 months after the first dose and 2 months after the second dose administration. Immunogenicity of the vaccine was evaluated by measurement of specific serum antibodies using commercial ELISA kits. Results:Two months after the first dose injection, 52.9% of children were seroconverted (> 150 mIU/ml) against measles. The rate of seroconversion increased to 53.8% and 97.1%, 6 months after the first dose and 2 months after the second dose injections, respectively. For rubella, the seroconversion rate (> 4 IU/ml) was found to be 13.5%, 17.3% and 44.2% for the time intervals mentioned above. The seroconversion rate of mumps (> 20 RU/ml) was 69.9%,2 months after the first dose injection and 60.6% and 97.8%,6 months after the first dose and 2 months after the second dose injections, respectively. Conclusion: Immunogenicity of the measles and mumps components of the MMR vaccine seems to be similar to many other validated vaccines reported in the literature, but comparison between immunogenicity of the rubella vaccine component with the results reported for other commercial counterpart vaccines indicates a lower seroconversion rate for the local vaccine employed in this study.
Keywords: Vaccine, Measles, Mumps, Rubella, Children, Immunogenicity

911. Evaluation of the Effects of New Formulation of *Leishmaniamajor*Antigen Post Challenge in Balb/c Mic

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Background: Cutaneous leishmaniasis (CL) is a zoonotic disease transmitted between rodents and canines,mainly by phelebotomus sand flies. In southern Iran, the incidence of this protozoan disease has doubled over the last decade.Human leishmaniasis is distributed worldwide, but mainly in the topics and subtropics, with a prevalence of 12 milion cases and an approximaite incidence of 0.5 milion cases of VL and 1.5 milion cases of cutaneous leishmaniasis (CL). The aim of this study was to compare the protective effects of a candidate cocktail vaccine encoding various *leishmania major* antigens in highly susceptible (Balb/c) mice after challenge with live leishmania. In this regards we selected two previous study's successfully doses (100 & 200 µg/o.1ml), three injection groups: Leishmania plus BCG (LB), Leishmania plus new adjuvant (TeucriumPolium)[LT], leishmania plus BCG and TeucriumPolium (LBT),and one susceptible mice(Balb/c) and measure expansion of white pulp size after challenge with live leishmania. Materials andMethods: Anew formulation antigen was evaluated in susceptible mice (Balb/c).*Leishmania major*promastigotes was cultured and harvested at different growth stages, and a cocktail maide from the harvested organisms. The preparations were tested for sterility and contamination. Five different methods were utilized to produce a crude antigen preparation, previously. The protein levels of the antigen preparations were measured using Lowry method, and the antigens were intradermally

injected to Balb/c mice. After first injection we have two same booster doses which its interval was one week. Past20 days after third leishmania injection or second booster dose, challenge is caring out with live Leishmani. The protective response was evaluated by the challenge and almost three times for every week all mice. Evaluated for inducing lesion, and survival and anothercritical signals,over 70 days. After this time all mice were euthanized with diethyl ether, and spleens were removed. Histological sections were prepared, stained with hematoxylin and eosin and changes to the splinic white pulp (SWP) were studied microscopically. Results: Compared with control groups, there was a change in white pulp structure. The size increases of the SWP were dependent on the injection group .There was a remarkable expansion of lymphoid follicles in the treated groups in Balb/c mice. Conclusion: This new formulation antigen is able to stimulate and expand the lymphoid constituents of spleen tissue after challenge with live leishmania. The SWP is where immune responses and antibodies are produced .Therefore, the effect of antigen preparations on secondary immune responses,adaptive immunity,and antibody production is important in determining the susceptibility of mice to cutaneous leishmaniasis and the induction of immunity and subsequently achieve productivity encounter to challenge with live Leishmania major.

Keywords*: Leishmania major*antigen, challenge, spleen

912. Safety Evaluation of DNA Vaccines: Integration into the Host Genome and Bio-distribution in Reproductive Organs Following Different Delivery Routes

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Background: DNA immunization with plasmid DNA encoding bacterial, viral, parasitic and tumor antigens has been reported to trigger protective immunity. The use of plasmid DNA vaccination against many diseases has produced promising results in animal and in human trials. However, there are several safety concerns about the use of a DNA vaccine which include the possibility of integration into the host genome and the elicitation of adverse immune responses. Materials and Methods: In this study, we examined the potential integration and bio-distribution of pcDNA3.1+PA, a new vaccine against anthrax with GenBank accession number: EF550208 encoding the PA63 gene, in reproductive organs of mice, ovaries and uterus in female and testis in male mice. Animals of both sexes were injected with pcDNA3.1+PA in three different delivery routes, intramuscular, intradermal and subcutaneous. Host genome integration and tissue distribution were examined using PCR and RT-PCR methods, twice a month during 6 months. Results: The results confirmed that pcDNA3.1+PA was not integrated into the host genome and they could not enter to reproductive organs. Indeed, these study shows that the pcDNA3.1+PA is safe to use. Conclusion: This finding has important implications for the use of pcDNA3.1+PA vaccine and opens new perspectives in DNA vaccine area.

Keywords:DNA Vaccines, Host Genome, Bio-distribution, Reproductive Organs

913. Non-allele specific Humoral Immunity to PfMSP-1_{19}: Implication for Multivalent Malaria Vaccine Design

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Background: Development of an effective vaccine against *Plasmodium falciparum* malaria has been a main goal for malaria elimination and eradication program. Since genetic diversity in protective antigens is responsible for challenging in development of an effective malaria vaccine, having information on vaccine candidate antigens such as Merozoite Surface Protein-1 of *P. falciparum*(PfMSP-1_{19}) genotype and study on specific immunity to its variant forms in malaria endemic areas are important for selection which allele to be included in multivalent vaccines. Therefore the main objective of this investigation was to determine the frequency of PfMSP-1_{19} variants followed by investigation of cross-reactive antibody responses to these antigens. Materials and Methods: To define PfMSP-1_{19} variant forms, 50 blood samples from *P. falciparum* infected individuals were collected from Sistan & Baluchistan province during 2006-2008. Genotyping was performed in samples using sequence analysis. Based on sequence analysis 4 GST-PfMSP-1_{19} (E/TSR/L, E/TSG/L, E/KNG/F and Q/KNG/L) variants were expressed in *E. coli* and IgG specific antibody to these antigens was evaluated by using ELISA. To determine the cross-reactivity of antibodies against each variant in *P. falciparum* infected human sera, immuno-depletion ELISA was performed. Results: Genotyping of PfMSP-1_{19} gene showed 4 distinct variants, among which Q/KNG/L (26%) and Q/KNF/F (26%) were the predominant haplotypes. In ELISA assay, the prevalence of IgG antibodies to all PfMSP-1_{19} variant forms was equal (84%) among patients' sera. Results of Immuno-depletion ELISA showed the induction of cross-reactive antibodies against different variants of PfMSP-1_{19} since the sera depleted with one antigen did not have positive response to any of four examined variant forms.Conclusion: The present results showed the presence of cross-reactive antibodies to PfMSP-1_{19} variant forms in patients and as a result, sequence polymorphism of PfMSP-1_{19} is of minor importance. Therefore, one of the examined variants could be sufficient to be included in PfMSP-1_{19}-based vaccine. Keywords:Humoral Immunity,PfMSP-1_{19}, Multivalent Malaria Vaccine

914. Live Recombinant *leishmania*Vector Expressing HPV16 E7 linked to N-terminal Fragment of gp96 as a Candidate Vaccine against HPV

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Background:High risk *humanpapillomaviruses* (HPV) such as HPV 16 andHPV 18 areassociated with the development of cervical cancer.The HPV E6 and E7 oncoproteins are ideal target antigens for a therapeutic vaccine since these proteins are constitutively expressed in cervical cancer cells. Various live bacterialand viral vectors have been used in HPV therapeuticvaccines. In recent study, we focused on generation of a novel, non-pathogenic live recombinant HPV vaccine, *Leishmania tarentolae* (*L. tarentolae*) expressing E7 linked to N-terminal fragment of gp96 as an adjuvant.Materials andMethods: The E7-NT (gp96) was cloned into the *Leishmania* expression vector (pLEXSY-neo2). Then, promastigotes of *L. tarentolae* were transfected with linearized pLEXSY-E7-NT(gp96) by electroporation. The clones highly resistant to neomycin were selected on Noble agar plates and further propagated in liquid culture medium. Confirmation of genomic integration was done by diagnostic PCR using genomic DNA of transgenic strains as template. Protein expression was analyzed by SDS-PAGE and western blotting. Finally, two mice groups were immunized subcutaneously with live recombinantpromastigotesexpressing E7-NT (gp96)andE7, respectively. The control groups were injected with PBS and *L.tarentolae*. The humoral and cellular immune responses were evaluatedin C57BL/6 tumor mice model. Tumor volumes were measured for two months following tumor challenge.Results: The data showed that the linearized expression cassette containing the encoding region for E7-NT (gp96) integrated into the chromosomal *ssu* locus of *L. tarentolae* through homologous recombination. Western blot analysis revealed E7-NT (gp96) stable expression in *L. tarentolae*. The recombinant *L. tarentolae*could significantly enhance humoral and cellular immune responses in comparison with control groups.Conclusion: The successful expression of the full-length E7-NT (gp96) protein was observed in the non-pathogenic *L. tarentolae*. This novel live recombinant vector may represent a promising approach for improving the effectiveness and safety of candidate live vaccines against HPV infections.

Keywords: Recombinant *leishmania* Vector, HPV16 E7, gp96, Vaccine

915. Evaluation of Immune Response to MMR Vaccine in Iranian Children Nonresponders to DTP Vaccine

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Background: Vaccination with multiple bacterial and viral vaccines may result in modulation of the immune response to some vaccine components. In this study, the immunogenicity of viral MMR vaccine was assessed in 18 months old children who failed to mount antibody response to the whole cell pertussis component of the bacterial DTP vaccine. Materials and Methods: In this prospective study, 114 children who had received 3 doses of DTP vaccine within six months after birth, and were nonresponders to pertussis component of the vaccine, received the first dose of MMR vaccine at 12 months and subsequently received their 4th dose of DTP vaccine, when they were 18 months old. The antibody response to DTP and MMR vaccines was evaluated 1-2 months after administration of the last dose of DTP and MMR vaccines. Serum antibody levels were measured using commercial ELISA kits. Results: After administration of the 4th dose of DTP vaccine, 61 children were nonresponders and 53 children developed immune response to pertussis. Comparison of the antibody response to MMR vaccine between responders and nonresponders to DTP vaccine, showed that there were no significant differences between antibody titers against measles, mumps and rubella between the two groups. The geometric mean titers (GMT) of antibodies produced against measles, rubella and mumps were 1571.27 mIU/ml, 19.17 IU/ml and 61.38 RU/ml in nonresponders and 1428.53 mIU/ml, 28.14 IU/ml , 40.91 RU/ml in responders to pertussis vaccine, respectively. Conclusion: The immune response to MMR vaccine was similar in responders and nonresponders to whole cell perussis vaccine, implying that lack of response to the bacterial pertussis vaccine is antigen specific and does not negatively influence the immune response to the viral MMR vaccine.

Keywords: Immune Response, MMR Vaccine, DTP Vaccine

916. A New Perspective on Vaccine Design

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Background: vaccination has proven to be the most successful medical intervention ever developed. However, the vaccines that are currently available still fail to protect against certain pathogens. These failur consequences of vaccination have received a considerable amount of attention, both from an empirical and a theoretical standpoint. Our theory is about bacteria that have a strong immune-stimulating antigen but due to low expression or non-surface expression of an antigen cannot be considered as a candidate vaccine. This theory is based on a vaccine designed to have three advantages:(a) because large amounts of these antigens are exposed to immune system so the immune system responds to them. (b) These antigens are separated from bacteria and act as a secretory antigen that immune system does its best response to them. (c)If antigen be a good immunogens, can eliminate the needs for adjuvant.We'll practice this theory on *legionella pneumophila*. *L. pneumophila*, the etiological agent of Legionnaires' disease, is an important cause of both community-acquired and nosocomial Pneumonia. The cell envelope of *L. pneumophila* has several interesting antigens, including 19 kDa peptidoglycan associated lipoprotein (PAL) protein. The PAL protein is an immunodominant component of *L. pneumophila* and is a highly conserved antigen among Legionella species. Materials and Methods: According to our theory the antigen should be cloned, purified and compound with Killed Bacteria (KB) in different concentrations of antigen. Because immunogenic doses of KB without Purified antigen stimulate immune responses efficiently, we purpose a sub-immunogenic dose of KB, as a model vaccine, to evaluate the immunogenic activity of the antigens. Results: About *L. pneumophila* the pal gene of *L. pneumophila* serogroup 1 was amplified with specific primers, cloned and expressed under *pelB* signal sequence and T7 lac promoter in pET26b+ plasmid. The cloning was confirmed with digestion and sequencing of recombinant pET- 26b-pal plasmid. The expression of r-PAL protein in cytoplasm and periplasmic space of *E.coli* was approved by SDS-PAGE and western blotting. Conclusion: We will expect combination of r-PAL antigens and Killed Legionella pneumophila (KLP) have a good immunogenic effect compare to KLP or r-PAL antigens separately.

Keywords: vaccine design, recombinant pET- 26b-pal plasmid, Legionella pneumophila

917. Influenza Vaccination in Pregnant Women: a Necessity or Advice?

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Background: Women are at increased risk for morbidity and mortality from influenza during pregnancy. Vaccinating pregnant women for influenza can protect both the women and their infants. It is recommended inactivated influenza vaccine for all women who are pregnant during influenza season, regardless of trimester. In this study, the efficacy and importance of the Influenza vaccine is reviewed in pregnant women.Materials and Methods:It was written with searching keywords such as Influenza vaccine, pregnancy and efficacy in databases include pubmed and google scholar.Results: One potential approach to protecting young infants against influenza infection is to vaccinate their mothers during pregnancy. The studies support the possibility of protecting the offspring against influenza by immunization of the mother. IgG cross the placenta via active transport from the mother to the fetus, particularly in the final weeks of pregnancy. Additional IgA is transferred from the mother to the infant via breast milk. Some studies show that inactivated influenza vaccine given to pregnant women is highly effective (91.5%) in preventing hospitalization.Other results also reveal high effectiveness trivalent inactivated vaccine (TIV) in pregnant women.Conclusion:According to previous studies TIV is recommended for all pregnant women except for persons with a serious allergy to egg protein. So It is benefit that Iran Ministry Health informs for pregnant women Influenza vaccination in fall and winter season and administer them free to reduce morbidity and mortality during pregnancy.

Keywords**:** Influenza vaccine, pregnancy and efficacy

918. Immune Characterization against a Mini Vaccine Candidate from HIV-1 after Co-administration with *Pseudomonas aeruginosa* FLiC Molecule: Role of Bioconjuagation on Vaccine Immunogenicity

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Background: Since past decades, HIV infection has been one of the most important factors for threatening global health. An effective vaccine candidate against HIV-1 is highly demanded. The majority of antigen vaccines that are currently under investigation include recombinant molecules that are very pure or subunit vaccine from pathogens. Due to weak immunogenicity, these vaccines could not induce strong immune responses. Therefore, application of adjuvant is useful for increasing vaccine efficacy. Recent studies indicate that Flagellin has adjuvant activity and can induce immune responses by TLR5 engagement and thereby can improve the immunogenicity of some vaccine candidate. In the present, we compared the adjuvant activity of FLiC molecule in conjugated and mixture form with a peptide vaccine from HIV-1 P24-Nef. Materials and Methods: Balb/c mice (n=6) were immunized intradermally with 20 μg of HIV-1 P24-Nef peptides in both conjugated and mixture form with FLiC, adjuvanted in Montanide ISI-70, three times with 2 weeks intervals. Two weeks after the last immunization, lymphocyte proliferation was measured with Brdu, IL-4 and IFN-γ cytokines with ELISA, total antibody and IgG1, IgG2a isotypes with indirect ELISA methods. Results: Immunization of mice with HIV-1 P24-Nef conjugated and mixture form with FLiC molecule led to a significant increase in lymphocyte proliferation and IFN-γ cytokine responses. Total antibody titer increased in both conjugated and mixture form, although not significantly.

Conclusion: FLiC molecule could be used as adjuvant in Conjugated and mixture form with vaccines candidate against HIV-1, and Conjugation of vaccine candidate is more potent for increasing vaccine immunogenicity compared to mixture form.
Keywords: Mini Vaccine, HIV-1, *Pseudomonas aeruginosa*, FLiC Molecule, Bioconjuagation

919. Evaluating the Adjuvant Activity of Novel Adjutants *Astragalus Adscendens* Extract in Mice Compared to Quil A and Allum

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Background: Objectives- adscendens is a perennial plant of family *Astragalus* that its polysaccharide extract is usually used as a popular sweetie in Iran for years called GAS ANGABIN. Several studies demonstrated that *Astragalus* membranous extracts could be safely used as an adjuvant with low or non-hemolytic effect. As there is no study assessing such potential ability in *Astragalus Adscendens* the current study was designed to evaluate the hemolytic and adjuvant activities of this plant in mice. Materials and Methods: 4 groups of ICR mice were subcutaneously immunized with OVA 100 µg alone or OVA and *Astragalus* extract (ASE as a new adjuvant), QuilA and Allum on Day 1 and 15. Two weeks later (Day 28), concanavalin A (Con A), lipopolysaccharide (LPS) and OVA-stimulated splenocyte proliferation and OVA-specific antibodies in serum were measured. Hemolytic activities of ASE was evaluated using 0.5% rabbit red blood together with its adjuvant potentials on the cellular and humoral immune responses at both 100 and 200 µg/ml doses. Results: ASE showed no hemolytic effect, at the concentration of 100 and 200 µg/ml. ASE significantly enhanced the Con A, LPS, and OVA-induced splenocyte proliferation in the OVA-immunized mice at both doses of 100 and 200 µg. The IgG total and IgG sub-class responses in the serum of mice were significantly higher using ASE as adjuvant compared with QuilA, Allum and control group. Conclusion: This study demonstrated that ASE has a considerable adjuvant activity with non-hemolytic effect at both 100 and 200 µg/ml doses superior to Allum and QuilA.
Keywords:*Astragalus Adscendens*, Extract, Hemolytic, OVA, Adjutants

920. Investigation Protection against *Pseudomonas aeruginosa* white an Alginate –ExotoxinA conjugate vaccine

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Background: One of the serious problems worldwide in hospital infection is control and prevention from *Pseudomonas aeruginosa* infection. Drug therapy especially with approving of Antibiotic because of appearing resistance is failed. So, using an effective vaccine against all different serotypes is one of the solutions to overcome this problem.Materials andMethods*: Pseudomonas aeruginosa* strains were isolated from pulmonary patients suffering to Zanjan city. Mucoid strains of this bacterium were isolated after assistance test. Alginate was isolated by cold ethanol precipitation, dialysis, proteinase and nuclease digestion ultracentrifuge and chromatography.To increase immunogenicity ,Alginate was join to ExotoxinA with ADH as a spacer and EDAC as a linker .Assistance tests losting of poisonousness antigen, taking fever in rabbit, toxicity and sterility is also done. After molecular evaluation, protective activities and safely of the Alginate were determined in mouse models.Results: present resistance indicates that, purified Alginate of *Pseudomonas aeruginosa* considerable protective activity in Balb/c mice. Plus, it was no pyrogen and did not produce any detectable abnormal toxicity in rabbits and mice.
Conclusion: the results showed that conjugate of ALG-ExoA because of protection true form can be used as a profit and protective immunogenic in vaccine therapy against infections caused by *Pseudomonas aeruginosa* .
Keywords:*Pseudomonas aeruginosa*,Alginate,vaccine

921. Preparation of Sodium Alginate Microsphere Containing Leptospiral Antigen as a New Vaccine in Pilot Scale

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Backgrounds: Leptospirosis is a world-wide zoonosis caused by the genus *Leptospira*. Leptospiral vaccine has been used as effective vaccine to elicit specific antibodies for protection. Vaccine failed to induce long-lasting immunity. Thus, there is a need of more effective vaccine that not only elicits immunity across the heterologous *Leptospira* spp. serovars but also induces long-lasting immunological memory. During decade scientists have been focused in the design of a better way for antigen and drug delivery in animal and human that could increase immune response and decrease side effects. They discovered use of polymeric microspheres for an appropriate vaccine. In this research we tried to find the best microsphere with consideration to the size and morphology and loading leptospiral antigen as a new vaccine in pilot scale. Materials and Methods: The preparation method of alginate microspheres was emulsification method. An aqueous solution containing sodium-alginate was dispersed in an iso-octane solution containing a lipophilic surfactant (Span85) by using a mechanical stirrer. An aqueous solution containing an hydrophilic surfactant (tween85) was then added and the emulsion was stirred. A calcium chloride solution (8.0%w:v) was added and the dispersion was mixed. After centrifuge, the sediments were aggregated. The alginate concentration (3, 3.5, 4%w:v), iso-octane solution concentration (0.15, 0.2, 0.3 %v:v) and Tween 85 (3, 3.75 , 4.5 %v:v) were studied. After preparation the best concentration of each material has been choosen. Optical microscope has been used for evaluation the shape and mastersizer 2000 to determine the size of microspheres. After centrifuge and lyophilization, weightening showed the yield of each batches. Microscopical studies define the best shape and dispersion. Mastersizer(particle analyzer) determined the diameter of each microspheres. Results: After analysis the batch with these properties has been selected: yield: 1.1mg in 39ml of soloution, concentration: alginate 3.5%w:v, iso-octane 0.2%v:v, tween 3.75% v:v and diameter(0.9): 548.128 µm(Vol. Weighted Mean(4,3) D:293.96). Conclusion: To prepare a better vaccine for leptospirosis encapsulated antigen could be used. In this study we chose the best concentration of three solution for encapsulation with the analysis that are above and in the future studies we are going to load leptospiral antigen to this microsphere that has high yield and good diameter.
Keywords: sodium alginate, microsphere, vaccine, *leptospira*, pilot scale

922. Subunit Vaccine against Influenza Viruses: AVaccineBased-on *Influenza Virus M2 Protein and Leishmania major HSP70 Gene*

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Background Influenza is a major cause of morbidity and mortality each year in the world. Vaccination has been available since the late 1960s.Influenza is widely recognized as an important target for prevention by vaccination because of the considerable yearly burden of death, hospitalization and medically attended illness associated with influenza epidemics.Nowadays, researchers are focusing on conserved antigens such as matrix protein 2(M2) for influenza vaccine development. The M2 protein is a proton-selective ion channel, integral in the viral envelope of the influenza A virus and allows the virus to enter and cause an infection in the host cells. M2 is conserved among influenza A subtypes. Vaccines based on these proteins offer the potential for increasing the breadth and duration of protection against diverse subtypes. Immunization with purified M2 protein has also been shown to ameliorate infections in animal and elicit cross-protective antibodies in a murine model. In this study M2 protein fused to leishmania major heat shock protein 70 (HSP70) to prepare an effective vaccine against influenza A viruses. The HSPs are an important part of the cell's machinery for protein folding, help to protect cells from stress and induce cellular and humoral immunity.Materials andMethods: The gene of influenza matrix protein 2(M2) was amplified by PCR using specific primers, digested by appropriate Enzyme, and then cloned into expression plasmid pQE30 upstream of Leishmania major HSP70. The resulting plasmid pQE30-M2-HSP70 was transformed into E.coli (M15) and a single colony of transformants was inoculated in LB broth containing 50µg/ml ampicilin and

50µg/ml kanamycin. Expression was induced by addition of IPTG 1mM, and then confirmed by SDS-PAGE and western blot analysis. Result and Conclusion: The result of sequencing revealed that the M2 gene was properly cloned into pQE30-HSP70 in the right frame to 6xhis tag. The confirmed construct by then expressed in E.coli (M15) and determined by SDS-page and Western blot analysis. The chimer protein will be purified by Ni-NTA column chromatography and evaluated in animal models. In influenza infection, CD8 cytotoxic T lymphocytes (CTL) recognize epitopes from internal protein M presented on MHC class I molecules. Depending on their antigen specificity, CTLs may be subtype-specific or, in case they recognize internal antigens, broadly cross-reactive with influenza A. Animal experiments using adoptive transfer of CTLs revealed their proliferation and migration pattern during infection and their potential in mediating recovery from influenza infection. Influenza infection results in the weakly systemic production of antibody to M2 proteins such as IgG subclasses. It has been shown that binding of HSP to the desired antigen induce increased level of immune responses. Hence the purified chimer protein prepared in this study could be an appropriate vaccine candidate to prevent influenza infection.
Keyword: Influenza virus A, M2-HSP70 protein, subunit vaccine

923. Cloning, Sequencing and Expression of *helicobacterpylorihpaA* gene in *E. coli* host with vaccination target

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Background: *Helicobacter pylori* is a widely distributed gram negative bacterium that infects the human stomach and duodenum. *HpaA* is a *Helicobacter pylori* specific lipoprotein that has been shown to be an effective protective antigen for mucosal vaccination against *H. pylori* infection in mice. *hpaA* of *helicobacter pylori* as a vaccine antigen is fully competent for stimulation of immune responses and here we report the expression of the protein in a recombinant form. Materials and Methods: The *hpaA* gene was inserted into pET28a (+) as cloning and expression vectors respectively. The recombinant plasmid (pET-hpaA) was subjected to sequencing other than PCR and digestion analysis. Protein expression was induced by adding 1mM isopropyl-β-D-thiogalactoside to cultures of *E. coli* strain BL21 transformed with pET-hpaA. Protein expression assessed with SDS-PAGE analysis. Results: The restriction endonuclease digestion, PCR amplification analysis showed that the *hpaA* gene of 730 bp was amplified from *helicobacter pylori* DNA and Sequencing analysis of the pET-hpaA confirmed the cloning accuracy and in frame insertion of *hpaA* fragment. SDS-PAGE analysis showed the expression of an approximately 29000 Dalton protein. Conclusions: sequencing results along with SDS-PAGE analysis confirms the expression of recombinant hpaA in the heterologous *E.coli* BL21.
Keywords: recombinant hpaA, *helicobacter pylori*, *E.coli* BL21, SDS-PAGE

924. Mixture of Naloxone and Alum Induced Cellular and Poly-isotypic Humoral Immune Response for a Peptide Vaccine against HIV-1

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Background: Following the inefficacy of AIDS vaccines, some new approaches have been suggested by utilizing adjuvants for increasing the immunogenicity of vaccines. In previous studies, the adjuvant effect of Naloxone on the HSV1, Killed-Listeria Monocytogenese and Salmonella Typhimurium vaccines has been confirmed. In the present study, for the first time, the adjuvant effect of Naloxone in combination with HIV1-P24-Nef fusion peptide as a vaccine model has been evaluated. Materials and Methods: Female Balb/c mice were divided into five groups. Group 1 was immunized subcutaneously on the day 0 with 20 µg of P24-Nef fusion peptide with Alum adjuvant and 6mg/kg of Naloxone, and on the days 21 and 42, they were boosted with the final volume of 200 µl of vaccine. Group 2 was immunized with fusion peptide and alum. The control groups received Naloxone, alum and PBS under the same conditions, respectively. At the day 42, the proliferative responses of lymphocytes and the secretion rates of IL-4 and IFN-γ cytokines were determined using MTT method and commercial ELISA kit, respectively. Also the quantity of IFN-γ producing lymphocytes was evaluated by ELISPOT method. Finally, the specific antibody titers as well as IgG1, IgG2a, IgG2b, IgG3 and IgM isotypes were assessed by ELISA method. Results:Immunization of mice with Naloxone led to a significant increase in the proliferative responses of lymphocytes, IFN-γ cytokine and total antibody titer with poly-isotypic form in comparison with the control groups. Conclusion: The results suggest that Naloxone drug could be useful in HIV vaccine research.
Keywords: Naloxone, Alum, Humoral Immune Response, Peptide Vaccine

925. Immunity to Hepatitis B Vaccine amongHealth Care Workers

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Aim: The aim of this study was to determine the level of anti-HBsAg (hepatitis B surface antigen) in vaccinated high risk group.Materials andMethods: We measured anti-HBsAg concentration in blood sera of adult students aged from 19 to 37 years old. Five milliliters (5ml) of blood sample was taken from 210 cases four months after the second dose and 126 out of 210 cases three months after the third dose of hepatitis B vaccination. All blood samples were analyzed for anti-HBsAg by ELISA method.Results: 125 out of 210 samples (59.5%) showed anti-HBsAg concentrations higher than 20mIu/ml and considered immune after the second dose of hepatitis B vaccination. Also, 99.2% of samples had anti-HBsAg higher than 20mIu/ml three months after the third dose of the vaccination. Non-immune cases in males were more than females (41.2% vs.40.1%).Conclusion: our results reinforce the importance of hepatitis B vaccine in adolescents and suggest that three dose of hepatitis B vaccine is necessary to increase the seropositive rate of anti-HBsAg in adults.
Keyword:HBsAg, Vaccination, Yazd student

926. Immunogenic Potentials of *Acinetobacterbaumannii*Phospholipase D; An *in silico*Approach

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Background*: Acinetobacterbaumannii*is an emerging bacterial pathogen of considerable health care concern. However, little is known about the organism's virulence factors. During the evolution, microbes develope different mechanisms topenetrate into the host cells. One of such mechanisms is the production of enzymes which destroy cell membranes. The term "phospholipases" is referred to a heterogeneous group of enzymes capable to hydrolyze one or more ester linkage in glycerophospholipds. According to the specific bond cleaved in the phospholipid molecule they are indicated as A, B, C and D. With the increasing bacterial resistance to multi drugs and evolution of new strains, there is a need to attend the matter urgently and find appropriate vaccine candidates. Materials and Methods: In this study, keeping the above in mind,protein sequence availability and similarity search was done. Related sequences obtained from genebank, were aligned. Secondary structure prediction and 3D structure modeling was done after appropriate sequence selection. The humoral and cellular immunity induction potential was analyzed with different bioinformatics tools, in order to assess immunological points.Prediction of allergens and mapping of IgE epitopes were also carried out.Results: Antigenicity and solubility predictions showed that phospholipase D possess plentiful B cell epitopes in both linear and 3D structure. Thus it represents effective antigen for antibody production.This protein had the potential to induce CD^{4+} and CD^{8+} immune responses against the pathogen. In addition it is not allergent. *In-silico* analysis revealed that phospolipase D of *Acinetobacterbaumannii*can serve as an appropriate target for development of a novel class of vaccines with divergent mechanism of action.
Keywords: *Acinetobacterbaumannii, in silico,* Phospholipase D

927. Co-administration of Interleukin 12 Gene Adjuvant Can Boost Immune Response of Hepatitis C Core DNA Vaccine

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Background: Hepatitis C viral infection is the major cause of acute hepatitis and chronic liver disease and remains the leading cause of liver transplants. but an effective vaccine is not yet available. DNA vaccines represent a promising means for HCV vaccination because they tend to induce a Th1-biased cell-mediated response in the host cell. Since the strength of the immune responses induced by DNA vaccines has been relatively weak, it is necessary to develop novel methods for circumventing this limitation, such as codelivery of novel cytokine adjuvants. Thus, immunostimulatory cytokines as interleukin IL-12 has been studied as genetic adjuvants. Materials and Methods: In the present study, we are going to administrate HCV Core DNA vaccine with Interleukin 12 (IL -12) adjuvant, then evaluate cell immune response. For this purpose, we were inserted gene of HCV Core gene into pCDNA 3.1 eukaryotic expression vector. Female C57BL/6 mice were immunized intramuscularly with three doses of 90μg DNA vaccines on Days 0, 14, and 28. Two weeks after the last immunization, HCV specific cytotoxic T lymphocyte (CTL) activities were measured by LDH CTL cytotoxicity assay, MTT Lymphocyte proliferation assay. IFN – γ and IL - 4 cytokine assay were detected by ELISA assay. Results: Obtained results showed enhanced lymphoproliferative response and cytotoxic T-lymphocyte activity compared with negative controls. LDH, MTT and cytokines assay demonstratedthatthe co-injection of IL-12 can enhance immune responsesof HCV core DNA vaccine alone. Conclusion: Our study demonstrated that administration ofIL-12 adjuvantwith core gene enhancedcellularimmuneresponsesinmice. The study warrants further investigation as a potential vaccine against HCV infection so Intramuscular co-injection HCV Core-IL-12 DNA vaccine induced strong and significant cellular immune responses in mice.
Keywords: Hepatitis C virus, DNA Vaccine, Core, IL-12; C57BL/6.

928. Comparison between Episomal and Stable Transfection of *Leishmaniatarentolae* using Vaccine Candidate Antigens

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Background: *Leishmania*as an intracellular protozoa, transmitted to their mammalian host by the bite of infected sand flies and cause a group of diseases known as Leishmaniasis. Despite attempting different vaccination strategies, no human vaccine is yet available against this disease. Among different species of *Leishmania*, *Leishmaniatarentolae* is a lizard parasite which is non-pathogenic to humans and could be used as an expression system for producing virulence proteins or epitopes as well as to be used as an efficient and safe recombinant live vector in vaccinology.Cysteine proteases (CPs) are among virulent factors in Leishmania and acting as candidate antigens for vaccine development. Previously it has been shown that CPs immunization with two genes or recombinant proteins of CPA and CPB individually or fused together with various adjuvant are able to elicit a protective immune response against *L. major* in BALB/c. Here, we presented two different approached in order to have recombinant *L. tarentolae* expressing cpa*/cpb/egfp*. MaterialS and Methods: In this study, knock-in *L. tarentolae* line was generated by transfection method to express a heterologous triple fusion gene encoding *cpa/cpb/egfp*. For this purpose, two different expressions approaches, episomally bearing rDNA (ribosomal DNA) promoter and integratively to rRNA locus of genome, were used and two lines of recombinant parasites were generated. The intensity level of fluorescent was monitored in different phases of parasites and *ex vivo* system by both fluorescence microscopy examination and Flow-cytometry analysis. Western blot and RT-PCR analysis were also performed to show specifically the expression of cpa*/cpb/egfp* fused gens in both lines of transgenic parasites. Results:The presence and correct location of genes inside the transgenic parasite genome was done at molecular level (both by DNA and RNA). The expression of triple-fusion(CPA/CPB/EGFP) was confirmed at protein level using western blot and EGFP intensity by flow cytometry in both recombinant parasites. In logarithmic phase, the intensity of EGFP was proportional to amplification of parasites and was 20 fold higher in compare to stationary phase. After 5 days when the culture is near to stationary phase, the level of intensity decreased to 2.56-11.04% in different concentration of G418. One of the hallmarks of episomally expression is their instability without any drug pressure *in vivo* and copy number of plasmids decreases rapidly. In addition, the copy number of plasmids between transfectedparasites are unequal and expression rate within parasites are severely different. Conclusion:Recombinant *L. tarentolae* expressing *cpa/cpb/egfp* fused genes with two different approaches were established. Our data suggest that it is more secure to use the stably transfected*L. tarentolae* for further vaccine studies.
Keywords: *Leishmaniatarentolae*,Episomal, Stable Transfection,Vaccine Candidate Antigens

929. *In silico* Analysis of Chimeric Recombinant Vaccine Against Causing Diarrhea Agents

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Background: Entericinfectionresultingindiarrheal diseases remains health problem worldwide. *Shigella* and *Escherichia* causes the most diarrheas in the world. *Shigella* can cause bacterial dysenteries and shigellosis through invasion. One of the most effective proteins for pathogenesis is invasion plasmid antigen C (IpaC). Other bacteria like ETEC and EHEC are also causing diarrhea. Most of the travelers' diarrheas are resulted from ETEC infection. Colonization factor antigen I (CFA/I) is critical virulence protein for these infections with two subunits called CfaB and CfaE. Another major pathogen is EHEC which produces intestinal disorders. Attachment of bacteria is the main step of infection andthe protein intimin plays the key role in this function. Protection against the vast majority of responsible pathogens of diarrheas, requires the developed the polyvalent vaccine against *Shigella*, ETEC and EHEC. *In silico* techniques are best tools to study, design and evaluate of new vaccines. In the present study, we designed a multisubunit protein that could be a suitable vaccine candidate against thesepathogens. Materials and Methods: A synthetic chimeric gene (CII) containing immunologically significant parts of CfaB, IpaC and Intimin proteins was designed. After codon optimization for *Ecoli*, modeling was done to study the 3D structure of the protein. To assess immunological points, the humoral and cellular immunity was analyzed. Prediction of allergens and mapping of IgE epitopes was obtained. Results: The bioinformatic analysis showed that each domainfoldedseparately in protein structure. CII had many T and B cell epitopes in both linear and 3D structure. This prediction chimeric construct had the potential to induce CD^{4+} and CD^{8+} immune responses against these pathogens. In addition CII could be accessible to surveillance by the immune system in mouse and human. Conclusion: *In-silico* analysis showed that this chimeric protein can be used as a candidate vaccine against *Shigella*, ETEC and EHEC.
Keywords: *In silico,* Chimeric Recombinant Vaccine, Diarrhea Agents

930. Immunological Evaluation of a Cocktail Vaccine againstEnterotoxigenic *Escherichiacoli*

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Background:Enterotoxigenic*Escherichia coli* (ETEC) is the most significant agent leading to childhood diarrhea and death in developing countries. Due to its prevalence as well as difficulties in its treatment, designing effective vaccines against ETEC is a goal of World Health Organization. Colonization factors such as CfaB and CfaE as major and minor subunit of fimberiae, respectively; have a vital function in bacterial binding to epithelium cells.Heat labile enterotoxin (LT) B subunit is nontoxic subunit of LT molecule that plays an important role in ETEC pathogenesis. Hence, these molecules alone or together with other candidate molecules have been considered in vaccine design.In this investigation, we produced recombinant LTB, CfaB and CfaE in *E. coli* with the mean of examine its immunogenicity as a cocktail vaccine.Materials andMethods:The cfaB, cfaE and ltb genes were isolated from a local isolated ETEC, separately cloned and expressed using pET28a expression vector. The recombinant proteins was purified and used as antigens for mice immunization and in immunological tests.

Results:The immunological analyses showed production of high titer of specific antibody in immunized mice. Anti LTB Antibody could bind to whole toxin and neutralize the toxin through inhibition of its binding to the Ganglioside M1 receptor. Relying on agglutination inhibition experiment, anti-CfaB and anti-CfaE serum was able to block the binding of CFA/I fimbriated ETEC to erythrocytes.Conclusion:Considering the LTB, CfaB and CfaE roles in ETEC pathogenesis, they can be use ascocktail vaccine against ETEC.
Keywords: Cocktail Vaccine, Enterotoxigenic *Escherichiacoli*

931. Vaccination with Dendritic Cells Loaded with Tumor Apoptotic Bodies (Apo-DC) in Patients with Chronic Lymphocytic Leukemia
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Background:We previously demonstrated that autologous dendritic cells that have endocytosed apoptotic bodies of chronic lymphocytic leukemia (CLL) cells (Apo-DC) can stimulate antileukemic T cell responses in vitro. In this phase I study, we vaccinated 15 asymptomatic CLL patients at five time points with Apo-DC administered intradermally either alone (cohort I), or in combination with subcutaneous granulocyte-macrophage-colony-stimulating-factor (GM-CSF) (cohort II) or with GM-CSF and intravenous low-dose cyclophosphamide (cohort III). Aim of the study was to evaluate the safety and immunogenicity of Apo-DC alone or in combination with GM-CSF and low-dose cyclophosphamide in CLL patients. Materials and Methods: All patients completed the vaccination schedule without dose-limiting toxicity. No objective clinical responses were seen. Vaccine-induced leukemia-specific immune responses were evaluated by IFN-γ ELISpot and proliferation assays over a 52 weeks observation period and immune response criteria were defined. According to these criteria, 10/15 patients were defined as immune responders. Results: The frequency of immune-responding patients was higher in cohorts II (3/5) and III (5/5) than in cohort I (2/5). In order to further characterize the induced immune response, estimation of secreted cytokines and CD107-degranulation assay were performed. Clustering of T and CLL cells was observed in CD107-degranulation assay and visualized by confocal microscopy. Additionally, assessment of regulatory T cells (T(regs)) revealed their significantly lower frequencies in immune responders versus non-responders ($P < 0.0001$). Cyclophosphamide did not reduce T(regs) frequency. Conclusion: vaccination with Apo-DC + GM-CSF and cyclophosphamide was safe and elicited anti-CLL immune responses that correlated inversely with T(regs) levels. Lack of clinical responses highlights the necessity to develop more potent vaccine strategies in B cell malignancies.
Keywords: Vaccination, Apo-DC, Chronic Lymphocytic Leukemia

VETERINARY IMMUNOLOGY

Oral Presentation

932. MicroRNAs; Novel Interferon-Induced Gene Regulators
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Background: MicroRNAs (miRNAs) are a new class of 18 23 nucleotide long noncoding RNAs that play essential roles in a wide spectrumofbiological processes. Recent reports also clarify the role of miRNAs ascritical effect or sin the complicated host pathogen interaction networks. Emerging evidence suggests that miRNAs play a key role in the regulation of immunological functions, including innate and adaptive immune responses. Materials and Methods: In the present study, we use microarray technology to identify up regulation of miRNAs in Rainbow Trout after exposure to the fish pathogenic rhabdovirus causing Viral Haemorrhagic Septicaemia (VHS) which is an economically important disease in freshwater aquaculture.
Results & Conclusions: we discuss the mechanism of interferon induced miRNAs in Rainbow Trout infected by thisvirus.
Keywords: micrornas, Interferon, Rainbow Trout, Viral haemorrhagicsepticaemia Virus.

933. Molecular cloning and expression of the constant region of chicken µ immunoglobulin chain in *Escherichia coli*
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Background: Anti-antibodies are among the most valuable reagents for use in immunodiagnostic assays. Chicken antibodies are increasingly being used as diagnostic and therapeutic tools. Materials and Methods: In present study, a cDNA fragment of 843-bp, encoding the constant region of chicken µ immunoglobulin chain was amplified from the spleen cells of a healthy one-week-old chicken using RT-PCR. The gel-purified product was cloned into the prokaryotic expression vector pMALc2x. This allows us to express this fragment as an N-terminal MAL (Maltose binding protein) fusion protein in *Escherichia coli*. The construct was transformed to competent *Escherichia coli* strain TG1 and the transformants were grown on Luria-Bertani (LB) agar medium containing ampicillin. Results: SDS-PAGE analysis of IPTG induced bacteria revealed the expression of a protein slightly above 73kDa in size. This was in good agreement with the calculated molecular mass for the protein. To further reveal the nature of the expressed protein, the proteins were transferred to PVDF membrane following SDS-PAGE and probed consecutively with a mouse anti-chicken serum and a goat anti-mouse IgG, conjugated to peroxidase. Conclusion: The gene expression of MAL fusion protein was stable and appeared to be strong enough for using in further analysis.
Keywords: pMALc2x, RT-PCR, SDS-PAGE

934. Universal Vaccine Candidate: Overexpression and Characterization of Influenza Virus M2 Protein in Prokaryotic System
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Background: Influenza A virus causes respiratory disease in avian and mammalian species. The M2 protein forms a proton channel, which is essential in uncoating the virus during the initial stage of infection. This protein is conserved among all influenza A viruses and so, is an appropriate target for the development of influenza vaccine with broad-spectrum protection. In this study,M2 proteinof influenza virus A was expressed in a prokaryote system and purified. Materials and Methods: M2 gene of influenza virus A/NewCaledonia/20/99(H1N1) was amplified by PCR using specific primers digested with appropriate enzymes andligated into the prokaryotic expression vector PET28a.The E. coli (BL21) competent cells were transformed with recombinant plasmid (PET28a-M2) and grown in LB broth media for 4 h after inducing by IPTG. The media were supplemented with 50 mg/ml kanamycin. Expression of the M2 protein was approved by SDS-PAGEand western blot analysis. The expressed protein with 6xHis-tag at the N-terminus was purified usinga Ni-NTA resin column. Results: The results of sequencing showed that M2 gene was cloned in PET28a properly in frame to Histidine tag and immunoreactions of anti-his antibody to recombinant M2 in western blotting confirmed it. Conclusion: These vaccines significantly enhance the immune response and protect against a lethal challenge with

influenza A virus to some extent and induce broad spectrum protection against multiple influenza subtypes. In order to design an effective and protective vaccination regimen, we are going to use M2 protein with different adjuvant and/or DNA vaccine in animal model.
Keywords: M2 protein, Influenza Virus, Vaccine, E.coli, pET28a

935. Expression of *Iss* Protein from *Escherichia Coli* Strain X1378 (O78:K80) as New Antigen for Control and Detection of Poultry Colibacillosis

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Background: Avian pathogenic *E. coli* (APEC) causes colibacillosis in chicken, turkeys and other avian species, is one of the main causes of economic losses in all poultry farms. Certain virulence factors have been proposed as means of controlling APEC infections, including some proteins to be used for vaccination. In the study we report, one of the major virulence factors, the *iss* (increased serum survival) gene, from *E. coli* strain χ1378, isolated from poultry colibacillosis in Iran, was cloned to construct a prokaryotic expression vector, in order to analyze the *iss* gene and its protein product. Materials and Methods: The *iss* gene from *E. coli* strain χ1378 was amplified and cloned into the pTZ57R / T vector, sequenced and then the *iss* gene was successfully cloned into the pGEX-3X vector. The construct was transformed into *E. coli* BL21 to express the Iss protein under induction. The Iss protein was expressed as a glutathione-S-transferase (GST) fusion protein. GST::Iss protein was sequenced by MS/MS MALDI-TOF techniques to confirm its amino acid sequence. Results: BLAST analysis showed Iss from strain χ1378 had 100% identity with other *E. coli* serotypes and isolates from different origins and also 98% identity with *E. coli* O157:H7. Phylogenetic analysis showed no significant different phylogenic groups among *E. coli* strains. Conclusion: Overall, it seems that the Iss protein from strain χ1378 could be used as a good antigen to vaccinate against poultry colibacillosis. Currently, GST::Iss protein is being used as recombinant protein in SPF chicken models with the goal of evaluating the immune response for APEC control.
Keywords: APEC, pGEX-3X vector, pTZ57R/T vector

936. Comparison of the Best Conditions for Preparation of Fab2 Fragments from Rabbit and Bovine Immunoglobulin

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Background: Using fab2 fragments of antibody is powerful tool for providing the best results in immunological application and particularly in therapeutic methods. In this study we investigated the optimum condition for production of Fab2 fragment from rabbit and bovine immunoglobulin. Materials and Methods: Rabbit and bovine immunoglobulin G were prepared from serum by precipitation with Na2SO4 followed by chromatography on DEAE-Cellulose. The samples at concentration of 5 mg/ml were incubated with pepsin at protein / enzyme ratio of 40/1, 30/1, 20/1 in 0/2 M-Sodium acetate with PH 3.6, 4, and 4.5. Incubation time at 37̊ C was varied between 2 and 36 hours. The reaction was stopped by adjusting to PH 7.5 with Tris buffer. Fab2 was isolated by precipitation procedure with Na2SO4. Test for purity were conducted by SDS-PAGE method at different stage of process. Results: The optimum PH, ratio and time were 4, 3.6, 30, 20 and 24h, 36h for rabbit and bovine respectively. Conclusion: This result indicates that bovine IgG is more resistant to pepsin digestion than rabbit IgG is.
Keywords: Fab2, bovine, rabbit.

937. Monoclonal Antibodies for Vibriosis in the Shiraz Ornamental Fish

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Background: monoclonal antibodies (Mab) proximate versus *Vibrio salmonicida* were produced and partially characterized. The bacterium is the causative agent of 'Hitra disease' or coldwater vibriosis (CV) and varies from all other *Vibrio* bacteria tested so far with respect to an individual surface antigen (VS-P1). Materials and Methods: Thirteen hybridoma clones produced antibodies (Ab) which exclusively reacted with this antigen in ELISA. Results: conclusion: The fragment four clones reacted against undefined determinants and were partly cross-reactive to *V. anguiilarum* and *V. fischeri*. Fifteen Mab were of IgG1/kappa and two of the IgG3/kappa isotypes. Eleven of the IgG1 plus the two IgG3 Mab reacted with the VS-P1 molecule.
Keywords: *Vibrio salmonicida*, monoclonal antibodies, coldwater vibriosis, hybridoma, ELISA, shiraz

Poster Discussion Presentation

938. Effect of Vitamin E and Selenium Administration during the Late Period of Pregnancy on Phagocytic Ability of Peripheral Blood Mononuclear Cells and Milk Cell Counts in Parturient Ewes

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Background: The role of vitamin E and selenium in immunity system have been demonstrated in previous studies. On the other hand, ruminants are more sensitive to new intra-mammary infections in pre-parturient period. The aim of this study was to determine the effects of treatment with vitamin E and selenium during the late period of pregnancy on phagocytic ability of peripheral blood mononuclear cells (PBMC) and somatic cell count (SCC) of milk in parturient ewes. Materials and Methods: Four week before lambing, ten pregnant 2 to 4 years old ewes were randomly allocated in two groups with equal numbers. Treatment with vitamin E and selenium (1cc per 50 kg of body weight) was initiated at 4 week before the estimated time of lambing and continued weekly to the time of parturition. Blood samples were collected after lambing and PBMC were tested for phagocytic ability by NBT test. Finally, the effect of Treatment on SCC of milk was measured. Results: Treatment significantly increased the phagocytic ability of PBMC in parturient ewes ($p<0.005$). Also,treatment reduced somatic cell counts during the subsequent lactation ($p<0.05$). Furthermore, the administration of vitamin E and Se was associated with differences in differential cell counts of milk samples (macrophages, 47.9% vs. 36.5%; neutrophils, 39.1% vs. 48.7%; and eosinophils, 0.6% vs. 1.3% for control ewes and ewes receiving treatment, respectively). Conclusion:Parenteral administration of vitamin E and Se to ewes during the late period of pregnancy inhibited the reduction of phagocytic ability of PBMC and increased Defensecapabilities ofmammary glands.
Keywords: vitamin E, selenium, ewe, phagocytosis, milk cell count.

939. Molecular Identification and Comparison of LEI0258 Microsatellite Variability in Khorasan, Marandi and Arian Broiler Chickens

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Background: The LEI0258 microsatellite marker is located within the B region of the chicken Major Histocompatibility Complex (MHC), and is surprisingly well associated with serology. It could be used as a genetic marker for drawing population genetic influence on immune responses. Here, LEI0258 microsatellite variability in three Iranian native chickens (Khorasan, Marandi and Arian) was investigated and comparison of LEI0258 microsatellite alleles frequency between three populations was analyzed. Materials and Methods: A total of 242 blood samples from

three Iranian chicken populations (including 142 Khorasan, 42 Marandi and 58Arian chicken) were examined. Genomic DNA was extracted from the whole blood. The LEI0258 alleles were determined using polymerase chain reaction (PCR) as described by Fulton et al. (2006). Genotypic frequencies, number of alleles, Expected homozygosityand heterozygosity and Deviations from Hardy–Weinberg (HW) equilibrium was estimated using The Popgene 1.32 software. Results: Collectively, 25 alleles were found for three Iranian chicken ecotypes (including 15 alleles in Khorasan, 23 alleles in Marandi and 11 alleles in Arian population) . We identified 79 different genotypes; 46 in Khorasan, 37 in Marandi and 12 in Arian chickens. The observed heterozygosity was 81% in Khorasan and Marandi, and 34% in Arian chickens. Conclusion: Our results indicate that LEI0258 diversity in Marandi chicken is higher than the other populations. Allelic diversity in Iranian chicken is relatively higher than the local chicken breeds reported for Brazilian and Vietnam.

Keywords: LEI0258, PCR, MHC

940. Immunohistomorphological Study on the Fetal Spleen of Iranian one Humped Camel (*Camelus Dromedarius*)

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Background: The vertebrate spleen has important functions in immunity and haematopoiesis and many of which have been well studied. The aim of this study was to recognition of the different immunohistomorphological compartments and biometrical parameters in developmental processes of Iranian one humped camel (*Camelus dromedarius*)fetuses during fetal period. Materials and Methods: The immunohistological structure and cells of 16 females and 14 males spleens of Iranian camel fetuses (81/9 to 259/6 dayes old) were studied. Lymphoid follicles diameterthe splenic capsule thickness, splenic trabeculae thickness were determined on thirty camel fetuses. Results: The white pulp area was large and irregular shape, and the (PALS), lymph follicles and the marginal zone were very clear. The lymphoid follicles were spherical in shape and their diameter measured. The cross section of the PALS contained 2-3 arteries; the artery was tortuous and branched in PALS. A wide marginal zone surrounds the white pulp and it was contained sheathed arteries and smooth muscle cells. Conclusion: The results revealed that spleen structure of fetal camel is similar to reports in adult camel spleen.

Keywords: Immunohistomorphological study, spleen, Iranian camel fetuses

941. Immunofluorescence Diagnosis of Rabies in Camels in Iran

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Introduction: Rabies is fetal disease for warm-blooded vertebrates, which causes central nervous system infection, paralysis and death. Camelids are susceptible to rabies and the disease has been occasionally occurred in Iran. In this Article we report diagnosis of rabies in some Cases of Iran. Materials and Methods: During 2008-2011 in Sistan va balochestan, Semnan, Kerman and Mazandaran provinces, 9 camels were suspected to rabies and then, samples were taken from their nervous system. After an incubation period of 10 days the following clinical signs were seen in suspected camels: increased sensitivity, ferocity, biting faces and lips of other camels, blat, restlessness, limb paralysis and hypersalivation. When rabid camels became paralyzed, they lie on their sides and flail with their limbs and yawn continuously that led to death. Brain samples were taken from all affected camels soon after death and were tested by fluorescent antibody technique (FAT). Results: All samples of affected animals were positive for rabies. Conclusion: The standard method of making a diagnosis of rabies is to demonstrate rabies virus antigen in impression smears of fresh brain by immunofluorescence. In all Rabid camels that tested by FAT, massive viroplasms of rabies virus antigen conglomerates of varying size were seen immunofluorescently in the brain.

Keywords: fluorescent antibody technique, Rabies, camel

942. A Study on the Effects of the Estrous Cycle on Uterine Fluid and Blood Serum Immunoglobulin G (IgG) Content in the Cow

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Background: Stage of the estrous cycle is an important determinant of resistance of the uterus to infection, with females of several species being more susceptible to infection during the luteal phase of the estrous cycle. The uterus and its luminal fluid components are of great importance in animal reproduction, and some attempt has been made to investigate its composition. Materials and Methods: To investigate the uterine fluid (UF) IgG content and its variations during the estrous cycle of the cow and to compare them with those of the blood serum (S), 6 pairs of serum and UF samples for each phase of the cycle selected out of 240 bovine genital tracts and blood samples collected in Urmia abattoir. The UF samples were collected by gentle scraping of the endometrium by a curette after uterine incision and their IgG content and those of the serum were measured by single radial immunodiffusion (SRID) assay. Results: Serum IgG values (Mean ± SEM) were generally higher than the UF values throughout the cycle except in di-estrus (S: 38.5 ± 0.9, UF: 51.6 ± 2.1mg/ml), in which the highest values observed in UF samples. In met-esturs the difference was not significant (S: 34.8 ± 1.8mg/ml, UF: 30.8 ± 5.2mg/ml), but in estrus the mean UF IgG value (12.5 ± 1.1mg/ml) was lower than that of the serum (31.3 ±1.2mg/ml). In pro-estrus, the lowest values (S: 27.8 ± 1.3mg/ml, UF: 9.1 ± 1.5mg/ml) were obtained. Conclusion: The results show a lower IgG values in the bovine UF than those of the serum in the follicular phase of the cycle, while in di-estrus the UF IgG content was the highest, suggesting some IgG production in the uterus at this phase.

Keywords: Cow; Estrous cycle; Uterine fluid; IgG.

943. Effects of *SpirulinaPlatensis* on Some Immunological and Hematological Factors in Common Carp (*CyprinusCarpio*)

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Background: *Spirulinaplatensis*is a photosynthetic, blue-green, multicellular microalga. This alga has been studied as a human food source due to its enriched protein and micronutrient sources and immunostimulant activity.In the current study, possible immunostimulant effects of *S.platensis* on immunology and hematology factors in common carp (*Cyprinuscarpio*) have been investigated. Materials and Methods: One hundred 150–200 g fish were fed with 5, 10 and 15 g algae per kg diet for two weeks. The blood samples were taken at the end of the first and the second weeks post-trial. Lysozyme activity, total and differential white blood cell counts, total serum protein, albumin, alpha-globulin, beta-globulin and gamma-globulin were assessed.Results: After the first week, total serum protein and gamma-globulin in fish fed 5 and 15 g algae were significantly increased ($P < 0.05$) compared to control one. Serum lysozyme activity in fish fed 10 g algae and alpha-globulin in 5, 10 and 15 g algae groups were also increased at the end of the second week ($P< 0.05$) compared to control group.Conclusion: The findings of this study indicate that the use of *S.platensis* as a food additive can improve some immunophysiological variables of carp, therefore its consumption in fish food is recommended.

Keywords:*Spirulinaplatensis, Cyprinuscarpio,* immunophysiological

944. Design of rPOMP90-ELISA for Detection of Enzootic Abortion of Ewes

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Background: Enzootic abortion of ewes (EAE) caused by *Chlamydophila abortus* is one of the most common chlamydial diseases in animals, and has significant economic implications. *Chlamydophila abortus* (*Chlamydia psittaci* serotype 1) is a gram-negative obligate intracellular bacterium . This bacterium newly classified as a new genus *Chlamydophila* in the family *Chlamydiaceae* . severe economic losses it causes in field of veterinary and also represents an emerging zoonotic risk for pregnant women. Though direct evidence of the pathogen is the ultimate diagnosis, serological assays are more suitable for screening large number of animals. Several experimental assays have been developed in order to improve chlamydial serology. More specific assay are based on the polymorphic outer membrane proteins (POMP).The aim of this study, was development of an ELISA test with three segments of POMP90 protein. Materials and Methods: For this purpose the coding regions of the segments(POMP90-3 and POMP90-4 and POMP90-3,4) *of Chlamydophila abortus* isolate S26/3 DNA gene were amplified by PCR and cloned into *E. coli* BL21(DE3) with a prokaryotic expression vector (pGEX-4T-1). The recombinant proteins GST-segments was expressed in *E. coli* and were purified using glutathione Agarose chromatography column and used as the diagnostic antigens to develop a POMP-based type specific indirect ELISA for detecting antibodies *to Chlamydophila abortus* for ruminant sera. The ELISA with this segment was compared with a commercial *Chlamydophila abortus* ELISA kit (Institut Pourquier-France). Results: Excellent agreement (92.91%) was obtained between POMP90-3- ELISA and commercial ELISA kit (k= 0.858). Conclusion: Results indicated that in house POMP90-3-ELISA can react similar to commercial ELISA for detecting the antibodies in ovine inflected with *Chlamydophila abortus* and lower indication of positive serua with inhouse ELISA may be resulted from higher specificity, not from its lower sensitivity.

Poster Presentation

945. Effects of Alcoholic Extract of Garlic on Immune Response of Broiler Chickens

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Background: Many favorable biological and pharmacologic effects with consumption of garlic preparations have been reported experimentally and clinically. garlic extract has been reported to have an array of pharmacologic effects, including immunomodulation.The recent study was conducted to investigate the effects of alcoholic extract of garlic (Allium sativum) in drinking water on immune response of broiler chickens. Materials and Methods: In a completely randomized design, one hundred and twenty 1-days-old broiler chicks (Ross308) were assigned to 4 treatments with 3Replicates :1) control, 2) 0.2% alcoholic extract of garlic, 3) 0.4% alcoholic extract of garlic, 4) 0.6% alcoholic extract of garlic, used in drinking water. Feed and water were provided ad libitum. In the end of experiment period, two birds of each replicate were randomly selected and the blood samples were taken from wing vein with syringes. Results and Conclusion: Moreover, consumption of garlic (Allium sativum) extract in water increased the bronchitis antibody titer as compared to the control birds in orthogonal comparsions (P<0.05). But between treetment groups there was no significance difference.
Keywords: garlic, broiler chickens, bronchitis antibody titer

946. The Effects of Different Levels of Dietery Lysine on Serum Antibody Titers against Avian Influenza Vaccine (H9N2) in broiler chickens

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Background: Amino acids are essential for correct immune system function. Lysine is the second limiting amino acid in corn-soybean meal diets. Avian influenza viruses are classified in the family Orthomyxoviridae, genus Influenza virus A. The purpose of this research was to investigate the effect of different levels of lysine in ration on serum antibody titers against AI vaccine in broiler chickens. Materials and Methods: One hundred and eighty day-old broiler chicks, divided in three groups (each group contain 60 chickens in 3 replicates). Group 1 (control) received base ration with 1.2%, 1.1% and 1% lysine in Starter (days: 1-14), grower (days: 15-28) and finisher (days: 29-42) ration respectively. Group 2 and 3 received ration with 1.3%, 1.2%, 1.1% and 1.4%, 1.3%, 1.2% lysine in Starter, grower and finisher ration respectively. Results: Chicks were raised on floor-pen under standard condition until day 42 of age. At day 7 of age all groups inoculated via subcutaneous with killed AIV vaccine (subtype H9N2). At day 14, 28 and 42 of age, 10 chicks of each group bleed via wing vein and the sera referred to laboratory for HI test. Antibody titers mean of in Groups 1, 2 and 3 at days 14 of age were 2.3, 3.2, 2.9 and at day 28 and 42 were 3.1, 4, 3.5, and 2.8, 4.6, 3.4 respectively. The results showed that antibody titers were higher in groups 2 and 3 compare to the control group. The highest mean of Antibody titers was seen in group 2 in starter, grower and finisher period that had significant differences with control group at day 42 of age.
Conclusion: It can be conclude that supplementation of lysine in poultry diet over than advised level by NRC can be useful and have positive effect on the antibody response against Avian Influenza disease vaccine.
Keywords: H9N2, broiler chickens, NRC

947. The study of dietary *Aloe vera* feeding effects on some factors in nonspecific immune responses of Common carp (*Cyprinus carpio)*

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Background: The effects of aloe-supplemented feeds on some factors of nonspecific immune responses were determined in Common carp (*Cyprinus carpio*). Materials and Methods: Experimental fish (300 fish) were randomly divided into 4 equal groups. First group was immunized with *Aeromonas hydrophila* bacterin (A.h) and was fed a diet containing 0.5 % Aloe vera extract. A second group was immunized with A.h and fed an Aloe free diet (immunized control group). The third group was not immunized but fed a diet containing 0.5 % Aloe. The fourth group was neither immunized nor fed Aloe. Blood samples were taken every 14 days during 8 weeks. Samples were analysed for immunological parameters (lysozyme activity, serum bactericidal activity, complement activity). Results: At the end of treatment, 20 fish in each group were challenged with *A. hydrophila*. The Relative Percent Survival (RPS) was increased in Aloe fed fish. lysozyme and bactericidal activity were significantly increased in serum of Aloe treated fish (P<0.05). Conclusion: No significant differences were seen in complement activity among groups (P>0.05). The results of the present study showed that feeding with *Aloe vera* can help to non-specific immune responses in controlling bacterial infectious diseases in common carp.
Keywords: Nonspecific immune responses, *Aloe vera*, *Cyprinus carpio*, lysozyme, complement system, *Aeromonas hydrophila*,

948. Evaluation of *aloe Vera*-Supplemented Feeds on Specific Immune Responses against *Aeromonahydrophia*in Common Carp (*Cyprinuscarpio)*

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Background: Recently, immunostimulants in aquaculture has been becoming popular for enhancing the activity of specific and nonspecific defence mechanisms and protection against disease.In this study the effects of *Aloe vera*crude extract in Common carp were investigated. Materials and Methods: A total of 300 fish were randomly divided into 4 equal groups. Blood samples were taken every 14 days during 8 weeks. Samples were analysed for IgM concentration and specific *A. hydrophila* antibody. At the end of treatment the Relative Percent Survival (RPS) was increased in Aloe fed fish. Results: The WBC value, antibody levelwere significantly increased in serum of Aloe treated fish ($P<0.05$). Conclusion: This study indicates that oral administration of *Aloe vera*in moderate doses can help to control andenhance some specific immune responses against infectious diseases in common carp.
Keywords: *Aloe vera*, *Cyprinuscarpio*, immunostimulant, Specific immune responses, *Aeromonashydrophila*

949. Production, Characterization and Neutralizing Potential of Chicken Egg Yolk Antibodies against *Escherichia Coli* K99

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Background: *Escherichia coli* K99 is the main cause of diarrhea in calf. For the disease incidence prevention due to this bacteria, many methods such as vaccination and passive immunity transmission could be used. For this purpose, using immuned eggs in sensitive calf's feed has been recently considered. Materials and Methods: In order to prepare antigen, *Escherichia coli* K99 was killed by adding 1% formalin solution after culture on Nutrient Broth media for 24 hours. The antigens were refined and made ready to use after three times washing by phosphate buffer saline (PBS). The amount of antigen in each dosage was set based on 1×10^9 CFU/ml. The layer chickenes were incubated (injected) 4 times with 2 weeks intervals, the first one with Freund's complete adjuvant and the next ones with Freund's incomplete adjuvant. Various sampling has been done once in each 2 weeks, and then antibody titers were assayed in serum and egg specimens by direct agglutination method. Results: The results have been assessed by statistical analysis (Mann-Whitney test and Repeated Measure ANOVA) and the significance has been proved. Conclusion: The results indicate that antibodies generated in chicken by IgY technology could be used for prophylaxis, diagnosis and therapeutic purposes in case of new born calves Clibacillosisdiarrhea. Also, may be an attractive alternative in treating and preventing bacterial digestive diseases.
Keywords: *Escherichia coli K99*, diarrhea, egg yolk antibody (IgY), prophylaxis, immunized egg

950. Protein Expression of Infectious Laryngotracheitis Virus Thymidine Kinase Gene in Bacterial Cell

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Background: Avian infectious laryngotracheitis (ILT) is a severe clinical respiratory disease of chickens and causes the clinical symptoms of difficulty in breathing and bloody coughing and as if involves laying hens affect the egg production. Materials and Methods: In order to cloning of the coding region of *TK* gene of ILT virus , PCR product of the open reading frame of the gene from DNA extracted of infectious tissue of involved poultry farms in different parts of south west of Iran was amplified by PCR. A 1141bp PCR product of the *TK* gene with *BamHI*, *Xho*I restriction sites were subcloned of pTZ57R/T and digested by the mentioned endonucleases. Digested insert cloned in to pGEX-4T-3 and transfected in *E.coli* cells. For the expression of TK protein, the pGEX-4T-3 recombinant vector was transformed and then induced in BL21 (DE3) strain of *E.coli* competent cells using IPTG, the presence of TK expressed protein was shown in immunoblotting and SDS-PAGE system. Results: Analysis of the partial ILT virus *TK* gene sequences obtained from insert and was carried out. The Iranian ILT *TK* sequences were compared to 11 other corresponding sequences of ILT isolated in different countries. Nucleotide analyzing of the sequences were shown a variation of 0–62.8% and constructing phylogenetic tree revealed two clusters in it. Conclusion: Most of agreement was related to the known sequences in the *TK* gene in USA (S83714.1), China (DQ522947.1, AF435453.1), Switzerland (EU360946.1) and most of differences were related to strain of this virus in Australia (GQ180115.1).
Keywords: Avian infectious laryngotracheitis, *Thymidine kinase*, pTZ57R/T, pGEX-4T-3, Phylogenetic analysis, Protein expression

951. Antibody Detection against Brucella Spp. in Small Ruminant Flocks of Iran in 2009

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Background: Brucellosis is a highly contagious zoonotic disease affecting terrestrial mammals. Prevention of human brucellosis relies on the control of the disease in animals, which has traditionally consisted of a combination of test-and slaughter and/or vaccination. National control program in Iran based in kid and lamb vaccination and the slaughtering of positive reactors. In this study we report seroprevalence of brucellosis among sheep and goat flocks in Iran. Materials and Methods: Serum samples from 27796 sheep and goats of 17 provinces in Iran were analyzed with the Rose Bengal test and tube agglutination test, and positive results were confirmed with the 2-mercaptoethanol test. These tests were carried out according to Alton *et al* (1975) and Brinley Morgan *et al* (1981).
Results: Out of the 27796 serum samples, 629 cases 2.26% were serologically positive for brucellosis. Conclusion: Prevention is dependent upon increasing public awareness through health education programmes and safe livestock practices. Active co-operation between health and veterinary services and animal movement control should be promoted.
Keywords: Brucella Spp., Rose Bengal test, agglutination test

952. Effect of Medicinal Plants on Immune System of Broilers

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Background: This study was carried out to investigate the effects of dietary administration of some medicinal plants to improve immune responses efficiency in broilers. Materials and Methods: Total of 180 d-old broilers (Arbor Acres Plus) were randomly distributed in 6 dietary treatments as follow: control; 4 groups of medicinal plants (dried powder of peppermint; thyme; basil and garlicat 1.5%); and one group fed diets containing antibiotic (15 ppm). Each treatment was replicated 3 times. Blood samples were collected from 3 birds at random from each group on d 25 and 46 of the experiment for evaluation of immune responses, including humoral immune response to sheep red blood cells (SRBC) and Newcastle disease vaccine (ND) and cellular immune response to phytohemagglutinin (PHA). Results: Results showed that supplementation of broilers diets with garlic and pepper mint increased humoral immune response significantly (4.33 ± 0.07 and 3.99 ± 0.03 for garlic and peppermint groups vs. 2.33 ± 0.11, 2.66 ± 0.08, 3.00 ± 0.13 and 3.33 ± 0.06 for control, antibiotic, basil and thyme treatments respectively) ($P < 0.05$). There were no significant differences among the treatments on cellular immune response. Also there were no significant differences in Newcastle disease antibody titer (HI) except garlic treatment ($P > 0.05$). Conclusion: Results indicated that use of medicinal plants as dry powder, had no significant effects on humoral immune responses, except for garlic and peppermint. However, garlic supplementation to broiler diets caused significantly higher HI titers against Newcastle disease at 46d compared with 25d.
Keywords: medicinal plants, immune system, broilers

953. Diagnosis of Leptospirosis in small ruminants by Microscopic Agglutination Test

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Background: Leptospirosis is a common zoonotic disease which can occurs throughout the world. It is characterized in sheep by fever, jaundice, anorexia, depression, dyspnea, hemorrhagic and anemic syndrome. Reproductive problems may occur, including spontaneous abortion in the final third of gestation, birth of weak lambs, stillbirth and infertility. This disease is endemic in Iran and it can create heavy economic losses to ranchers. As sheep breeding in Iran is performed by rural and nomads and this is a zoonotic disease, providing health in sheep means providing health in human society. This abstract describes diagnosis of leptospirosis in 2 provinces' sheep flocks of Iran by using Microscopic Agglutination Test (MAT). Materials and Methods: Base of leptospirosis diagnosis is serological tests and MAT is one of these tests which is still used today in reference laboratories. We had been suspected to leptospirosis in Ardabil and Markazi provinces. So, we extracted 100 sera samples from sheep and goats which had symptoms among 1520 sheep and goats. Then we use MAT to diagnose. MAT has high specificity and we performed it according to WHO recommendation. Results: The results of MAT showed that 37% of total samples were positive. Furthermore, this study shows harjo, canicola, grippotyphosa and pomona as endemic serovars. Conclusion: Although MAT is a raliable test, there are other alternative tests like CFT, MCAT, ELISA, FAT and PCR. The most effective measure to prevent leptospirosis outbreak is vaccination. Since serovars don't cause cross-immunity and livestock mostly get involved in endemic serovars, we need to identify endemic serovars in each region to have successful control and prevention programs.
Keywords: leptospirosis, MAT, endemic serovars

954. Detection of Peste Des Petits Ruminants'antigenby Immuno-Capture ELISA inLymph NodeSamples of Small Ruminant

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Background: Ruminants' Peste des petits is an acute, febrile, highly contagious viral disease of small ruminants with great economic importance. The serological tests that are routinely used consist of the virus neutralization and the competitive ELISA. Competitive ELISA(c-ELISA) was used to analyze samples because this method has some advantages compared to standard agar gel immunodiffusion (AGID) and precipitinogen inhibition test (PIT) for the diagnosis of PPR. These advantages include the best sensitivity and specificity and can be used for samples which are not kept under ideal conditions and performed for the identification of PPR virus in different biological fluids and tissue samples. This study reports observations from outbreaks of PPR in Fars province and presents details of the existence of PPR virus antigen in lymph node samples. Materials & Methods: Samples were taken from small ruminants with mucosalsymptoms, high body temperature (up to 107 ◦F, severe mucopurulent, nasal and ocular discharges, necrotic stomatitis and respiratory distress. Diarrhea was also present but abortions were reported in a few cases that wesuspected the PPR disease. SampleswereanalyzedbyThe Immunocapture ELISA test kit from CIRAD / EMVT, Montpellier (France). Results: Eighty seven Samples were taken from sheep that IC ELISA showed presence of PPR antigen in 30 samples (34.5%). Conclusion: Cases with mucosal symptoms should be differentiated from contagious ecthyma and foot and mouth disease.The rapid detection by suitable and appropriate methods of antigen detection in infected animals will help in early diagnosis of infection. Vaccination strategy and cross-border transmission control will reduce theincidence of the PPR disease in Iran.
Keywords: PPR disease, c-ELISA, lymph node

955. Measurement of IgG In Colostrum of Sarabi Native Cows by Single Radial Immuno-Diffusion Assay (SRID)

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Background: The aim of the present study was measurement of IgG in Sarabi native cows and comparison of it with other breeds in Iran and world. Materials and Methods: This study was done in the support Sarabi native cows' center, that was located in 30 km of Sarab, East Azerbaijan province. Measurement of IgG was done in the first milking colostrum after calving by a SRID kit (Kents Lab, USA). Twenty (20) samples with 200 ml volumes were taken from cows with different ages in a plastic container and preserved in -20°C temperature until experiment time. Characteristics of the cows and calving were recorded in a sheet. Statistical analyses by SPSS, version 16, were used for evaluation of results (one and two way ANOVA, t- student). Results: The results were present that, the minimum and maximum of IgG in the colostrum was 2000 and 17000 mg/dl, respectively, and Mean± SE of IgG was 8825± 1206 mg/dl. The age of dam, season of calving, single or twining, gender of calves (male or female), weight at birth, normal calving or dystocia of dam, and duration of dry period was not affects of IgG in colostrum ($P>0.05$). Conclusion: Based on literature, IgG have different values in different breeds. The type of placenta in ruminants (syndesmochorial) cause infants not taking IgG during pregnancy and the neonate depends to immunity that taking via colostrum after birth. Failure of passive transfer (FPT) is refer to the above point. These calves will take pneumonia and diarrhea syndrome, lower body weight; and high percentage mortality during its growing. The lowest IgG in the colostrum to prevent FPT is 1000 mg/dl. Therefore if taking colostrum were done by calves as soon as possible after birth (less than 6-12 hours) could protect them from FPT syndrome.
Keywords: Colostrum, IgG, Sarabi, SRID, Cow

956. Polymorphism of BOLA-DRB 3.2 Gene in Iranian Holstein Cattle by Use of PCR-RFLP Method

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Background: The MHC is an organized genetic cluster which has immunologic and non-immunologic fanctions. One of its important functions is the encoding of the molecules which present antigens. MHC proteins, class Ia & II, are receptors which are binded to antigenic peptides and present them to T-cytotoxic and T-helper lymphocytes. Thus these molecules are necessary to modulate immune responses against pathogens. The diversity of MHC is important in the immune diversity in the population and allelic variations of the MHC contributs in host's resistance and immune response to diseases. Therefore, the MHC should be considered in genetic improvement programs in order to increase resistance to a disease. Materials and Methods: blood samples (n=85), containing EDTA, were taken from Holstein cows of Ziaran slaughterhouse located in Qazvin province. After DNA extraction, second exon of BOLA-DRB3 was amplified by the seminested PCR method. The fragments produced by amplifying second exon were digested by three enzymes, RsaI, PsuI and HaeIII. Restriction fragments were revealed by polyacrylamide gel electrophoresis. Statistic analysis carried out by using Pop GENE software ,Levene& Nei method and chi-square test. Results: The number of 40 alleles were identified when result were being studied. Among them, 8 RFLP combinations (8 alleles) were not reported until now. Alleles DRB3*24,*16, *23, *09, *08,*22, *51 with total frecuency of about 52.5% were introduced as the most frequent alleles of the present studied population. Conclusion: This study showed that second exon of BoLA-DRB3 locus is very polymorphic and typing with PCR-RFLP method is a highly appropriate way for studying of polymorphism of above-mentioned locus.The high presence of alleles that are associated with sensitivity mastitis and leukemia was a remarkable point in this study. It should be noted that these alleles could be seriously paid attention in the management of genetic improvement programs.

Keywords: BOLA-DRB 3.2 Gene, PCR-RFLP, Polymorphism

957. Immunological Diagnosis of Bovine Tuberculosis in Iran

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Background: Bovine tuberculosis is an infectious disease of cattle caused by *mycobacterium bovis* and exposure to this organism can result in a chronic disease. This disease is a considerable threat because it causes economic loss by its effects on animal health, prodactivity and by international trade restriction. It is also a zoonotic disease and human can be infected through close contact to infected animals or consumption of contaminated raw milk. One of the important tests used to diagnose bovine tuberculosis is comparative cervical tuberculin test. That is based on cell mediated immune responses against tuberculosis infection. This study reports status of bovine tuberculosis in 10499 industrial husbandry units in Iran. Materials and Methods:Control strategies for bovine tuberculosis in Iran have been based on the use of comparative cervical tuberculin test and slaughter. So in this study in 2009, the comparative cervical tuberculin test was conducted in 1220310 cows which belong to 10499 industrial husbandry units. This test involves the interadermal injection of purified protein derivatives and the subsequent of swelling and induration at the injection site, 72 hours later. Defined increase in skin thickness at the injection sites is used to identify cattle positive to the test. Results:The results revealed that 2733 of these cows were positive. Cattle movements play an important role in the spread of bovine tuberculosis. Conclusion: To control the disease affected herds are re-tested periodically to eliminate cattle that may shed the organism. Quarantine, sanitation and disinfection may reduce the spread of the agent. Currently, there are no tuberculosis vaccines licensed for veterinary use. Because of these, ongoing economic and public health concerns, there is an urgent need for improved methods to combat bovine tuberculosis.
Keywords: bovine tuberculosis, comparative cervical tuberculin test, Iran.

958. Immune Response of Mice Subsequent Genetic Immunization with DNA Vaccination Construct Containing *Mycobacterium Avium* Subsp. *Paratuberculosis* Heat Shock Protein 70/Ahpc

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Background: Paratuberculosis (Johne's disease) is chronic and incurable granulomatous enteritis of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis as an intracellular pathogen.*Intense investigation of immunogens and immunization strategy is a key issue to elicit effective host immune responses against the disease.Effective control requires the development of acellular vaccines offering a better protection than the current available vaccines without side effects and allowing the discrimination between infected and vaccinated animals. In this regard, DNA vaccines stand out due to many of their advantages over protein-based vaccines or other approach of vaccination. Whereas the potency of DNA vaccines in larger animals and human is considerably low, Optimization of them such asformulation of plasmid in adjuvant helps to overcome this hurdle. The aim of this study was evaluation the DNA vaccines potential of C-terminal domain of Mycobacterium avium paratuberculosis heat shock protein70 (MAP-Hsp70) and Alkyl Hydroperoxide Reductase C (MAPAhpC), major antigen constitutively expressed by *Mycobacterium avium* subsp. Materials and Methods: *Paratuberculosis* genetically fused to C-terminal domain of MAP-Hsp70 (AhpC-MAPHsp70) in mice. Results: We have found that immunization of mice with this constructs elicits specific humoral (total IgG) and cellular immune responses (gamma interferon) against antigens. Also, in the absence of any adjuvant, MAP-Hsp70 in combination with Alkyl Hydroperoxide Reductase C induced more humoral and cellular response toward MAP AhpC antigen. Conclusion: In conclusion, this study demonstrates adjuvant properties of MAP-Hsp70 as DNA vaccine and good immunogenicity of this construct. The results also suggests that further testing of these plasmid cocktails in laboratory animals and field evaluating of them in susceptible animal is warranted.
Keywords: Mycobacterium paratuberculosis; DNA vaccine; Heat shock protein70; Immune response

959. Comparison of PLGA Nanoparticle and Freund Adjuvant in Immunization of Laying Hens against *Salmonella Entritidis*

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Background: In poultryindustry there are differentapproaches to preventbacterial infectionsandthe subsequentreduction ofthese infectionsinhumans. Vaccination is one of themost effective strategies toprevent theinfectionsandactiveimmunizeof humanandanimals. So a successfulvaccination depends on an appropriateand safeadjuvant which couldprovideeffectiveimmunization. In this study the adjuvant effect of PLGA nanoparticles in immunization of laying hens against *Salmonella entritidis* was evaluated and compared with the effect of Freund adjuvant.Materials and Methods: 12 leghorn hens were categorized in three groups based on type of adjuvant and method of treatment. In order to immunize, in group one, two and three, *salmonella* antigen with fround adjuvant, *salmonella* antigen with PLGA and microencapsulated *salmonella* antigen with PLGA nonoparticles were used, respectively. Control group does not receive any antigen. On days 15, 30, 60, 75 and 90 after injection, immunogenicity of different treatments was evaluated by ELISA method.Results: The results showed that the group received the bacteria with PLGA nanoparticle once administration exhibited more immunity compared to the other groups ($p<0.05$).Conclusion: It seems that usingPLGAwith appropriateformulationandplanning antigenreleasetime,is an effective wayto designvaccines whichcan beadministeredwithout booster.
Keywords: PLGA, *Salmonella Entritidis*, ELISA

960. Effects of Immunosuppression Caused by Bovine Viral Diarrhea Virus (BVDV) Infection on the Bovine Leucosis Virus (BLV) Seroprevalence in Dairy Herds in Iran

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Background: Bovine leukaemia virus (BLV) is an oncogenic member of the genus Deltaretrovirus of the family Retroviridae that is an important agricultural problem with high costs to the dairy industry. Roughly one-third of the infected cattle show persistent lymphocytosis (PL). Bovine viral diarrhea (BVD) is also a contagious disease of domestic and wild ruminants and one of the most economically important diseases in cattle. BVDV belongs to the genus Pestivirus, within the family Flaviviridae. Infection of cattle by BVDV can cause generalized or specific immunosuppression. Exact mechanism of immunosuppressive activity is vague but it is speculated that could be a property peculiar to certain isolates of the virus. Materilas and Methods: In the present study, blood samples of 263 cows over 2 years old from 13 province of Iran in different regions and environments were taken and examined for differential count of white cells and then, persistent lymphocytosis (PL) were detected. Sera samples were also examined for antibodies against BLV and BVD by reliable blocking ELISA. Results: The seroprevalence of BLV, BVD and both BLV and BVD was 38.4%, 63.4% and 28.5%respectively. Among BLV seropositive, the rate of PL was 42.5% and a little higher than the standard value in the word. Significant interaction between the BLV and BVD was evidenced by the chi-square method ($p < 0.01$) for the association of BVD with BLV. The percentages of BLV positive sera were significantly increased by the BVDV presence in population. Conclusion: PL status (linked with immunosuppression) is a little higher than the standard of the world and due attention to this fact that certain

isolates of BVDV have significant immunosuppressive activity in cows, our data suggests that the BVD infection in Iran may markedly affect the susceptibility of cattle to the BLV infections.
Keywords: BVDV, BLV, PL

961. Feasible Use of PediococcusAcidilactici as Probiotics for Improvement of Growth Factors and Water Quality in AequidensRivulatus (Green Terror)

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Background: The objective of this experiment was to test the probiotic *pediococcusjaacidilactici* during60-day feeding experimental period.Materials and Methods: Three experimental groups were considered as follow: The first group of controls (A), the second group fed with fish oil and feed (B) and The third group fed with 1gr*kg-1 bacteria (*pediococcusacidilactici*) and fish oil(C).At the initiation of experiment 60 pcs of fisheswere introduced for the treatments. For each experimental unit, average fish weight was 0.388±0.002 at the beginning of breeding period. Meanwhile, physicochemical factors of water including temperature, PH, EC, ammonia, nitrite and nitrate were measured during the breeding period.Along with theexperiment; water-soluble ions (ca2+, cl-, na+, k+ and mg2+) were measured. Results: Results showed that fish fed with probiotic*pediococcusacidilacticihada* higher growth compared with the other treatments ($P<0.05$). Conclusion: No significant differences were recognized between growth factor treatments (A), (B) ($P>0.05$). Measurement of water's Ammonia, nitrite and nitrate showed no significant differences between treatment of (C)and other treatments ($P<0.05$). However, water-soluble ionsresulted inno significant difference among different treatments ($P>0.05$). Given what is occluded, utilizing this bacterium is likely to have positive effect on growth and water quality for green terror fish.
Keywords:*Pediococcusacidilactici*, Aequidensrivulatus, probiotic, growth performance,water quality

962. Rabies in Sheep and Goats in Iran

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Background: Rabies is a neurological disease of mammals and is also azoonotic diseases. In sheep and goats, rabies has remained a public health problem of considerable importance in most countries. The disease is transmitted by the bite of an infected animal, or exposed to its saliva.This abstract describes rabies diagnosis in small ruminant in Iran in 2010. Materials and Methods: Fourteen Sheep and goats from 9 provinces were suspected to rabies. Two brain samples were taken from all affected animals soon after death. One portion of each sample was fixed in 10% formalin and the other was refrigerated and transported fresh on ice to the laboratory. Impressions of tissue samples from brainstem, thalamus, cerebellum, and the hippocampus (Ammon's horns) were examined for rabies infection using fluorescent antibody technique (FAT). Another portion of brain samples used for histopathological examination. Results: Using both techniques, both samples of all affected animals were positive for rabies. The results of histopathological examination revealed the Negri bodies in the cytoplasm of neurons. In FAT aggregates of nucleocapsid protein were seen by specific fluorescence of bound conjugate. Conclusion: Final goal of rabies control in animals is the reduction or elimination of human rabies. There are two useful techniques for control of animal rabies: prevention of exposure and pre-exposure vaccination.There is a little document about sheep and goat rabies in Iran which it could also be attributed to the poor reporting of owners about health situation of these animals.
Keywords: rabies,FAT, histopathological examination

963. Bovine Brucellosis Vaccination in IRAN

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Background: Brucella vaccination is one of the important measures for the prevention and control of brucellosis. In Iran, bovine brucellosis was first recognized in 1944 and is now endemic throughout the country.
Materials and Methods: In a retrospective study data were collected from Iran Veterinary organization.
Results and conclusion: In the 1950 the first sample of S19 vaccine at the dose rate of 400 was injected to some of the cattle around Hesarak by Razi institute. This vaccine was injected to adult cows in addition to calves but when the numbers of reactors were gradually reduced, vaccination was limited to4-8months calves. From 1959, a file was formed for every cattle around Tehran and milk ring test on the samples of livestock`s milk had been done. In these years after isolation of infected cows, S19 vaccine was injected to calves and non pregnant adult and vaccinated cows were marked by tattooing on ear. From 1973 vaccination of adult cows with full dose of S19 vaccine was deleted from program of vaccination and was injected only to 4-8 months calves and healthy adult cows were vaccinated with K45/20A. Injection of S19 vaccine to female calves in 4-8months age and injection of K45/20A vaccine to upper than 8 months and adults that had not been injected before. It should be noted that from the 1981 using of K45/20A vaccine in adult cows had been stopped.
From 1983 all of the 3-6 months male and female calves had been vaccinated with full dose of S19. In the 2005 entrance of reduced dose of S19 vaccine in the program, this vaccine was injected to adult cows in rural and nomadic regions and from the next year, means 2006, test and slaughter program in this population was stopped. In 2008, S19 vaccine was completely removed and now all industrial and rural cows are vaccinate with RB51 vaccine.
The control and prevention of brucellosis is far more complex than vaccination, testing and slaughtering of the reactors. A financially well-supported control and eradication program and joint efforts between the farmers and governmental authorities are needed as a means to prevent the spreading the disease. Without these, even a very good strategy will fail.
Keywords:Bovine Brucellosis, S19 vaccine, K45/20A vaccine, RB51 vaccine

964. Evaluation of Different Levels of Dietary Methionine on Serum Antibody Titers against Avian Influenza Vaccine (H9N2) in broiler chickens

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Background: Methionine is the First limiting amino acid in corn-soybean meal diets. Viruses of the family Orthomyxoviridae are responsible for Avian Influenza (AI) disease. Infection with AI viruses as well as immunization with vaccines elicits a humoral antibody response. The aim of this research was to study the effect of different levels of methionine on serum Antibody titers against AI vaccine in broiler chickens. Materials and Methods: One hundred and eighty day-old broiler chicks (Ross 308) divided in three groups (each group contain 60 chickens in 3 replicates). Group 1 (control) received base ration with 0.5%, 0.38% and 0.32% methionine in Starter (days: 1-14), grower (days: 15-28) and finisher (days: 29-42) ration respectively. Group 2 and 3 received ration with 0.6%, 0.5%, 0.4% and 0.7%, 0.6%, 0.5% methionine in Starter, grower and finisher ration respectively. Chicks were raised on floor-pen under standard condition until day 42 of age. Food and water were supplied *ad libitum*. At day 7 of age all groups inoculated via subcutaneous with killed AIV vaccine (subtype H9N2). At day 14, 28 and 42 of age, 10 chicks of each group bleed via wing vein and the sera referred to laboratory for HI test. Mean titers of Antibody in Groups 1, 2 and 3 at days 14 of age

were 3, 3.2, 3.5 and at day 28 and 42 were 3.1, 3.9, 4.7, and 3, 3.9, 4.6 respectively. Results: The results showed that antibody titers were higher in groups 2 and 3 compare to the control group. The highest mean of Antibody titers was seen in group 3 in starter, grower and finisher period that had significant differences with control group in 28 and 42 days of age. conclusion: So supplementation of methionine in poultry diet over than advised level by NRC, have positive effect in prevention of AI in broiler chickens.
Keywords: H9N2, Antibody titers, NRC

965. Serotyping and PCR-based Restriction Fragment Length Polymorphism for Subtyping of Salmonella from Chicken Isolates in Arak

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Background:Salmonella is the major cause of foodborne illness in poultry and its products. Avian salmonellosis are the most common avian diseases that are communicable to humans. Genotyping techniques such as PCR have been applied as alternative methods for Salmonella subtyping. PCR-based RFLP is considered a rapid, cost-effective approach with good reproducibility for molecular typing in bacterial epidemiologic studies. The flagellin genes such as *fliC* used for Salmonella subtyping. This gene encode proteins onto the Salmonella surface. Materials and Methods: A total 50 Salmonella isolates from poultry were subjected for serotyping. Genomic DNA was extracted by the standard phenol-chloroform method. The specific primers for amplification of *fliC* gene were designed and PCR was standardized. Amplification products were detected by electrophoresis. The *fliC* PCR products were directly digested by the restriction endonucleases *Sau3AI* and *HhaI*.Results: 5 serovars of salmonella were isoloated. All salmonella strains tested produced a single 1.5 kbp band. The RFLP analysis with *Sau3AI* revealed 5 discriminated restriction profiles. There were 4 distinct profiles obtained when *HhaI* endonuclease was used. The combination of these two restriction profiles could differentiate all of the serovars from each other.Conclusion: comparing the results of PCR-RFLP with serology experimentations showed that this molecular technique has a direct and significant relationship with serotyping and its result is approximately approving the result of serotyping. These surveys revealed that PCR-RFLP method based on *fliC* gene was a simple method with a high discriminatory power and it can be used to determine Salmonella serotypes.
Keywords: Salmonella, PCR_RFLP, Molecular detection, fliC gene, Serotyping

966. Serologic Monitoring of *Mycoplasma Gallisepticum* and *Mycoplasma Synoviae* in Poultry Industry Farms of Tehran, Semnan and Ardebil Provinces

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Background: *Mycoplasma gallisepticum*(*M. gallisepticum*) and *Mycoplasma synoviae*(*M.synoviae*) are the most important poultry pathogens that cause serious economic losses in the poultry industry. One of the diagnostic methods for identify them are serologic tests. Rapid Serum Agglutination (RSA) and Enzyme Linked Immunosorbent Assays (ELISA) are the most important serologic tests that use for diagnosing *M.gallisepticum* and *M.synoviae*. The aim of this study was serogical monitoring of *M. gallisepticum* and *M.synoviae* in poultry industry of Tehran, Semanan and Ardebil provinces. Materials and methods: A total of 1391 serum samples were collected from 50 industry poultry farms of Tehran, Semnan and Ardebil provinces which were sent to the Mycoplasma reference laboratory in Razi research vaccine and serology institute and then used RSA and ELISA for diagnosing *M. gallisepticum* and *M.synoviae*. Results: Of 1155 serum samples that were collected from poultry industry farms of Tehran province for diagnosing *M.synoviae*, 842 of them were positive in RSA test and 662 samples were positive in ELISA, of 81 serum samples were collected from poultry industry farms of Tehran province for diagnosing *M.synoviae*, 65 of them were positive in RSA test and 62 samples were positive in ELISA, of 155 serum samples were collected from poultry industry farms of Ardebil province for diagnosing *M.synoviae*,147 of them were positive in RSA test and 77 samples were positive in ELISA of 1155 serum samples that were collected from poultry industry farms of Tehran province for diagnosing *M. gallisepticum*, 274 of them were positive in RSA test and 292 samples were positive in ELISA, of 81 serum samples that were collected from poultry industry farms of Tehran province for diagnosing *M. gallisepticum*, 16 of them were positive in RSA test and 1 sample were positive in ELISA, of 155 serum samples that were collected from poultry industry farms of Ardebil province for diagnosing *M. gallisepticum*,83 of them were positive in RSA test and 25 samples were positive in ELISA. Conclusion: This study showed that the industry poultry farms of Tehran, Semnan and Ardebil had higher prevalence of *M.synoviae* than *M. gallisepticum* infection,and this study suggested that ELISA as an additional test for definitive diagnosis of *M.synoviae*and *M. gallisepticum* in industry poultry farms of Iran.
Keywords: *Mycoplasma gallisepticum* - *Mycoplasma synoviae* - RSA - ELISA

967. Small Ruminants Brucellosis Vaccination in IRAN

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Background: Brucellosis, especially caused by Brucella melitensis, remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually. In Iran, brucellosis was first recognized in 1932 and control of small ruminant brucellosis began in 1963. Materials and Methods: In a retrospective study data were collected from Iran Veterinary organization.Results and conclusion: Production of REV1 vaccine had been started in Iran from1963 with cooperation of WHO. First vaccination of kid and lamb`s population was done in 1963 which lambs and kids upper 3 months until one month before their first breeding and also non pregnant adult sheep at the end of their lactating period until one month before fertilization were vaccinated by full dose vaccine with cut the peace of their ears. From 1974 vaccination of adult female sheep and goats had been stopped because of causing standard serumal antibody and difficulty in diagnosis and just female lambs and kids upper 3 months until one month before fertilization and also males that are kept for fertilization are vaccinated with full dose and for recognition, one part of their ear is cut. From the beginning of 2004 in addition to vaccination program of lamb and kid, vaccination of adult animals with reduced dose of Rev1 vaccine has been done and test slaughter program of sheep and goat had been stopped. In high risk regions, adult female sheep and goats are vaccinated with reduced dose vaccine (RDRev1) for every two year. Conclusion: In Iran B.melitensis biotype 1 in sheep, goat and man are the predominant infective biotype. The disease reported in sheep, goat, cattle,horse, camel and human. Brucella vaccination is one of the important measures for the prevention and control of brucellosis and has been receiving more attention.
Keywords: Small Ruminants Brucellosis, Vaccination, RDRev1

968. Evaluations of Hilyses®, Fermented *Saccharomyces Cerevisia*, on Rainbow Trout (*Oncorhyncus Mykiss*) Growth Performance, Goblet Cells of Intestine and Immune Responses

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Background: A feeding study was conducted to determine the effect of dietary nucleotides (NT) on gastrointestinal structure, growth performance and immune responses of rainbow trout. Materials and Methods: A basal diet supplemented with 0 (control), and 1 g NT kg-1 to formulate two experimental diets. Each diet was randomly allocated to triplicate groups of fish with initial average weight of approximately 110 g for 50 days. Results: Results of this study showed that nucleotides supplementation significantly increased weight gain, specific growth rate and feed intake and decreased feed conversion ratio compared to control ($P<0.05$). The serum lysozyme activity of NT group was significantly higher ($p < 0.05$)

than the control group. Light microscopy demonstrated that both groups of fish displayed normal morphology of proximal intestine and pyloric caeca. In NT treated group, higher percentage of goblet cell was shown in proximal intestine and pyloric caeca. Conclusion: Present study suggests that NT effectively promotes growth performance, serum lysozyme and gastrointestinal structure in rainbow trout.
Keywords: Nucleotide _ Rainbow trout _Histology _ Lysozyme _ Growth performance

969. Effect of Dietary Ergosan on Gastrointestinal Structure, Hematological Parameters and Immune Responses of Rainbow Trout (*Oncorhynchus Mykiss*)

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Background: Present study examined the effects of Ergosan on gastrointestinal structure, hematological parameters and immune responses of rainbow trout (*Oncorhynchus mykiss*). Materials and Methods: Rainbow trout (mean weight 100–110 g) were fed basal diet (control) and diet treated with Ergosan (5 g kg-1 of diet) for 50 days. Results: Light microscopy demonstrated that Ergosan treated group, higher percentage of intestinal goblet cell was shown in proximal intestine and pyloric caeca (P<0.05). Leukocyte and erythrocyte count also increased in juvenile fish fed Ergosan-treated diet compared to control (P<0.05). Lysozyme activity was enhanced during Ergosan supplementation compared to control fish (P<0.05). Conclusion: Present study suggests that Ergosan effectively promotes Leukocyte, lysozyme and goblet cell count of intestine in rainbow trout.
Keywords: Ergosan _ Rainbow trout _goblet cell _ Lysozyme _ Leukocyte

970. The IgG Content in the Blood Serum and Uterine Fluid of Follicular and Luteal Cyst in the Bovine; a Comparison with Normal Cyclic Cows

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Background: Immunoglobulin G (IgG) is the predominant immunoglobulin in uterine fluid of the cow, plays an important role in physiologic regulation of uterine immunity during estrous cycle. Ovarian cysts are defined as anovulatory fluid-filled structures ≥ 25 mm in diameter that persist on the ovaries for more than 10 days. We have investigated the possible linkage between serum and uterine fluid IgG levels of the cow by determining the concentrations of IgG in blood serum and uterine fluid of cattle with ovarian/luteal cystand comparisonthese values with that of in normal cyclic cows. Materials and Methods: Reproductive tracts and blood samples were collected from 240 adult dairy cows slaughtered in Urmia abattoir, northwest of Iran. Among these cows 3 Luteal and 3 follicular cystic ovaries were distinguished. The uterine fluid was collected by gentle scraping the endometrium by a curette. The blood was allowed to clot and the serum separated by centrifugation. All uterine fluid and blood serum samples were stored in -20°C until analysis. IgG levels in serum and uterine fluids were estimated by using single radial immunodiffusion. Results: Mean serum IgG concentrations were 35.5 and 45 mg/ml for cows diagnosed as having luteal and follicular cysts, respectively. Mean uterine fluid IgG concentrations were 50.5 mg/ml for cows diagnosed as having luteal cyst which was similar to that of luteal phase of normal cyclic cows (41.2 mg/ml). In follicular cystic cows, it was 10 mg/ml which was similar to follicular phase of normal cyclic cows (11.25 mg/ml).
Conclusion: Results suggest that the ovarian steroids may be involved in regulation of uterine IgG levels. Accordingly, the prolonged estrogenic and progestagenic periods in cows with follicular and luteal cyst (respectively), impair appropriate function of reproductive system via irregularity in uterine immunoglobulins secretion.
Keywords: Cow; Luteal cyst; Follicular cyst; Uterine fluid; IgG.

971. Effect of Different Levels of Vitamin C on Immune Parameters of *Barbus Sharpyie*

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Background: Present study was carried out to assess the effects of various levels of vitamine C on immunological indices of *Barbus sharpyie* fingerlings.Materials and methods: Experimental phase of the study was conducted from September to November 2010. Five experimental treatments (T1 control; T2 500; T3 1000;T4 1500; and T5 2000 mg vitamin c/kg diet) in triplicates and based on complete random design were set up to carry out the study. In each treatment 300L tanks containing 20 experimental fish (7.5±0.33 cm and 6.8±0.3 g) were used. Tanks were provided with aerators and water temperature was maintained at 25.6 ±1.74 ^{0}C. Experimental fish were fed to satiation twice daily. At the end of the experimental period blood samples were taken and divided to two parts. One part was immediately centrifuged (5 min at 5000g, Hettich D7200) at room temperature serum separated and stored at -80°C until analysis. Serum lysozyme activity was assayed based on the lysis of the lysozyme sensitive Gram positive bacterium, *Micrococcus lysodeikticus*. WBCs counts was done after dilution of blood with dacie's fluid.Results: Lysozyme activity was significantly (P < or = 0.05) enhanced by increasing doses of vitamin C supplementation. Leukocytes were increased significantly till 1000 mg vit. C/Kg diet but after that decreased in 1500 mg vit. C/Kg diet. Conclusion: Based on the results, 1000mg vitamin C per kg diet is recommended for rearing of *Barbus sharpyie* fingerlings.
Keywords: Barbus sharpeyi, Vitamin C, Immunity

972. Effects of Dietary *Echinacea Purpurea* on Growth Indices and Immune Parameters in Rainbow Trout (*Oncorhynchus Mykiss*) Fingerlings

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Background: An appropriate approach in preventing fish disease and therefore increase benefit in aquaculture is application of immunostimulants. *Echinacea purpurea* (EP) or purple coneflower is a member of the Asteraceae (daisy) family that has paramunity-inducing and non-specific immune responses stimulating effects. Materials and Methods: A basal diet was supplemented with 0 (control), 0.25, 0.5, 1 and 2 g EP Kg^{-1} to formulate five experimental diets. Fish were fed 8 weeks with these experimental diets. The blood samples were immediately centrifuged to separate plasma or serum and stored at -80°C until analysis. The plasma samples were used for immunoglobulin M (IgM) analysis and the serum samples for determining lysozyme and alternative complement activity.Results: The serum content of IgM, C3 and C4 and lysozyme activity increased in experimental group compare to control group (P < 0.05). Conclusion: Our finding showed that EP has a significant effect on *O. mykiss* immune parameters and could be used to improve fish immunological parameters.
Keywords: *Echinacea purpurea*, *Oncorhynchus mykiss*, immunity, growth

973. Seroprevalence of Bovine Viral Diarrhea Virus, Bovine Herpesvirus 1 and Bovine Leukemia Virus in Iranian Cattle and Associations among Studied Agents

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Background: Bovine viral diarrhea virus (BVDV), bovine herpesvirus type 1 (BoHV1) and bovine leukemia virus (BLV) are among the most important pathogens of dairy cattle which cause significant economic burden around the world. The aim of this study was to investigate the prevalence and concurrent presence of antibodies against BVDV, BoHV1 and BLV in dairy cattle in Iran. Materials and Methods: A total of 882 animals were sampled and the presence of antibodies against BVDV, BoHV1 and BLV was determined by commercial ELISA kits. Results: The overall BVDV, BoHV1 and BLV seroprevalence was 64.4%, 31.9% and 16.2%, respectively. The prevalence of antibodies against mentioned pathogens varied among different provinces studied. In addition, no correlation was observed between BLV seropositivity and either BVDV or BoHV1 seropositivity($P>0.01$) while there was a positive correlation between BVDV and BoHV1seropositivity($P<0.01$). Conclusion: The results of the current study revealed that BVDV, BoHV1 and BLV infections are present in different regions of Iran and thus it seems necessary to implement control programs to prevent further spread of mentioned pathogens. Moreover, it was demonstrated that a previous infection with either BVDV or BoHV1 might be a risk factor for future infection with BoHV1 or BVDV, respectively.
Keywords: BVDV, BoHV1, ELISA

974. Effects of Ewe Parturition Number and Litter Type on Colostral Immunoglobulin G Concentrations in Fat-tailed Sheep

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Background: Successful transfer of colostralimmunoglobulins is the only way to provide protection in neonate ruminants. It has been suggested that colostralIgG concentration varies between and within sheep breeds and also could beaffected by maternal factors. Accordingly, we investigated the possible effects of litter type and ewe parturition number on colostralIgG concentrations in two Iranian fat-tailed sheep (Shaul and Lori Bakhtyari). Materials and Methods: ColostralIgG concentrations of 30Shaul and 57 Lori Bakhtyari ewes were measured by single radial immunodiffusion (SRID), zinc sulfate turbidity (ZST) and Biuret methods. Results: Lori Bakhtyari ewes had significantly lower colostralIgG level than Shaul ewes ($p < 0.05$). Although there was a significant difference, in colostralIgG level, between first and third parturition of Lori Bakhtyari ewes, Litter type and ewe parturition number overall had no significant effects on colostralIgG concentrations. The amount of colostralIgG was not associated with ewe serum IgGlevel. The Bland-Altman graph showed thatthere was an agreement between SRID and Biuret method (Pitman's Test of difference in variance: $r = -0.164$, $p = 0.235$).No agreement was observed between SRID and ZST (Pitman's Test of difference in variance: $r = 0.717$, $p < 0.01$). Conclusion: While colostralIgG concentrations vary widely between individuals and may influence the health of newborn lambs, it seems necessary to monitor colostralIgG levels in colostrum to ensure adequate transfer of maternal antibodies to offspring. Moreover, the results of this study revealed that measurement of colostral total protein by Biuret assay can be used as an inexpensive and easy method to predict immunoglobulin content of sheep colostrum.
Keywords: SRID, Ewe Parturition, ColostralIgG, zinc sulfate turbidity (ZST), Biuret methods.

975. Effect of Dietary Garlic on Serum Antibody Titers against Avian Influenza Vaccine (H9N2) in broiler chickens

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Background: Garlic (*Allium sativum*) is widely used as either a flavoring agent for food or as a medicinal agent for the treatment of a variety of diseases, ranging from infections to heart diseases.Avian Influenza (AI) disease is caused by a virus of Orthomyxoviridae family. Stimulation of immune response has important effects on the protection levels achieved by vaccination against AI. In this research the effect of different levels of garlic on serum antibody titers against Avian Influenza disease vaccine was investigated in broiler chickens. Materials and Methods: One hundred and eighty day-old broiler chicks (Ross 308) were purchase and divided in four groups (each group contain 45 chickens in 3 replicates). Group 1 (control) received base ration without any additive. Group 2, 3 and 4 received base ration plus 0.2%, 0.6% and 1% garlic powder throughout the experiment. Chicks were raised on floor-pen under standard condition until day 42 of age. Food and water were supplied *ad libitum*. At day 7 of age all groups inoculated via subcutaneous with killed AIV vaccine (subtype H9N2). At day 14, 28 and 42 of age, 10 chicks of each group bleed via wing vein and the sera referred to laboratory for HI test. Results: Mean titers of antibody in Groups 1, 2, 3 and 4 and days 14, 28 and 42 of age were 2.7, 2.6, 3.1, 3.2 and 2.7, 2.7, 2.9, 3.2 and 3.1, 3.4, 3.6, 3.4 respectively.The results showed that antibody titers were higher in 0.6% and 1% garlic treated groups compare to the control group but these differences were not significant. Conclusion: The results of this research showed that use of garlic in ration of broiler chicken has no significant positive effect on the antibody response against Avian Influenza disease vaccine.
Keywords: Garlic, antibody titers, Avian Influenza

976. The Effects of Rev-1 Vaccination of Sheep and Goats on Human Brucellosis in IRAN

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Background: Brucellosis from Brucella melitensis is an important zoonosis that constitutes a serious hazard to public health. Control and eradication programmes have been implemented in many countries where brucellosis exists. Vaccination is the only suitable method for controlling the infection and this must be the first step for the elimination of the disease. In Iran, brucellosis was first recognized in 1932 and control of small ruminant brucellosis began in 1963. Our aim was to investigate the effects of the measures implemented in Iran from 1990 to 2009 on the vaccination coverage with the incidence in humans. Materials and Methods: The relationship between the vaccination coverage with the incidence of brucellosis in humans was assessed with the use of rank correlation. Results: It was a positive rank correlation (-0.6) among these variables. Mass-vaccination campaign of animals of all ages seemed to have decreased the human incidence. Conclusion: The control of brucellosis can be achieved if the population's resistance to disease would be increased by vaccination. It is accepted that vaccination is more acceptable and effective than other methods applied for this purpose. Also the vaccines used practically eliminate the clinical signs of brucellosis, so contamination of the environment and exposure of population at risk to the infectious agent are reduced.
Keywords: Rev-1 Vaccination, Brucellosis, Mass-vaccination

977. Effects of Oral Administration of Silver Nano Particles on the Function of Immune System and Inflammatory Responses on Male Rat

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Background: Since use of nano combinations in different products and industries is increasingly growing,studying effects of these substances on environment and body organs is very important. Toxicity of nano particles,depends on different parameters such as:size, shape, chemical composition, surface charge and so on.Silver nano particles are widely being applied in different products, because of their unique antimicrobial activity. Materials and Methods: In this study, we considered the toxicity effects of silver nano particles of 71nm in different doses, administered orally for one month, in male rats. We put forty matured male rats in four groups of ten, including: 1.contorol group.2.treated with AgNPs orally, for thirty days in a 0.25 mg/kg dose with food.3.treated with AgNPs orally for thirty days in a 0.5 mg/kg dose with food. 4. treated with AgyNPs

orally for thirty days in a 1 mg/kg dose with food. After one month kept under the same labrotary conditions, samples of complete blood, liver and kidney of all rats were collected for biochemistry and histopathology experiments.
Results: According to the experiment results, the levels of IL-4, IL-6,IL-1,IL-12,TGF-B and IL-10 in treated groups were significantly increased in comparison to control group. This dose-dependent increase of silver nanoparticles was increased with the increase of the dose received, so that the most increase occurred in treated group with 1mg/kg dose. Study of histopathology of internal organs showed accumulation of AgNPs in internal organs, especially kidney and liver. These particles, cause necrosis, degeneration and death of hepatic and kidney cells. The extent of lesions increased with increasing dose administered. Concloution: According to the results, toxicity of AgNPs is dose-dependent and the greater the mass absorbed, the greater the effect. Also, oral administration of AgNPs continuously, exerts inflammatory responses and induces internal organs toxicity.
Keywords: Silver Nano Particles, AgyNPs, inflammatory responses

978. Anesthetic Effect of Clove Oil on Some Innate Immune Response in Rainbow Trout *Onchorhynchus Mykiss*

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Background: Practices that require fish handling are a common source of stress in aquaculture operations and research activities. Therefore, application of anesthesia have important role in aquaculture industry. However, the types of anesthetic that affect the physiology of the fish are the important points. The present study aimed to study effect of Clove oil (relative to non-anaesthetized controls) on some innate immunity response. Materials and Methods: Sixty rainbow trout weighting (Mean weight 100±10g, mean length 25±2cm) were held in a recirculation system of the aquatic animal health department of University of Tehran. Experimental groups of fish were established, each containing 15 fish. One group was maintained without anesthesia as a control, the other group was anesthetized with 25-ppm clove oil. The experiment was triply repeated for treatment and control. Respiratory burst activity (NBT test), ACH50 (available methods Matsuyama et al. 1988) and Lysozyme activity (turbidimetric method assay), 1 h post anesthesia measured, to recognize any impacts of clove oil in narcosis stage. For data analyses, T student test (SYSTAT 16.0 software, SPSS) was used. Results: Recovery time for anesthetics was 180±12 sec clove oil. No statistically significant difference was noted in Lysozyme activity 113.8±14.00 (µg/ml), ACH50 (18.13±11.99 Unit /ML) and NBT test (۰.۲۵۲±0.011nm) of treatment in comparison with control, 1 hour post anesthesia (p>0.05). Conclusion: it seems that due to our results 1 h post anesthesia clove oil doesn't suppress innate immunity in rainbow trout and therefore might be suitable for aquaculture operations.
Keywords:Clove Oil, NBT test, *Onchorhynchus Mykiss*, Innate Immune Response

979. Immune Response Stimulation of Levamisol Compared with Yoghurt in Some Birds

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Background: Levamisole is one of the antihelminths drugs with immunostimulatore activity, also Yoghurt is an enriched domestic lactative dairy probiotic . Materials and Methods:Here levamisol and Yoghurt have used in birds garden as immunostimulation for ND vaccination in 5 groups of birds : 2 poultry (Included Fowls and Peacocks)and 2 aquatic birds(Included Pelicans and Ducks) and a single group of Fowl for yoghurt test ,and the control groups.The birds remain in the Isfahan birds garden and marked and remained in 5 test groups as A(8 Fowls),B(8 Peacocks),C(8 Pelicans) and D(8 Ducks), and a single group for Yoghurt (E:8 Fowl) meanwhile control group (F) , included 32 birds of test groups without Levamisol and/or Yoghurt (8 Fowls,8 Peacocks,8 Pelicans and 8 Ducks).Test groups of A,B,C,D were treated for 4 mg of levamisol orally/day for a week, The birds of E groups have feed for a week with a mixed diet of 50g/kg (M/M) , and then Newcastle Disease Lasota vaccination achieved for all the birds as oral routs and 10 days post vaccination the blood samples of all of the birds of test and control groups were collected via wing vein and tested for serological HI test and WBC counting.Results and conclusion:Regarding to the results and statistical analysis (P<0.05)the HI titer in all of test groups increased comparing with control groups, however the WBC counting shows a little increasing of Hetrophils and Lymphocytes in test groups of Levamisol treated ,but not important changes in the E group.
Keywords: Immunostimulation, levamisol, Yoghurt, Birds,

STUDENTS' SYMPOSIUM

Oral Presentation

980. Evaluation of PPV in Diagnosis of Systemic Lupus Erythematous by Physicians in Compare with Laboratory Evidence in Esfahan from April 2010 to October 2010

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Background**:** Systemic Lupus Erythematous is an autoimmune disease that can involvevariousorgansbecause of immunecomplex-mediated due to auto-antibodiesboundtothe cells. Global prevalence of 0.00015% until 0.00050% is a variable. Ten criteria are helpful in diagnosing SLE includes: rash manner, discoed rash, sensitivity to light, mouth ulcers, arthritis, Serositis, kidney disorders, neurological disorders, hematological disorders and immunological disorders. If there areat least4 criteriaofthediagnostic criteriaabove, the riskoflupustaking considerandtestpositiveofanti-nuclearantibody(ANA),lupusdiseasecan beconfirm. This test, Despite the low detection sensitivity (75%), but has very high specificity (95%). Use of other diagnostic tests such as anti-ds DNA (sensitivity and specificity approaching 70%) is helpful in diagnosis. **Materials and Methods:** In this study, with cluster sampling method, 162 patients (10 males and 152 females) with mean age of 23.8 years (age range 15-30 years) with at least four diagnostic criteria, physician diagnosis of lupus and to confirm the diagnosis had been referred to the laboratory, studied by the ANA test. ANA assay method of Indirect Fluorescent Assay (IFA) and the method by *Euroimmune* kits with a sensitivity of 76% and specificity 94% were done. Results**:** Among the 162 individuals tested, 22 (including 20 women and 2 men) were positive. The Catching rate mean, of 75% suspected cases with clinical observation are referred just 13.5% were definite SLE correctly. Interestingly the average age with a positive test (26.3) was determined according to statistical analysis using SPSS 18 software and statistical test were independent t-test, significant relationship between infection and diagnosis of lupus correctly (P value <0.05). Point in the calculation of the statistical correlation between gender and the correct diagnosis of lupus is based on laboratory methods and clinical examinations were seen (P value> 0.05). Discussion: According to theresultsobtained inthisstudy, it seems that the PositivePredictive Value(Ratio of true positive casestototalcasessuspected ofreference) for thediagnosis oflupusbyIranianPhysicians (5/13%) than thebenchmark(56%) isasignificant difference. Itis recommendedto avoidthe enormouscostsassociated withthewrongdiagnosis, the appropriatesolutionto be consideredbythe health caresystem.
Keywords**:** Lupus, clinical diagnosis, indirectimmunofluorescence, Ppositive Predictive Value

981. A Single Intramuscular Injection of Mega Doses Vitamin DReduced the Inflammatory Factor TNF-α and Increases Serum Adiponectin after Delivery in Mothers with Gestational Diabetes Mellitus

Hoseinzadeh M

Background: In the past, it was mainly focused on the role of vitamin D in biological activities such as maintaining mineral homeostasis and bone metabolism. But recently identified vitamin D receptors in various tissues, including T lymphocytes, macrophages, thymus tissue and in

pancreatic beta cells, more than ever the importance of this vitamin has increased. The purposes of this study were to determine the effect of single dose administration of 300,000 units of vitamin D after delivery in women with gestational diabetes after 3 months follow-up on the status of inflammatory factors influencing insulin resistance, including TNF-α, IL-1, protein acute phase reactant (CRP) and adiponectin.Materials and Methods:This is a randomized clinical trial study with the follow-up period of 3 months. Totally 45 women who diagnosed for the first time in their recent pregnancy were randomly divided into intervention group (IG) and control group (CG). The IG received an IM of a dose of 300,000 units of vitamin D while the other group did not. After an overnight fasting, 6ml blood was taken from all the patients up to 10 days after delivery and at the end of intervention. Glycosylated hemoglobin A1C (HBA1C), 25 -OH vitamin D, and two-hour glucose, TNF-α, interleukin -1, CRP, and adiponectin were measured. For data analysis software package SPSS version 11 was used.Results:Totally 45 patients including 24 with the age average of 30.7±6.2 in the IG and 21 with the age average of 29.5±4.0 in the CG participated in the study. Mean adiponectin before and after intervention between the two groups showed no significant difference, but only in IG after the intervention compared to before its results can be significantly increased. Median concentration of 25 - OH vitamin D levels in the intervention group before and after 24.25 and 62.1 nmol/L, respectively (P-value<0.001), while the figures in the CG was 25.3 and 24.1, respectively (P-value=0.02). Median of TNF-α in IG of 6.2 pico grams/L at the start of the intervention was decreased to 3.05 at the end of intervention, while in CG of 1.25 to 3.95 has increased. Median of IL-1 at the beginning and end of intervention between the two groups showed no significant difference, while it was significantly increased at the end of intervention in both groups.Conclusion: This study showed that IM administration of mega-dose supplements of vitamin D 300,000 units to women with gestational diabetes after delivery, improves vitamin D status, reduces the inflammatory factor TNF-α and increases adiponectin.
Keywords: Gestational Diabetes Mellitus, Inflammatory Factors, Adiponectin, IL-1, TNF-α

982. Association of TLR2 Protein with Serum ALT in Chronic Hepatitis B Patients Harboring Pre-core Mutant

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Background:Chronic hepatitis B patients infected with pre-core mutant variant of hepatitis B virus may show elevated or fluctuating of ALT and HBV DNA levels with histologic activity on biopsies indicative of inflammation and injury. The Toll-like receptor 2 (TLR2) has important rolein the innate immune response.The aim of this study was to investigate the association of serum TLR2 with clinical findings of HBeAg negative chronic hepatitis B patients.Materials and Methods: Fifty one HBeAg negative patients with detectable HBV DNA were examined for the presence of mutations in pre-core region of HBV genome. Concentration of serum TLR2 was measured by enzyme-linked immunoassays. Interaction of tertiary structure of truncated HBeAg and TLR2 (2Z80 A) was evaluated with molecular docking.Results:Serum TLR2 showed higher concentration in patients with G1896A mutation compare to patients without this mutation (4.8±2.9 vs 3.4±2.2 ng/mL, P=0.03). Estimating regression equations showed with increasing serum TLR2 concentration bigger than 6, serum ALT raises sharply. Computational molecular docking studies showed an interaction between truncated HBeAg and TLR2. Conclusion: Matching with previous studies, the present results suggest that increase of serum TLR2 in pre-core mutant patients reflect higher expression of membrane-bound TLR2 and its pro-inflammatory effect for hepatocyte damage and elevations in the serum ALT.
Keywords: Chronic hepatitis B,Pre-core G1896A mutation, Toll-like receptor (TLR2)

983. Construction and Expression of Candidate Vaccine fimH/fliC against *Escherichia coli*Urinary Tract Infection

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Background: Urinary tract infection (UTI) is one of the most common infections in the world. UropathogenicEscherichia coli (UPEC) are the most frequent cause of cystitis and pyelonephritis. Type1 pili by having the adhesion FimH and flagella by having flagellin (FliC) are the important virulence factors of UPEC. To date, any ideal vaccine against UTI has not been approved for human use and thus we need to test new target antigens to develop an ideal and safe vaccine against UTI. In this study, we constructed recombinant fusion fimH/fliC of UPEC as a novel candidate vaccine against UTI. The immunological properties of the fusion protein are in progress.Materials and Methods:Uropathogenic Escherichia coli were isolated from the UTI patients. The fimH and fliC genes were amplified by PCR. Construction of fimH-fliC fusion protein was performed by overlap PCR with fusion primers and the genes were cloned in pET28a vector. The confirmation of expression of the proteins was done by SDS-PAGE and Western blot.Results:The fliC and fimH genes were amplified in all of the UPEC strains tested. The fimH and fliC sequences showed significant homology with the sequences in Genbank. We generated a fusion protein consisting of the fimH protein linked to the N-terminal end of fliC. Sequencing of the fusion fimH-fliC by internal and universal primers showed that fusion was constructed precisely. SDS-PAGE and western blot confirmed the expression of the proteins.Conclusion: Urinary tract infections (UTI) are the second most common infection. Some of the virulence factors of UPEC have been tested as vaccine targets against UTI that have limited success. Recombinant fusion protein strategies offer a significant advantage in inducing enhanced antigen specific cellular and humoral responses.
Keywords: fimH/fliC, *Escherichia coli*, Urinary Tract Infection

984. Evaluation of PPV in Diagnosis of Systemic Lupus Erythematous by Physicians in compare with Laboratory evidence in Esfahan from April 2010 to October 2010

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Background: Systemic Lupus Erythematous is an autoimmune disease that can involve various organs because of immune complex-mediated due to auto-antibodies bound to the cells. Global prevalence of 0.00015% until 0.00050% is a variable. Ten criteria are helpful in diagnosing SLE includes: rash manner, discoed rash, sensitivity to light, mouth ulcers, arthritis, Serositis, kidney disorders, neurological disorders, hematological disorders and immunological disorders. If there are at least 4 criteria of the diagnostic criteria above, the risk of lupus taking consider and test positive of anti-nuclear antibody (ANA), lupus disease can be confirm. This test, Despite the low detection sensitivity (75%), but has very high specificity (95%). Use of other diagnostic tests such as anti-ds DNA (sensitivity and specificity approaching 70%) is helpful in diagnosis. Materials and Methods: In this study, with cluster sampling method, 162 patients (10 males and 152 females) with mean age of 23.8 years (age range 15-30 years) with at least four diagnostic criteria, physician diagnosis of lupus and to confirm the diagnosis had been referred to the laboratory, studied by the ANA test. ANA assay method of Indirect Fluorescent Assay (IFA) and the method by *Euroimmune*kits with a sensitivity of 76% and specificity 94% were done. Results: Among the 162 individuals tested, 22 (including 20 women and 2 men) were positive. The Catching rate mean, of 75% suspected cases with clinical observation are referred just 13.5% were definite SLE correctly. Interestingly the average age with a positive test (26.3) was determined according to statistical analysis using SPSS 18 software and statistical test were independent t-test, significant relationship between infection and diagnosis of lupus correctly (P value <0.05). Point in the calculation of the statistical correlation between gender and the correct diagnosis of lupus is based on laboratory methods and clinical examinations were seen (P value> 0.05). Conclusion: According to the results obtained in this study, it seems that the Positive Predictive Value (Ratio of true positive cases to total cases suspected of reference) for the diagnosis of lupus by Iranian Physicians (5/13%) than the benchmark (56%) is a significant difference. It is recommended to avoid the enormous costs associated with the wrong diagnosis, the appropriate solution to be considered by the health care system.
Keywords:Lupus, clinical diagnosis, indirect immunofluorescence, Ppositive Predictive Value

985. Evaluation of LIVIN Protein Expression, as Promising Marker for Infiltrating Background and Malignant Cells in Hodgkin Lymphoma, Compared to Non-neoplastic Lymph Node

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Background: A novel human inhibitor of apoptosis protein (IAP) family member termed Livin, was demonstrated in pathogenesis of different human malignancies, and also is being investigated as potential treatment targets in cancer patients. However there is no report on Livin expression in Hodgkin Lymphoma.Materials andMethods: In this study, we evaluated Livin expression in 78 paraffin embed block including evaluated Livin expression in 39 staged cases of HL in comparison with 39 control subjects(normal and reactive hyperplasia lymph nodes) which are randomly selected. Tissue Microarray-based Semi-quantitative Immuno-flourecent Staining was applied for protein expression profiling in control subjects and also both infiltrating non-neoplastic cells (preferentially Lymphocytes) and neoplastic cells (Hodgkin and Reed-Sternberg) of cases. Results: At this study the mean ratio of Livin /GAPDH expression was significantly increased between infiltrating background cells in hodgkin Lymphomas and control cases (0.54596 Vs 0.50827,P<0.001). Also a significant difference was found in mean ratio of Livin /GAPDH expression between neoplastic cells (HRS) and major background cells in tumor microenvironment (0.59024 Vs 0.54596 , p<0.001), Furthermore, this study confirmed significant increase of livin expression in Early-stage toward Advanced-stage in HL (0.52888 Vs 0.580146 , P<0.01).Conclusion:these findings suggest that the Livin may have critical role in the pathogenesis of Hodgkin lymphoma and also could be a novel prognostic marker in this kind of lymphoma. In summary, Livin can be regarded as a promising target for experimental anticancer therapy in patient with HL.

Keywords: Hodgkin lymphoma, Reed-Sternberg cell, Inhibitor of Apoptosis Protein, Livin/BIRC 7

986. Naloxone Influences the Pathogenicity and Cellular Immune Responses of BALB/c Mice Infected with *Leishmania major*

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Background: Leishmaniases are caused by protozoan parasite of genus Leishmania. This parasite can cause a wide spectrum of clinical manifestations ranging from a subclinical or self healing cutaneous lesion to progressive fatal visceral disease, depending on the parasite species and immune status of the host. In experimental models, infection with *L. major* results in different manifestations in distinct inbred mice. While, C57BL/6 mice show resistance against L. major infection and cause a Th1 response, susceptible BALB/c mice suffer from progressive disease which associates with predominant Th2 response. In this study, the effect of Naloxone (an Opoid antagonist) was studied in BALB/c mice infected with *L. major* to evaluate the ability of Naloxone in modulation of immune responses in these susceptible mice. Materials and Methods: In the present study, two groups of mice inoculated each with 5×104L. majorpromastigotes from which one group treated with Naloxone (0.1 mg, one dose) and compared with other group by measurement of foot pad swelling and parasite burden in lymph node (LN) of mice. Likewise, the proliferative response and also cytokines responses were assessed in lymph node culture of mice by ELISA. Results: The results obtained showed significantly lower lesion size in treated mice than untreated mice, and all untreated mice died within 16 weeks, but treated mice survived until 35 weeks. Moreover, parasite burden in treated mice reduced at 4, 6 and 8 weeks post injection. On the other hand, T cell proliferative responses in LN of treated mice were higher than untreated mice at 8 weeks post injection. Likewise, the results of cytokine assay showed higher responses of IFN-g and IL-12 in LN of treated than untreated mice, two weeks after injection, but not after 4 and 8 weeks. Lower responses of IL-4 were shown in treated mice after 4 weeks and IL-10 level was decreased in treated than untreated mice after 2, 4 and 8 weeks post infection. Conclusion: Data indicated that one dose of Naloxone have ability to reduce pathogenicity of L. major infection by decrease of lesion size and reduction of parasite burden. Moreover, naloxone induced the tendency of immune responses towards a Th1 response by increase of IFN-g and IL-12 and decrease of IL-10 at early period (2 weeks) post infection, which continued by induction of a decrease in IL-4 and IL-10 level at late period (4 and 8 weeks) post infection.

Keyword: BALB/C mice, Naloxone, leishmania major,Th1 respons

987. The Effect of Zinc Supplementation on the Number of Lymphocytes in HIV-infected Patients: a Randomized Clinical Trial

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Background:Zinc is one of the elements that improves body's immune system and adequate zinc is critical for its function; however, zinc deficiency occurs in >50% of human immunodeficiency virus (HIV)-infected adults. Moreover, the prevalence of deaths is more among those with low serum concentrations of zinc. Studies have shown that zinc supplementation at nutritional levels delays immunological failure. This study was performed to further assess the efficacy of zinc supplementation on the number of lymphocytes in HIV-infected patients. Materials and Methods:In this interventional study participants attending "Navabsafavi" medical center in Isfahan, Iran in May and April 2009 were randomly assigned to receive zinc supplementation (zinc sulfate 45 mg per day) for about two months. The number of lymphocytes before and after the oral administration was counted and data was analyzed using SPSS 16 by chi-square and T-paired tests.Results:This Study was performed on 30 HIV-infected patients. The average lymphocyte counts before and after the administration was 33.8% ±7.3% and 35.6% ±8.2% respectively (P value> 0.05). Data was analyzed using t-paired test at a significant level of P<0.05.Conclusion:As the result was not significant, the effect of zinc supplementation on the number of lymphocytes was not justified. More studies with larger sample sizes, different doses of zinc supplementation and longer administration of zinc supplement are suggested. We would also like to acknowledge the national elite foundation that supported us attending the congress.

Keywords:Zinc, HIV, lymphocyte

988. Tick Effects on the Host Immunological Interactions and its Role in Designing Vaccines

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Ticks are one of the most important issues in medical and veterinary public health. Tick's damages are caused by blood-feeding and transmitting infectious agents which could be potential zoonotic disease threats.Ticks produce different kinds of biologically active molecules and tick-borne pathogens can involve innate and specific acquired immune responses in vertebrate hosts. The aim of these responses is to obtain acquired host resistance. The host acquired resistance to tick infestation involves different kinds of the immune system, including antigen presenting cell, B and T lymphocytes, cytokines, granulocytes, monocytes and complement system. In response to host immune system, tick-mediated host immunosuppressive countermeasures inhibit host immune defence. This suppression is caused by some bioactive molecules in ticks saliva such as; prostaglandin E2, interleuckin-2 inhibitors, T lymphocytes inhibitors, macrophage migration inhibitors, histamine binding proteins and anticomplement proteins. Tick saliva causes antibody response suppression and modulation of host cytokine and T-cell proliferation.Currently, because of acaricide resistance, drug resistance and lack of efficient vaccines, control of ticks and tick-borne pathogens are not an easy work and need to use novel methods and new sight of science. In this article we will discuss about tick immunobiology and ticks saliva pharmacology and vaccines that has been designed to protect hosts.

Keywords: Ticks, Tick-host-pathogen interaction, Immune suppression, Acquired immunity, Tick saliva.

Poster Presentation

989. A Survey on Different Types of Allergy Reactions in 25 Cats During 8 Months

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Background: In recent years, the prevalence of allergic reactions in the population of cats concerns the owners of these small animals and attracts more attention of veterinarians to the importance of this issue. An allergic reaction occurs when the immune system overreacts to the presence of a substance or material that is not harmful. These responses may involve skin, respiratory or digestive system. Allergic reactionsincatsareclassifiedinfourgroups: Food allergy, inhalation allergy, contact allergy and flea allergy. It is important for veterinarians to identify the types of allergy and select the correct way of treatment. Determining the prevalence of any type of allergy among cats is the main topic in order to increase the awareness of veterinarians and the owners and finally reduce the increasing prevalence of allergic reactions.

Materials and Methods: This present paper is a case study on 25 cats which were transferred to the Specified clinics (three clinics in Tehran and two clinics Karaj) from 90/01/30 to 90/09/30. Some prepared questions were asked from the owners of the cats about history of the cats, clinical signs, maintain conditions, patients' diet, the type of detergents. Veterinarians do further examinations and diagnostic tests such as blood test, urinary test, fecal test, sampling of skin, then necessary actions were done, finally the type of allergy were detected in patients. Results:Following statistical analysis, the highest incidence was related to food allergy (48%) and about the others, inhalant allergy (28%), contact allergy (20%) and flea allergy (4%). **Conclusion:** Specializing veterinarians who were involved in this statistical project have stated that some factors may increase the risk of allergies such as preparation of diet without paying attention to physiological structure of cat body, no attention to pollutants in the maintain environment, use of inappropriate detergents, failure to observe the basic points about the maintenance of cats.

Keywords: allergy, cat, clinic, Tehran, Karaj

990. Frequency of Cyclic Citrulline Peptide Antibody (Anticcp) and Rheumatoid Factor (RF) in Individuals Referring to Tooba Special Clinic in the First Half of the Year 1390

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Background:Rheumatoid Arthritis (RA)is one of the most common autoimmune diseases which can cause cartilage damage. RF (Rheumatoid Factor) is highly sensitive but not specific for the diagnosis of RA. Today, AntiCCP (cyclic citrulline peptide antibodies) test is used a marker which is highly specific for rheumatoid arthritisand97%specificityforthedisease. In this study, we examined the frequency of AntiCCP and RF in individuals referred to special Tooba clinic of Sari city, in order to find a new horizon in diagnosing Rheumatoid Arthritis.Materials and Methods:In a cross - sectional study, we studied available results of l patients who referred to Tooba special clinic, in the first 6 months in year (1390). Demographic data including the age and sex of the available files were extracted of Tooba clinic and statistical analysis were accomplished with SPSS (ver.15).Results: In this research, 207 individuals participated, 34 subjects (16.4%) were male with mean age of45.74 ± 18.09and173 were females (83.6 %) with mean age of 43.47 ±14.84. 179subjects(86.5%) had negative RF and 22 subjects(13.5%) had Positive RF. 185subjects (89.4%) had negative for AntiCCP and 22 patients(10.6%) had positive AntiCCP. 132 subjects (63.8%) had negative CRP, and75subjects(36.2%)had positive CRP, and The greatest number of positive CRP was in theagerange25-50 years. There was no significant difference between age with a variable RF, AntiCCP and CRP. In addition, there was no statistical between AntiCCP results in two groups ($p>0.05$). There is complete correlation between RF and Anti-CCP($p<0.05$).Also, There is a perfect correlation between CRP and AntiCCp($p<0.05$) But there is no correlation between CRP and RF ($p>0.05$) Conclusion: The results of these research show that Rheumatoid Arthritis is an important and common disease in our country. AntiCCP is a specific and sensitivity test for RA diagnosis, especially in patients with hidden RA. Furthermore, we proposed that screening for detection of disease examine after 25 year. Also, it is recommended that molecular studies expand for knowledge and understanding of disease mechanisms.

Keywords: Rheumatoid Arthritis–AntiCCP– RF.

991. Molecular Epidemiology of Measles Virus in Iran 2009–2010: First Detection of Measles Genotype H1

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Background: Measles virus (MV) genotyping is an important component of measles surveillance in the context of monitoring immunization program effectiveness and documenting MV elimination. Materials and Methods: Specimens of blood, urine and throat swab from suspected cases of measles in 2009-2010 were collected from sentinel sites, hospitals and outbreaks in different regions of Iran. Detection of IgM specific antibodies to measles in sera was performed using a commercial ELISA kit. Virus isolation was performed on urine and throat swabs of confirmed serologically measles cases. For virus isolation, the specimens were inoculated onto Vero/hSLAM cell line. Partial nucleoprotein gene segments of MV including the 456 terminal nucleotides were amplified by RT-PCR. PCR products were sequenced in both directions. Sequenced data were analyzed by BioEdit and treeconw software. Results: A total of 130 cases of measles in 2009 and 86 until May 2010 were confirmed by ELISA. Thirteen sequences (31.7%) were obtained from viral isolates using Vero/hSLAM cell culture and the 28 (68.3%) were obtained directly from clinical samples. Conclusion: Although mass vaccination has decreased successfully the incidence of the disease, outbreaks continue to occur.This is the first description of the genetic characterization of sporadic MV genotype H1 cases in northern Iran. Cases were probably linked to MV importation from distant parts of Asia. In addition, both sequence analysis and epidemiologic data indicated that the more recently detected genotype D4 viruses in Iran were related very closely to viruses that were detected in Pakistan, suggesting that these viruses may have been imported from Pakistan.

Keywords: Measles virus, Vero/hSLAM, ELISA, IgM

992. Prevalence of Toxoplasmosis in Children with Cancer after Treatment

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Background: Toxoplasma gondii an obligate intracellular protozoan complex in epithelial branching which it can be called the world's most common parasites. Due to the ability to invade cells and the production of latent tissue cysts, usually in people with suppressed immune systems may develop severe and extensive. Today'sindustrialsociety andthe increasing useofcomplementaryandteratogenic, cancerrateshaveincreased dramatically, especially inchildren. Includesvarious typesofcancerin children,accordingtothetype, stageandadvanceddiseaseseverity, course oftreatmentis different.Cancertherapydue toweakeningorcompletelysuppress theimmune systemthatcausesvariouscomplications,includingtheunderlyingdiseaseofthe past ornewopportunisticinfectionnoted. Materials and Methods: A review and critical essays looking at the past 25 years with special attention to children about cancer, leukemia and lymphoma and review reported cases of toxoplasmosis after we've written this review. Results: After *Derouin* and colleagues in 1986 examined the prevalence of toxoplasmosis in BMT patients has been addressed and significant increase in titer serological Toxoplasma after transplantation reported a turning point in research on parasitic on patients with suppressed immune figure was report of 7 cases with toxoplasmosis after bone marrow transplantation and in 1992 carried out by *Derowin*, more can be found in this issue.Toxoplasma infection is estimated that according to forecasts published cases (2% of total cases), it seems that these groups are highly at risk. In 2001 *Demedrios* and colleagues reviewed 11 years of age on

bone marrow transplant patients, also reported 9 cases of disseminated toxoplasmosis, the prevalence of 14/1% in patients with allogeneic bone marrow transplantation reported. *Mele* in 2002 with a reported one death and one case of disseminated toxoplasmosis among 110 cases treated with bone marrow transplantation, it also announced that 47% of positive cases have been diagnosed after transplantation, the risk of toxoplasmosis, they re-appear.The risk of toxoplasmosis in 2008 and in two studies separated by *Derouin* and colleagues and also Gourishiankar and colleagues on a variety of links members conducted thoroughly investigated, resulting in the likely incidence and also severe, the proposed remedies prophylaxis after transplantation was to avoid getting toxoplasmosis. In2011, Furtadoin thearticlealluded to thehighprevalence ofToxoplasmaDespiteprogressinthe science ofparasitology, toxoplasmosisremainsathreat topeoplewithimmune deficiencystates, and onresearchto achieverapidmethodsinthe early stages ofinfection withtoxoplasmosisisemphasized.Conclusion **:**Seems to weaken the immune system can check the occurrence of Toxoplasma in the cases indicate the importance of vigilance against parasites will suppress the immune system. Prevalence of Toxoplasma infection also can be important in the maintenance care to prevent new cases noted.

Keywords: Toxoplasmosis, Leukemia , Treatment, Children

993. Prevention and Immunity to *Salmonella* Infection in Food Animal Species

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Background: Salmonellosis in domestic animal and poultry species is important in terms of animal welfare and productivity. Infection may lead to decreased yields of eggs or meat, and in certain cases loss of livestock. Salmonellosis in domestic species is also important for public health as the major reservoir and source of food-borne human infections. Materials and Methods: A number of *Salmonella* serovars can induce a systemic typhoid-like disease in healthy adults of a restricted range of host animal species. Other serovars colonise the intestine of the host and in some cases may induce severe enteritis. The severity of the disease will be dependent on the virulence and dose of the challenge and immune status of the host. Thus, some *S. enterica* strains that would normally induce enteritis in adult hosts are able to induce systemic disease in immuno-compromised hosts. Results:Immunity to *S. enterica* is dependent on the nature of the disease that different serovars induce in different hosts. Mucosal immunity is more likely to be important in protecting against serovars that induce enteritis, whereas systemic immunity would be more important in protecting against serovars that induce systemic disease. Interaction of the host's immune system with different *S. enterica* serotypes is still rudimentary. Effective control of salmonellosis affecting domestic animals species requires a greater of immunological mechanisms during such infections. Conclusion: This will provide the basis from which rational control measures, such as more effective vaccines, vaccination strategies, diagnostic tools or other non-immunological tools may be developed. The precise immune response to *Salmonella* is not completely understood. Because the symptoms appear relatively quickly after exposure, before the body can make antibodies, it is believed that the innate immune response is involved.

Keywords: Salmonella infectin , Immunity , Food animal

994. Sero-Prevalence of Anti-*Toxoplasma* Antibodies in Children with Leukemia in Tehran by Using ELISA Method

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Background: One of the most important infections in children with blood cancer is T.gondii cause parasitemia and death rarely. The awareness of role of this parasitic infection in etiology of unknown Malignancy in child with leukemia is essential for the prevention and management of disease. This study done to determine the sero-prevalence of anti-Toxoplasma antibodies in children with cancer and its correlation with some variables in comparison with the control group. Materials and Methods: The present study consisted of 66 sera which were collected from children with leukemia who referred to the selected Pediatric clinic of Tehran during November 2010 to February of 2012 in comparison with 25 sera as the control group. All of the samples were analyzed for anti-Toxoplasma IgG and IgM antibodies using the ELISA method (Acon Company, USA). The demographic information's of the test and control groups were collected through a questionnaire. The data were processed using the SPSS ver.18 software and analyzed by the Chi-square, t-student and the logistic regression model. Results: The patients aged 1-18 year old (mean 8.2 yr.). Out of 66 sera, anti-Toxoplasma IgG antibodies were found in 21 (31.8% vs. the control group: 28%). The logistic regression model showed a non-significant correlation between anti-Toxoplasma IgG antibodies and the variables (age, sex). In this study only in one sample anti-Toxoplasma IgM antibodies were found (1.5% vs. the control group: 0%). Conclusion: These findings indicate that T.gondii infections may have a potential role in some disorders of children with leukemia. On the other hand, we must consider Toxoplasmosis and its relapse in leukemic children and design effective health policies directed toward the promotion of awareness of the child about the infection and its transmission routes.

Keywords:Leukemia, Toxoplasma, Seroprevalence, ELISA

995. Survey of Antibiotic Resistance in Different Shigella Serotype and Its Relation with SRL PAI in Patients with Dysentery

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Background: The *Shigella* resistance locus (SRL), which is carried on the SRL pathogenicity island (PAI) in *Shigella*, mediates resistance to the multi antibiotics. Aims of this study is detect multi antibiotic resistance in *Shigella* strainsand role of SRL PAI in this resistance. Materials and Methods*:* In the present study, 16 strains of *Shigella* were analyzed for multi-antibiotic resistance, the presence of the 7 SRL PAI and mutations in the resistance determining regions. Isolates were tested for their susceptibilities to 15 antibiotics including third generation of Cephalosporin. Results:All ١١isolates were intermediate susceptible or resistant to at least 2 antibiotics. The highest rates of antimicrobial resistance were to Trimethoprim (%56). None of the 16 strains of *Shigella* tested were resistant toImipenem, Meropenem and Ciprofloxacin. All of the strains harbored at least one SRL PAI genesencoding resistance to the investigated antibiotics.The highest rates of antibiotic resistance detected in SRL PAI genes that encode TetR and TetA gene (%66). Antibiotic resistance was higher in the isolates carrying Cat and Aada. *Shigella* isolates were more mutated in Aada1 gene than other genes. Conclusion:PCR studies with these strains demonstrated that most of the SRL PAIs, are present in *Shigella* isolates consistent with the hypothesis that the SRL PAI may be involved in the spread of multiple-antibiotic resistance in these strains.

Keywords:SRL PAI, *Shigella*, Pathogenicity Island, antibiotic resistance

996. The Value of Interleukin-8 and Interleukin-6 in Cervical Secretions as Predictors of Preterm Delivery

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Background: Preterm birth occurs in 8% to 11% of all pregnancies and is responsible for 75% to 80% of all neonatal deaths. Cytokines may be involved in the etiology of preterm birth through their stimulation of prostaglandin synthesis. Cytokines may be released into cervicovaginal fluid during the breakdown of the chorio-decidual adhesion or from an inflammatory reaction in the same area. The aim of this study was to determine whether the values of interleukin 8 and 6 in cervical secretions could predict spontaneous preterm birth in asymptomatic high-risk pregnant women. Materials and Methods: Levels of interleukin 6 and 8 in cervical samples, collected from 100 pregnant women between 22 to 28 weeks of gestation were measured by ELISA test in Mostafa Khomeini and hazrat-zeinab university hospitals in Tehran, from December 2006 to July 2007. Gestational age at time of delivery was recorded. Receiver operator characteristic curve analysis was used. Results: There were 4.8, and

4.4, -fold increase in cervical interleukin 8 and 6 concentrations in early preterm versus term delivery. A single interleukin 8 >751.25 pg/ml, and l interleukin 6 >157 pg/ml, was identified early preterm versus term delivery. Sensitivity , specificity, positive and negative predictive values of interleukin 8 in early preterm birth were 89% , 83%, 69% , 94% and for interleukin 6 as 89%, 78%, 63% , 88%, respectively. Conclusion: Cervical interleukin 8 and 6 is a sensitive and specific predictor preterm delivery.
Keywords: Interleukin 6, Interleukin 8, Preterm Birth, Preterm Labor

997. Trophoblast Stem Cells Equipped with HLA-G: Key to Immune Tolerance in Pregnancy or Invasion without Protection

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Background Multiple mechanisms underlie the surprising willingness of mothers to tolerate genetically different fetal tissues during pregnancy. Chief among these is the choice of HLA-G, a gene, for expression by the placental Trophoblast cells that interface directly with maternal blood and tissues. In this article, we first briefly discuss a number of conditions responsible for immune privilege and maternal tolerance for which scientific evidence is strong, then focus on a central feature-a unique capacity and function of placental Trophoblast cell cells for HLA-G expression and secretion. Materials and Methods: For this review, computerized literature searches of Medline was carried out to identify the trend of scientific production of original researches in the field of maternal tolerance during about two decades, between 1991 and 2012. Results HLA-G,was first identified in placental trophoblast cells, the unique lineage of cells derived from the trophectoderm layer of the blastocyst that interfaces directly with maternal uterine and blood cells. In particular, it has been learned that although membrane isoforms are present on invading cytotrophoblast cells, HLA-G5 soluble isoform is synthesized in placental villous cytotrophoblast (vCTB) cells. Recent studies indicate that suppression may be promoted by binding of soluble or membrane-bound HLA-G produced in placentas to inhibitory receptors on leucocytes known as LILRB1 (ILT2) and LILRB2 (ILT4). Immune cells driven into suppressive modes by HLA-G include $CD8^+$ lymphocytes, natural killer (NK) cells, activated macrophages and $CD4^+$ $CD25^+$ cells. Furthermore, Some studies demonstrated that HLA-G in cytotrophoblasts, may act as a key role in both genetic susceptibility to, and pathogenesis of pre-eclampsia(PE), but this pathway is obscure until now. So, We propose and illustrate this hypothesis for the first time that HLA-G in cytotrophoblasts provide CTB invasion in to deciduas tissues without any maternal immune responses or protection. Conclusion Additional studies are warranted to develop a more complete understanding of the mechanisms involved in the interaction between the HLA-G that placental Trophoblast cell express and fetomaternal tolerance. HLA-G expression, which appears to be a key component in trophoblast invasion and responsible in large part for the reprogramming of maternal immune responses into suppressive modes that characterize successful semiallogeneic pregnancy.
Keywords: Trophoblast cells, HLA-G expression, Maternal tolerance

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