Antimicrobial Activity and Antibiotic susceptibility of Lactobacillus spp Isolate From the feces of healthy infant against hospital, enteropathogenic and food-borne pathogens

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Introduction and Objective:

The human intestinal microflora is complex with total counts of $10^{11}-10^{12}$ bacteria per gram of stool. Among this vast number of organisms, are at least 500 species, within which lactobacilli are numerically a minority. Lactobacilli are a heterogenous, non-sporing, rod-shaped and catalase-negative group of Gram-positive bacteria. lactobacilli Isolate From the feces of healthy infant can be used as probiotics. The main aim of this study was Isolation, characterization of Lactobacillus spp from the feces of healthy infant and Determination of probiotic potential, Antibiotic susceptibility, Antimicrobial acyivity against hospital, enteropathogenic and food-borne pathogens including salmonella, E.coli, shigella, Bacillus Cereus, staphylococcus areus and Campylobacter spp (previously isolated in our division) and Aflatoxin B1 detoxification potential.

Materials and Methods:

Fecal samples were collected from healthy infants younger than 19 months. Approximately 1 g of each fecal sample was inoculated into 9 mL MRS broth and incubated at 37°C for 48 hr. The samples were then plated on MRS agar and incubated under anaerobic condition at 37°C for 48 - 72 hr. Gram-positive and catalase-negative rods were stored at -70°C in MRS broth with 20% (v/v) glycerol. The stock cultures were reactivated in MRS broth for 24 hr before each experiment. Identification of Lactobacillus isolates was performed by biochemical [Gram stain, catalase, fermentation of carbohydrates, hydrolysis of arginine, gas (CO2) production from glucose and growth at different temperatures(15°C, 45°C)] and 16S rRNA gene sequencing methods. And assessed For probiotic potential properties including acid and bile resistance, Adherence to HT-29 cells and antibiotic resistance. Etest, Disk diffusion and Broth microdilution used for Antibiotic susceptibility. An agar well diffusion assay was used for detection of antimicrobial activity of Lactobacillus isolates against salmonella, E.coli, shigella, Bacillus Cereus, staphylococcus areus and Campylobacter spp. The toxification of aflatoxin by lactobacilli spp isolate was quantified by Elisa method. Statistical analyses were performed with SPSS software (version16.0, SPSS). One-way ANOVA (Analysis Of
Variance) with post-hoc Tukey HSD (Honestly Significant Difference) was used for statistical analysis. Results were regarded as statistically significant at p< 0.05

Result:
The examined strains were identified as L. acidophilus, L. fermentum, L. brevis, L.rhamnosus, L. reuteri, L.plantarum. All Isolate showed good probiotic potential and Lb. fermentum showed adherence specificity to HT-29 cells and Lb. acidophilus showed good acid and bile Tolerance .Lb.brevis and Lb acidophilus exhibited the capability of reducing the amount of aflatoxin B1. The majority of the strains exhibited antagonistic activity towards Bacillus Cereus, salmonella, Campylobacter, E.coli, shigella, staphylococcus areus respectively. Antibiogram test showed that the isolate was sensitive to Chloramphenicol, Erythromycin, Amoxycilin, Clindamycin, Penicilin and moderately sensitive to Gentamycin. All isolates were resistant to streptomycine, Ciprofloxacin, Norfloxacain. The isolates except two isolates were resistant to vancomycine.

Conclusion
This study showed that Lactobacillus strains with good probiotic potential could be isolated from fecal of healthy infant and fecal microflora of healthy infants is a good origin for isolation of different Lactobacillus species with probiotic potential. Some Lactobacillus strains have Aflatoxin detoxification potential and antibacterial effect against hospital, enteropathogenic and food-borne pathogens and suggest Lactobacillus strains with probiotic potential may be useful for prevention or treatment of diarrhea and Supportive therapy in Aflatoxicosis but further in vitro and in vivo studies (clinical studies for human health, strain stability, bacteriophage resistance, viability in products) on these strains are still required.

Keywords:
Lactobacillus, Infant, Fecal flora, Probiotics, Antimicrobial Activity
OB-02

Investigation of the frequency of MDRStaphylococcus aureus strains in of hospital food and stool samples in patients with diarrhea in three hospitals of Tehran

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Introduction
We aimed to investigate the frequency of multidrug-resistant S. aureus (MDR-SA)in the hospital food and stool samples in patients with diarrhea.

Materials and Methods
A total of 258 faecal samples from patients withdiarrhea and 35 food samples were used to investigate infection with S. aureus. Methicillin-resistant S. aureus (MRSA) was characterized by the cefoxitin disk diffusion method in Mueller Hinton agar medium supplemented with 1% NaCl. PCR amplification of enterotoxin genes (sea, sec,and see) was carried out on all S. aureus. Susceptibility to 11 antimicrobial agents were analyzed by the standard disk diffusion method according to CLSI guidelines.

Results
S. aureus was detected in 22.09% (57/258) of the stool samples and 14.28% (5/35) of food samples. Nearly, 10.5% (6/57) and 8.7% (5/57) of the strains from stool samples and 20% (1/5) and 20% (1/5) of the strains from food samples were characterized as MRSA and MDR, respectively. Resistance to most of the antibiotics was <20%, while highest one detected against tetracycline (24.5%). Low frequency of MDR patterns (3DR, 4DR, 5DR, and 6DR) were detected in the fecal and food S. aureus isolates. Among them, penta-drug resistant S. aureus was detected in 3.5% of the patients’ isolates and triple-drug resistant phenotype was the only MDR pattern was detected in the food samples (2.8%). Nearly, 43.8% (25/57) of the strains carried the enterotoxin genes; the most common was sea⁺ (17.5%), sea⁺/see⁺ (5.2%), sec⁺(15.7%), sea⁺/sec⁺(3.5%), and sea⁺/sec⁺/see⁺(1.7%). These genes were significantly higher among MDR compared to non-MDR S. aureus strains isolated from the fecal or food samples (100% vs 39.2%).

Conclusions:
Involvement of MDR and enterotoxigenic S. aureus strains in the occurrence of gastroenteritis and their carriage in medical food samples highlighted the importance of food controls in prevention of gastrointestinal diseases, both in the community and clinical settings.

Keywords: MDR-SA, MRSA, Enterotoxigenic S. aureus, Diarrhea.
Study on pulegium effect of Aflatoxin in kefir probiotic containing *Lactobacillus acidophilus* or *Bifidobacterium bifidum*

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**Background:** Aflatoxins, the natural mycotoxin having mutagenic carcinogenic and teratogenic effects, are reported to be involved in various health complications including liver cancer. Many methods are suggested for control of aflatoxins such as chemical, physical and biological methods.

**Methods:** This study presents the effect of Mentha pulegium and *L. acidophilus* and *B. bifidum* separately and mix-culture style in reduction rate of aflatoxin in yogurt, so their lethal effects could be minimized. First, Milk contaminated artificially with aflatoxin M1 (AFM1) at a level of 232 PG, then after Mentha pulegium level of (2,4 and 6 gr) and the probiotic starters, Lactobacillus acidophilus (1×10⁸ cfu/g) and *B. bifidum* (1×10⁸ cfu/g) at the level of (1,3 and 6 g/l) were added separately and incubated at 38 °C. The AFM1 concentration of
sample was determined by ELISA at the day of 9 of refrigeration. The second step was about the effect of mix-culture of these probiotic and comparing the result with the separated style.

**Results:** The analysis of yoghurt during the 21st day of refrigeration using various treatment of Mentha pulegium (4gr) and Lactobacillus acidophilus (1gr) and Mentha pulegium (4gr) and *B. bifidum* (3gr) showed that the maximum level of binding about 145.8 and 148 at second day and minimum level of binding about 193.8 was for the sample Mentha pulegium 4gr at the 9th day of refrigeration time, respectively.

**Keywords:** Aflatoxin M1, Bifidobacterium bifidum, Lactobacillus acidophilus, Mentha pulegium.
Prevalence and genes expression of clfB and fnbA adhesions among Staphylococcus aureus by PCR and Real-time PCR

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Background and Aim: One of the most important causes of nosocomial infections is Staphylococcus aureus, which the process of infection is done by the MSCRAMM of this bacterium. clfB and fnbpA are one of the important factors in Staphylococcus aureus binding and invasion and play a key role in nose and skin colonization. The aim of this study was to determine the frequency and the rate of expression of these genes.

Method: Nasal swab specimens were collected from personnel of different departments hospital in Shahr-e-kord. Isolates strains in Mnitol Salt agar medium were identified using common microbiological methods including catalase test, tube coagulase test and manitol fermentation test. Then, the presence or absence of the desired genes and the expression of fnbA and clfB were investigated by PCR technique and real-time technique, respectively.

Results: Based on the results, 110 carriers of Staphylococcus aureus were identified. The frequency of clfB and fnbA genes were, 86.3% and 43.6% respectively. It was also observed that the fnbA gene showed no expression, but of 95 clfB-positive samples, 73 isolates (76.8%) were expressed clfB gene.

Conclusion: This study showed that the abundance of these genes varies in nasal colonization and varies in different geographic regions. It was also observed that clfB gene with a high frequency and high expression rate has an important role in nose colonization.

Keywords: Staphylococcus aureus, fnbA, clfB
The Role of Bacteria in the Inflammatory Bowel Disease Development: A Narrative Review

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Summary

Inflammatory bowel disease (IBD) is a general term used for the ulcerative colitis and Crohn's disease; in addition, IBD principally refers to a chronic disease of the gastrointestinal tract in which mediated by immune system. Consequently, IBD could progress in individuals who are genetically prone. Infections role in the development of inflammatory disease of the gastrointestinal tract has been studied by quite many clinical studies; furthermore, the possible role of some pathogens in the development and exacerbation of the inflammatory disease of the gastrointestinal tract have been described.

In conclusion, it was pertained the role of infections agents in inflammatory bowel disease progression has not yet identified conspicuously; however, based on the clinical and epidemiological evidences, the possible connection between some microorganisms and IBD development have been reported; Particularly, Mycobacterium avium subspecies paratuberculosis, Clostridium difficile, Escherichia coli, and Campylobacter Concisus. In addition, some viruses; including, cytomegalovirus, Epstein-Barr virus, and measles by various pathogenesis have promptly been indicated to be associated with the increase in the risk of IBD. While, Helicobacter pylori possibly by reducing inflammation in the intestines protect against IBD. Considering all available data Antibiotic treatment against specific organisms or FMT can be a promising outlook for IBD management.

Keywords: Inflammatory bowel disease, bacterial infection diseases.
Genotyping of CTX-M1 producing *Klebsiella pneumoniae* using Multiple locus Variable Number Tandem Repeat

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**Background:** Considering that CTX-M type have become the most prevalent and recognized as a rapidly growing family of Extended-Spectrum B-lactamase (ESBL) and the bla CTX-M1 has detected as the most common type of bla CTX-M. Genetic diversity of CTX-M1 *Klebsiella pneumoniae* circulating in Semnan, Iran was evaluated by VNTR-Multi locus variable tandem repeat (MLVA).

**Methods:** A total of 110 isolates of *Klebsiella pneumoniae* were collected from different clinical samples. The antibiotic susceptibilities and Double disk synergy test were determined by the Kirby-Bauer disk diffusion method. The PCR method for detection of CTX-M1 was performed and for genotyping, the eight VNTR loci selected along with the primers as described by Turton.

**Results:** Imipenem with 84.7% susceptibility was the most effective antibiotic against *K.pneumoniae*. Seventy (63.63%) isolates had ESBL positive results and 42 (60 %) out of 70 isolates were positive for *Bla*₅CTX-M₁* gene. Totally 27 MLVA genotypes were discriminated for *Bla*₅CTX-M₁*. Evaluation of diversity indexes for VNTR loci showed that VNTR-J with 6 different allels was the most polymorphic and the highest diversity index (0.807).

**Conclusion:** The finding of this study demonstrated that there was heterogeneity among CTX-M1 producing *Klebsiella pneumonia* isolates. The presence of CTX-M1 in several different MLVA type demonstrated that one specific clone was not responsible for spreading of isolates

**Keywords:** VNTR, MLVA, *Klebsiella pneumoniae*, CTX, *Bla*₅CTX-M₁, Antibiotic resistance, ESBL, genotyping, Imipenem
OB-07

Tick-Borne Borreliosis in western Iran

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Background and Objective: Tick-Borne Relapsing Fever (TBRF) is a zoonotic disease involving many species of rodents and small mammals in widely distributed areas throughout the world. We describe the epidemiological and demographic characters of found cases of TBRF in the Bijar County, and briefly review it for preventing this illness in the year of 2007–2008. Materials and Methods: A confirmed patient was defined as a person who had both febrile illness and detection of spirochetes by Wright-Giemsa or dark-field microscopy in a peripheral blood smear. All patients were asked to complete a questionnaire including demographic characteristics and clinical and epidemiological data of TBRF. Data was analyzed using SPSS. Eleven cases have been reported. Results: There were 5 cases (45.4%) of the patients younger than 10 years. Of the patients, 63.6% occurred in summer. All cases developed during the months of May to October. All of the reported cases were living in rural areas. Fever and chills, the most common symptoms, occurred in all patients. Recurrent fever occurred in 54.5% cases. All of the cases were cured according the national guideline for TBRF treatment. Only 18.2% of the patients were hospitalized. No patients in this study died of TBRF. Most (54.5%) of the cases were students. Approximately 72.7% of the patients were keeping cattle and sheep near or inside their homes. Conclusions: As demonstrated, TBRF is a considerable public health concern, especially for children and students living in Bijar County. Considering the epidemiology of the disease, new control measures should be established.

Key words: Epidemiology, Relapsing fever, Tick, Iran
Formulation a New Ointment with Herbal Plants for Burn Wound infection Treatments

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Introduction:

Pseudomonas aeruginosa is third hospital infection agent and is the second important factor wound infections. Despite many scientific advances in the treatment of burns, Burns likewise are one of the major public health problems worldwide, particularly in developing countries. According to the Medical Council from 1380 until the end of 1390 about 28,991 people died of burns. MDR and XDR strains of Pseudomonas aeruginosa and increased resistance to infection in burn patients recommend the issue of infection control. In this study, we decided that using herbal ointment inhibit virulence genes of Pseudomonas aeruginosa.

Materials and Methods:

herbal ointment ZOUSH prepared to ethanolic extracts of Satureja khuzestaniea, Zataria multiflora, Origanum volgarum, honey and polyurethane were formulated. The MIC and disk diffusion tests as separate, binary, tertiary and five compounds were examined.

The 20-day course of treatment with ointment ZOUSH, for mice with second degree burns on the back their bodies accompanied by bacterial infection by Pseudomonas aeruginosa were considered. An interval of 5 days from the liver, blood, wound cultures were done in four consecutive quarters and number of Pseudomonas aeruginosa were reported in the liver.

Also in this study were used silver sulfadiazine ointments and Akbar 1 was used as a positive control. PCR techniques carried to confirm the genes exoS, lasA and lasB and for the analysis of genes expressed exoS, lasA and lasB were used. qPCR and Real-time RT-PCR techniques. The genes gyrA and fabD reference were used for control. Real-time RT-PCR results were evaluated based on Livak (method ΔΔCt) as the comparative Ct method (known as ΔΔCt).

Results and Conclusion:

The MIC results showed that Satureja khuzestanie, Zataria multiflora, and Origanum volgarum have positive effects on growth inhibition of Pseudomonasaeruginosa. The antibacterial effects ZOUSH ointment compared with Gentamycine 30 μg, and Polymyxine B 300 u. The In vitro results indicated that wound infection (reducing the number of P.
*Pseudomonas aeruginosa* in the culture of the liver), as well as improvement in wound size in the treatment group compared to control treatment groups (topical silver sulfadiazine ointment and Akbar 1. In this research, the changes in gene expression were evaluated with molecular techniques using semi-quantitative RT-PCR and quantitative Real-time RT-PCR. The results showed downregulation of *exoS*, *lasA* and *lasB* after treatment with ZOUSH ointment. SPSS Analyses showed that the expression of *exoS*, *lasA* and *lasB* after treatment with ZOUSH ointment was significantly (*p*<0.05).

Key words:

*Pseudomonas aeruginosa*, Burns, *Satureja khuzestanica*, *Zataria multiflora*, *Origanum volgareum*, Honey, Polyurethane, Real-time RT-PCR, ZOUSH
OB-09

Effect of *Lactobacillus* on level of serum vitamin D in animal model of experimental encephalopathy

مريم كاظمي

**Background**: Probiotics are living organisms that have good beneficial effects for the host. One of the most important probiotics is Lactobacillus. The aim of this study was evaluated the effects of Lactobacillus isolated from traditional dairy products on the treatment of experimental animal encephalopathy and its effect on serum vitamin D level. **Methods**: Lactobacillus identification was performed based on gram staining, colony morphology, biochemical test and molecular identification. To determine the precision of the 16S rRNA Lactobacillus strain was amplified by PCR with specific pair of 190 FLb and 190 RLb primers and sequenced. The sequences after editing were nucleotide blast. Lactobacillus was gavaged with specific doses to rats (in 6 groups of 8) as follows: control group (healthy animals without disease), model group (animals of the EAE model without treatment), experimental group 1 (animals treated with Lactobacillus isolated from dairy products), experimental group 2 (animals treated with Lactobacillus isolated from dairy products and vitamin), experimental group 3 (animals treated with vitamin) and experimental group 4 (animals Prevented with Lactobacillus isolated from dairy products). After induction of EAE with cuprizone (to ensure induction of EAE motor tests), the rats were treatment with Lactobacillus during one month. The serum vitamin D level was determined ELISA test. **Results**: The results indicate that 19 isolated of Lactobacillus were selected for PCR sequencing. The frequency of L. plantarum, L. acidophilus L. sakei, L.parabuchneri, L. casei, L. pentosus and L. curvatus were 12.9%, 3.22%, 3.22%, 6.45%, 25.8%, 3.22%, 3.22% respectively in EAE models of MS Serum level of D3 vitamin in experimental group 1 was higher than the model group.

**Conclusion**: Lactobacillus bacteria decrease Multiple Sclerosis (MS) disease with increasing the serum levels of D3 vitamin. Therefore, using of Lactobacillus can be effective in MS treatment.

**Key words**: Probiotic, Lactobacillus, EAE, Multiple Sclerosis
OB-10

Evaluation of the antibacterial properties of *Achilleamillefolium* oil incorporated in liposomes

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**Background:** Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem. The development of microbial resistance has led the researchers to search the antibacterial activity of medicinal plants. Essential oils are chiefly used for the flavors and fragrances but they also possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties [1,2]. EOs from *Achilleamillefolium* have been used for their medicinal properties for centuries. It possess antibacterial, antifungal, antioxidant, anti-inflammatory and analgesic properties [3]. These applications require appropriate carriers like nanoliposome. The nanoencapsulation of these oils in drug delivery systems have been proposed due to their capability of improving the solubility and stability. The purpose of this study was to determine the antimicrobial properties of nanoliposomes containing essential oil.

**Methods:** minimum inhibitory concentration (MIC) of nanoliposomal *Achilleamillefolium* essential oil was determined against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, using broth tube dilution method and compared to MIC for free essential oil. Each exam was repeated three times. The results were analyzed by SPSS 16 software using one-way ANOVA and Tukey statistical tests.

**Results:** The inhibitory effect against *Staphylococcus aureus* was in concentration of 232 μg/ml and for *Pseudomonas aeruginosa* was 116 μg/ml while for free essential oil was inhighe concentration.

**Conclusion:** *Achilleamillefolium* incorporated in liposome has good antibacterial activity.

**Keywords:** Liposome, Essential oil, Antibacterial activity.
OB-11

MicroRNA based Molecular diagnostics strategies as a clinical suggestion for infectious diseases specialists

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Background:

As one of the leading causes of death in critically ill hospitalized patients, Infectious diseases are characterized with severe drug resistance, chronic nosocomial infections and mutations leading to re-emergence of new strains. Therefore, clinical researchers validate alterations in circulating microRNAs (miRNA) expression as small noncoding single-stranded endogenous RNA, in order to be consensused for early diagnosis and subsequent treatment. So, this study aims to evaluate clinical potentials of infectious diseases related miRNA.

Methods:

This systematic review was conducted to outline comprehensive studies published in PubMed, Scopus, Science Direct and Google Scholar databases from 2008 to August 2017 by using 4 keywords. 183 articles were screened and 76 were totally included.

Results:

There are various reports on miRNAs post-translational gene expression regulation and inflammatory responses. Changes in miRNA levels after physiological imbalance, makes miRNA implementation for infectious diseases diagnosis, logical. Diminution in time waste for pathogen identification and increase in the sensitivity and accuracy of detection rate up to 99% are considerable. It is highlighted that miRNA-146a and miRNA-223 can be utilized for sepsis diagnosis and miRNA-150 for high mortality rate prognosis instead of primary microbial cultures. Also, down-regulation of miRNA-7, 155, 505, and miR-940 have been involved in differential diagnosis of gram negative and gram positive infections. Pseudomonas aeruginosa infections also recognized with the modified expression of miRNA-302b and miRNA-233. On the other hand, antibiotic stewardship and differentiate between bacterial and viral infections will be feasible with miRNA strategy.
Conclusion:

Despite meticulously efforts for Health system improvement, Infectious diseases have been still remained common especially in developing countries. Therefore, optimistic insights on miRNA as stable, non-invasive low-cost and repeatable strategy, makes coordination between physicians and laboratory scientists obligatory, clarifying disease progression and assisting to diagnosis based clinical outcomes accreditation.

Keywords: Molecular diagnosis, Infectious diseases, microRNAs, Clinical advantage.
OB-12

Molecular characterization of extended-spectrum β-Lactamase producing enteroaggregative Escherichia coli isolates in Iranian children

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Background: Enteroaggregative Escherichia coli has been implicated as an emerging cause of traveller’s diarrhea, and persistent diarrhea among children and immunocompromised patients in both developing and developed countries. The objectives of the present study were
to investigate the extended-spectrum β-Lactamase (ESBL) production of EAEC isolates obtained from Iranian children with diarrhea.

**Methods**: In this cross-sectional study, within March 2015 and February 2016, 255 *E. coli* were collected from fecal samples of children aged <12 years with diarrhea attending two teaching hospitals Golestan and Abozar, affiliated to Ahvaz University of Medical Sciences, southwest of Iran. The specimens were cultured for *E. coli* on MacConkey agar and incubated at 37 °C for 24h. Subsequently, *E. coli* isolates were identified and stored using standard microbiologic methods. Genomic DNA was extracted from all *E. coli* isolates by boiling method. *E. coli* isolates were confirmed as EAEC by the amplification of pCVD and aggR genes. All EAEC isolates were tested for ESBL production using the double-disk synergy test using ceftazidime (30 µg) and cefotaxime (30 µg) disks, and combination with clavulanic acid (10 µg) disk as described by CLSI guidelines. Moreover, the presence of ESBLs resistance genes, SHV, PER, and CTX-M were determined by specific primers.

**Results**: Overall, 12.5 % (32/255) of the isolates were characterized as EAEC by pcr. Further analysis revealed that the rate of ESBL-producing isolates was 71.9% (23/32). PCR screening for the presence of ESBLs genes revealed that 28 (87.5%) of EAEC isolates were positive for TEM gene and 21 (65.5%) of isolates were positive for CTX-M gene, and 17 (53.1%) of isolates contained both TEM and CTX-M genes. PER gene not detected in any of the isolates by the absence of the desired amplicon.

**Conclusion**: In summary, the high detection rate of ESBL producing EAEC isolates highlights a need to restricted infection control policies to prevent further dissemination of the resistant and virulent EAEC strains.

**Keywords**: EAEC, Extended-spectrum β-Lactamase, Enteroaggregative *Escherichia coli*
OB-13

The biofilm formation and Phenotype expression of type 1 and 3 fimbriae among klebsiella pneumonia isolates from urinary tract infection

حديثه شيدابور

Introduction: Many bacterial extracellular structures have been described as essential for interaction with surfaces during early attachment steps for biofilm formation like fimbriae. K. pneumoniae can express several adhesive factors such as type 1 and type 3 fimbria. Type 1 fimbriae are related to type 1 fimbriae expressed by other species of Enterobacteriaceae. They confer a mannose-sensitive hemagglutination phenotype to ciliated hamster tracheal cells. Type 3 fimbriae are expressed by both clinical and environmental Klebsiella isolates. They mediate adherence to several cell types like human endothelial and urinary bladder cells, trypsinized buccal cells, tracheal cells and respiratory tissue.

The aim of this study was phenotypic investigation of type 1 and 3 fimbriae among K. pneumoniae biofilm producer isolates from urinary tract infection.

Methods: 62 klebsiella pneumonia strains isolated from urine specimens of patients were separated and identified based on biochemical tests. Microtiter plate method was used to determine the biofilm formation. The human and hamster red blood cell agglutination method was used in the presence and absence of mannose for the study of type 1 and 3 klebsiella pneumonia isolates.

Results: Among isolates, 80.6%, 16.1% and 0.03% had strong, moderate and weak biofilm formation capacity, respectively. Based on agglutination method 98.2% isolates had type 3 fimbria and 8% had type 1 fimbria. Only 4 strains had both types. Statistical analysis showed association between type 3 fimbria and biofilm formation. There was not relationship between biofilm formation and type 1 fimbria.

Conclusion: Type 3 fimbria was the most common fimbriae among klebsiella pneumonia isolates and the type 1 fimbria was related to biofilm formation. The more study of theses fimbriaes for bacterial pathogenesis is recommended.

Keywords: K. pneumonia, Type 1 fimbria, Type 3 fimbria, biofilm formation
OB-14

Frequency of Iron uptake proteins related genes among Klebsiella pneumoniae isolates

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Introduction:

Klebsiella pneumoniae as an opportunistic pathogen cause infections in immune-compromised patients including diabetes mellitus or patients with chronic obstructive pulmonary. Iron uptake systems after capsule are the second factors in pathogenesis of Klebsiella pneumonia. In the other hand because of important infections caused by Klebsiella pneumonia in this study prevalence of iron uptake coding genes among clinical and environmental isolates of Klebsiella pneumonia was investigated.

Material and Methods:

The clinical isolates were collected from Ilam hospitals and clinical laboratories during 14 months from October 2013 to January 2015. Environmental isolates were collected from waste water city, waste water of university and also environment of hospital during 9 months from April 2014 to January 2015. After culturing and identifying bacteria, 300 isolates of Klebsiella pneumonia including 150 clinical isolates and 150 environmental isolates were selected. Then DNA was extracted by boiling method and finally the frequency of iroN, iucD, kfu, hmuR, and ybt (yHPI) genes were detected by PCR method.

Results:

The frequency of kfuA, iucD, iroN, yHPI in clinical isolates were 33.3%, 16.7%, 24.7%, and 15.3% respectively and these genes among environmental isolates were 20.7%, 6%, 49.3%, and 0.7% respectively. Among clinical isolates, the most frequency genes were kfuA gene (50 isolates) and after that iroN (37 isolates), iucD (25 isolates) and yHPI (23 isolates), the most frequency genes among environmental isolates were iroN gene (74 isolates) and following that kfuA (31 isolates), iucD (9 isolates) and yHPI (1 isolate). There was not found hmuR gene among clinical or environmental isolates.

Conclusion:

The result of this study showed that because of high frequency of ferric iron system coding gene kfu among clinical isolates, this system might be important in survive of bacteria in host.

Key words: Klebsiella pneumoniae, iron uptake gene, siderophores, PCR
OB-15

Evaluation of therapeutic Carvacrole and thymol on pneumonic mice infected with Acinetobacter baumannii

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Background: Acinetobacter baumannii is an important pathogen in health care-acquired infections and has free existence of multidrug-resistance responsible for severe nosocomial and community-acquired pneumonia. Currently, mouse model for A. baumannii pneumonia is essential for designing novel therapeutic agents. Methods: In this report, we described a mouse model of A. baumannii using clinical isolate. Pneumonic mice were treated with carvacrol and thymol for evaluation of antibacterial effects of these two important components of Zataria multiflora on A. baumannii infected Balb/c mice. Ampicillin sulbactam was used as positive control. Results: Mice were infected intranasally by 1 ×10⁸ cfu/ml of A. baumannii. For treatment of pneumonic mice, one day after infection, mice were treated with 65mg/kg/day of carvacrol, 30 mg/kg/day of thymol and 35 mg/kg/day of ampicillin sulbactam by I.P. Injection for 7 days. Lung tissues of pneumonic mice were cultured daily on MHA medium and incubated in 37°C. Bacterial clearance of lung tissue of pneumonic mice was observed on day 4 after treatment with carvacrol. Bacterial clearance with ampicillin sulbactum was seen on the day 7, however, seven day-treatment with thymol showed two colonies of A. baumannii infection on MHA media. Discussion: Our data indicate that anti-bacterial effect of carvacrol is much higher than thymol and antibiotic.

Keywords: Acinetobacter baumannii, carvacrol, thymol, Zataria multiflora, Pneumonia, Balb/c mice
Detection of beta-lactamase genes (bla<sub>TEM</sub> and bla<sub>CTX</sub>) resistant to drugs and glutaraldehyde in samples of Acinetobacter baumannii isolated from surfaces of the medical device of intensive care units of hospitals of Tehran

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**Background:** Excessive consumption of antimicrobial materials in hospitals and the community is as the main encoder, led to the emergence, development and acquisition of new bacterial resistance to antimicrobials. According to the lack of the enough information about the mechanism of the resistant genes to disinfectants and receiving no report from the country about this study and with the aim of considering the resistance or sensitivity of the isolates of the Acinetobacter baumannii MDR in facing disinfectants, this study was taken place in the selected intensive care units if the hospitals of Tehran.

**Methods:** This study which was conducted over a period of 10 months, Acinetobacter baumannii species were separated by culture and biochemical tests. The resistance and sensitivity of the isolates to antibiotics is considered according to CLSI (2012) guidelines. By multiplex PCR method bla<sub>CTX</sub> and bla<sub>TEM</sub> were detected and finally, MDR strains were treated with 2% glutaraldehyde.

**Results:** In our study 131 isolates (22/28%) of Acinetobacter baumannii were isolated. The amount of the resistance to various antibiotics was in the range of the 69/4% to 100%. The percentage of frequency of the bla<sub>TEM</sub> and bla<sub>CTX</sub> was 3/2% and 19/4% respectively. And there was seen no resistance to glutaraldehyde.

**Conclusion:** It seems that beside variety and prevalence of bla<sub>TEM</sub> and bla<sub>CTX</sub>, enormous mechanisms like porin and leaking systems (efflux Pumps) are responsible in the making of the resistance of Acinetobacter baumannii to disinfectants. Also these results facilitated the study of phenotypic and genotypic resistance patterns of these antimicrobials and disinfectants in different parts of the world.

**Keywords:** Acinetobacter baumannii, lactamase genes, disinfectants, antimicrobial resistance
OB-17

Seroepidemiological Study of Helicobacter pylori Infection in Ekbatan Eastern Region of Tehran

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Introduction: Cardiovascular diseases are one of the most causes of morbidity and mortality in industrial and developing countries such as Iran. The epidemiologic pattern of Helicobacter pylori infection is differed between developed countries and developing countries. At the present study, the seroprevalence of Helicobacter pylori infection among residents of Ekbatan eastern region of Tehran was evaluated.

Material and Method: In this cross-sectional study of the blood of 96 volunteers who referred to clinical lab were collected. Using anti-Helicobacter pylori kit (IgG), the serum samples were tested for H. pylori infection. In other words, the titer of total IgG against Helicobacter pylori was evaluated by ELISA method. Data and different factors were analyzed by SPSS statistical software.

Results: The existence of total IgG against of H. pylori was detected in 61 (63.54%) of 96 volunteers and was negative in 35 people (36.46%). There was statistical meaningful correlation between positive result of serology test of IgG to some risk factors such as age and sex. Most infected volunteers were in the age range of 20-30 years old. In addition, statistics analysis showed in total infected individuals 39.35% was male and 60.65% were female.

Conclusion: With regard to high prevalence of Helicobacter pylori in this area and its presumptive effect in infected people, the necessary of hygiene education and precise control of infection is suggested.

Keywords: Helicobacter pylori, Cardiovascular Disorders, prevalence,
Obi-01

In vitro evaluation of immune responses to antigenic stimulation for experimental rabies vaccine produced at Pasteur Institute of Iran in comparison with the international human rabies vaccine

زینب سیدخان

Background and Aim: The aim of this study was to evaluate in vitro immune responses of lymphocytes stimulated by antigens of human rabies experimental vaccine in comparison with international human rabies vaccine.

Materials and Methods: Patients with documented human immunodeficiency, hepatitis or each chronic disease were excluded from our study. PBMCs have been grown in RPMI1640 medium with supplementation of 10% of FBS, 100,000 U penicillin and 100 μg/μl streptomycin. 150,000 cells are cultured into 96-well cell plate. Extraction of total RNA was performed from the BSR cell line using RNXTM plus solution (Cinnagen, Iran) according to the manufacturer’s instruction. mRNA expression level of the IL-4 and IFN-γ genes were estimated with the appropriate primers. The relative expression of the gene was assessed compared to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with specific primers.

Results: The stimulating lymphocytes with specific percentages of vaccines on the expression of IL-4 and IFN-γ was analyzed by qPCR. Data showed expression of IL-4 and IFN-γ cytokines mRNA in donors who have been already immunized with human Rabies vaccine and were re-stimulated with both testing vaccines as test groups in comparison with people who have not been immunized against rabies as a negative control.

Conclusion: The present study data showed increases in both type 1 and type 2 cytokines as indicators of cellular and humoral immunity responses considerably, and the muscle and intradermal routes of postexposure vaccination do not differ together.

Keywords: rabies, vaccine, Immune responses, antigenic stimulation, IL-4, IFN-γ
Efficacy of green tea in scavenging free radicals and trace elements regulation in the patients with severe Alzheimer’s disease: A randomized, double-blind, placebo-controlled clinical trials

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Background: Recent evidences indicated that free radicals and imbalances of metal ions such as iron (Fe), copper (Cu) and zinc (Zn) may have key roles in Alzheimer’s disease (AD). Forasmuch as green tea has remedial components that can scavenge free radicals and chelate metal ions, we examined its inhibitory effects on free radicals and trace elements.

Methods: In this randomized, double-blind, placebo-controlled clinical trial, sixty severe AD patients were involved according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria, with the Mini-Mental State Examination ≤10 (MMSE). The researcher registered the trial in the Iranian Registry of Clinical Trials, identifier: IRCT: IRCT201612063684N8. Patients were divided randomly into two equal groups to consume either green tea supplement or placebo (2g per day) for two months. Brain magnetic resonance imaging (MRI) and computed tomography (CT) scan and laboratory tests were used to exclude other causes of dementia. 2,2-Diphenyl-picrylhydrazyl (DPPH®) radical scavenging activity levels and levels of metal ions were determined before and after dietary intervention.

Results: In GT group, Fe and Cu levels were significantly lessened (P=0.04) and significant increases in Zn and DPPH® radical scavenging activity levels were found (P=0.01) after two months. In the placebo group, no significant changes were observed in the variables after intervention.

Conclusion: Repetitive consumption of green tea (2 g/day) prevent free radicals attack and regulate copper, iron, and zinc imbalances.

Keywords: Alzheimer's disease, free radicals, green tea, trace elements
Obi-03

The Association between Serum Thyroglobulin and Urinary Iodine Concentration in an Iranian Population
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Introduction: Iodine is an essential micronutrient for human health. An adequate dietary of iodine is essential for the synthesis of the thyroid hormones that they require for normal growth and development. Many populations based on their geographical location, suffer from iodine deficiency. Therefore, evaluation of the iodine status of the community is necessary to prevent the occurrence of iodine deficiency related thyroid disorders. Thereupon, functional markers are needed to assess the status of iodine in the population.

Method: Urine sample taken from the participants voluntarily and then based on the urinary iodine content, the samples were divided into two groups contain 25 iodine adequacy and 25 iodine deficiency subject and then were taken a Blood sample . A package of iodized salt was delivered to the participants to use . After the completion of the intake of salt (8 weeks), Blood and urine samples were taken again.

Result: The median of UIC before intervention in the low iodine group was 7.6 μg/l which increased significantly after intervention (15.5 μg/l), but there was no significant change in the iodine sufficiency group (Median before and after intervention respectively: 14.9 μg/l and 15.9 μg/l). Before intervention, serum thyroglobulin values was not significant difference between the two groups, however a slight increase in mean values of serum Tg was showed (6.34±0.14 ng/ml for iodine deficiency group & 4.55±0.14 ng/ml for iodine adequacy group), so there was no significant difference between the two groups after intervention (mean serum Tg in iodine deficiency group: 5.06 ng/ml and mean serum Tg in iodine adequacy group: 4.34 ng/ml).

Conclusion: This study showed that there was an inverse relationship between these two the markers. Therefore, both are an appropriate indicator for determining the status of iodine in a population.

Keyword: Iodine, Serum Thyroglobulin, Urinary Iodine Concentration, thyroid hormone
Obi-04

Rapid Diagnosis of Niemann-Pick Type C patients with Plasma cholestane-3β,5α,6β-triol and 7-ketocholesterol by LC-ESI-MS/MS

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Background: Niemann-Pick Type C (NP-C) is a rare autosomal recessive lysosomal storage disorder caused by impaired intracellular transport of unesterified cholesterol and glycolipids due to mutations in either NPC1 or NPC2 gene. NP-C is usually underdiagnosed due to a variable age of onset and heterogeneous age-dependent clinical manifestations. Moreover, definitive diagnosis is based on genetic investigations which are time consuming and not always conclusive. Development of novel therapies for NP-C in recent years emphasized the urgent need for a reliable biomarker in early laboratory diagnosis. Recently, colestane-3β,5α,6β-triol and 7-ketocholesterol that result from non-enzymatic oxidation of cholesterol have been shown to be elevated in plasma of NP-C patients. We explored the usage of plasma colestane-3β,5α,6β-triol and 7-ketocholesterol as powerful diagnostic biomarkers for rapid diagnosis of NP-C.

Methods: Immediately separated 50 µL plasma was sufficient for the analysis. Analyses were performed on a triple quadrupole mass spectrometer (Shimadzu 8040 LC-MS/MS, Japan) equipped with an ESI source and a reversed phase column after derivatization of oxysterols with dimethylglycine esters. Eight point calibrators and 3 levels of QC were used. Statistical analysis was performed with MEDCALC.

Results: Both colestane-3β,5α,6β-triol and 7-ketocholesterol levels in NP-C patients were significantly elevated than the healthy individuals (Figures). Mean colestane-3β,5α,6β-triol levels was 20.9±8.4 ng/mL and 7-ketocholesterol was 31.2±14.5 ng/mL for 70 healthy individuals. Mean colestane-3β,5α,6β-triol levels was 128.3±67 ng/mL and 7-ketocholesterol was 216.9±125.4 ng/mL for 8 NP-C patients. ROC analysis yielded AUC of 0.99 and 1.00 for colestane-3β,5α,6β-triol and 7-ketocholesterol, respectively. At the cut-off of 39 ng/mL,
coleste-3β,5α,6β-triol demonstrated a specificity of 98.6% and a sensitivity of 100%. Both a specificity and a sensitivity of 100% at a cut-off of 72 ng/mL was observed for 7-ketocholesterol. Diagnoses of NP-C patients were confirmed with genetic analyses (Table).

**Conclusion:** Our data demonstrates that plasma coleste-3β,5α,6β-triol and 7-ketocholesterol fulfills the need of rapid and reliable biomarkers for NP-C.
Does vitamin D deficiency in the northwest of Iran have a nutritional or sun-deprived cause?

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**Background:** With less than ten percent of skin exposed to the ultraviolet B radiation the onset point of synthesis of 25(OH)D in the body can be activated. People who cover their whole body, even hands and face, remain deprived of the radiation due to stopping the route of the activity. This study evaluated the cause of hypovitaminosis D among men and women in northwest of Iran.

**Materials&methods:** This was carried out on 3091 people, in different age groups, with 576 men and 2515 women in the year 2014.

**Results:** The median plasma level of 25(OH)D in developing ages among both male & female and also in child bearing age among veiled women was very severe. Unexpectedly, the average level of the vitamin was significantly higher in women, not covering their face and hands, (19.9 ±0.29 ng/ml) than men (16.71±0.34 ng/ml), P<0.001 and t (3089) = -5.087.

**Conclusion:** Not covering the hands and face in veiled women and the high level of the vitamin D in women rather than in men at the one hand, and not practicing food fortification in the area at the other hand, exhibited that the nutritional deprivation in terms of vitamin D deficiency outweighed the solar Ultraviolet B deprivation.

**Keywords:** Nutritional, women, solar, 25(OH) D, Ultraviolet B
Obi-06

Investigation the effect of Quercetin on macrophage polarization in RAW624.7 cell line

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Introduction: Macrophages are divided into two phenotypes (M1 and M2) based on their surface markers. M1 markers include F4/80, CD11, Mgl2, CD197.LY6G, iNOS, and M2 markers include CD206, CD163, Arginase, RetnlA, Mgl1. These markers’ activations are affected by several mechanisms such as ER stress, hypoxia, lipotoxicity, ROS production and NF-kB activation. Pro-inflammation cytokines are released by M1 macrophages like TNFα interfere many pathways include insulin signaling. Inhibiting these cells lead to reduce inflammation. M2 macrophages release anti-inflammatory cytokines: IL-4, IL-10, TGFβ1 that improve metabolic pathways. Furthermore, the balance between M1/M2 has an important role in inflammation mechanisms. High glucose concentration give rise to lipotoxicity and ROS products that result in M2 to M1 shift and lead to inflammation.

These days many studies are trying to reduce inflammation. recently have been shown polyphenols have anti-inflammatory potential and safety effects. Quercetin is a flavonol, one of the six subclasses of flavonoid compounds. This polyphenol possesses strong anti-inflammatory capacities; Through an increase of antioxidative activities, reduction of lipogenesis and macrophage polarization regulation. quercetin acts as a strong anti-inflammation flavonoid.

Method: This study has surveyed the glucose (53mM) modulation of RAW 264.7 macrophages activation and effects of quercetin (25µM) on high glucose-induced lipotoxicity and macrophage polarization. we have measured high glucose-induced lipogenesis by oil red O staining. For investigating macrophage polarization, we assessed M2 marker, CD206, as an anti-inflammatory factor and M1 marker, CD11c, as an inflammatory factor via flow cytometry.

Results: our results suggest that high glucose induce RAW264.7 lipogenesis and modify RAW264.7 morphology. Flow cytometry analyses showed that high glucose increase M1 marker, CD11c, significantly (about 80%) in vitro. RAW264.7 were treated with quercetin strongly reduce lipid droplet in oil red O staining and decrease M1 marker, CD11c to approximately 20%. Our data have shown quercetin caused a very slight increase in M2 marker, CD206 (4%) that isn’t signed.

Discussion and Conclusion: These results show that Quercetin produces a potential anti-inflammatory effect by modulating macrophage polarization and attenuate high glucose-induced lipogenesis in vitro. Decreasing M1 phenotype involved in the anti-inflammatory property of Quercetin.

Key Word: Inflammation, Quercetin, High Glucose, Macrophage polarization
Association of HaeIII single nucleotide polymorphisms in the SLC2A1 gene with risk of diabetic nephropathy; evidence from Kurdish patients with type 2 diabetes mellitus

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Aims: Given the growing rate of patients with type 2 diabetes mellitus, uncovering the effects of gene polymorphism on diabetes pathogenesis has attracted a lot of attention. Because glucose transporter 1 is involved in glucose uptake, the polymorphism of this gene may be an important risk factor in type 2 diabetes mellitus or in the progression of diabetes complications such as diabetic nephropathy. As far as the authors are concerned, this study is the first one aiming at evaluating the probable effects of solute carrier family 2 facilitated glucose transporter member 1 (SLC2A1) HaeIII polymorphism on clinical and laboratory outcomes of Kurdish patients with type 2 diabetes mellitus.

Methods: This study was conducted involving 126 diabetic nephropathy patients and 150 diabetic patients without renal involvement. Serum levels of Cystatin C, fasting blood glucose, creatinine and urinary albumin; levels of glycated hemoglobin and estimated glomerular filtration rate were measured. Moreover, the Hae III polymorphism of SLC2A1 gene was determined by PCR-restriction fragment length polymorphism (RFLP).

Results: The rate of CC genotype was higher (37%) in patients with diabetic nephropathy compared with controls. There were a significant correlation between the CC genotype and risk of diabetic nephropathy. There were significant correlations between genotypes, serum Cystatin C and estimated glomerular filtration rate in patients with diabetic nephropathy.

Conclusions: The results demonstrated the high frequency of C allele of SLC2A1 HaeIII in Kurdish patients with diabetic nephropathy. It was also found that this polymorphism is a significant risk factor for diabetic nephropathy. The effect of this polymorphism on clinical and laboratory characteristics of diabetic nephropathy patients was significant.
Obi-08

Generation of insulin-producing cells from human induced pluripotent stem cells on polyethersulfone scaffold

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Abstract

The studies have been done on patient-specific human induced pluripotent stem cells (hiPSCs) like a series of autologous growth factors and nanofibrous scaffolds (3D culture); perhaps it will have many benefits for regenerative medicine in type 1 diabetes mellitus (T1DM) patients in the future. For this purpose, we established a polyethersulfone (PES) scaffold and differentiation protocol that induce the hiPSCs into insulin-producing cells (IPCs). The Characteristics of derived IPCs in 3D culture were compared with conventional culture (2D) groups that evaluated at the mRNA and protein levels, the viability for induced pancreatic cells were 21 days. The in vitro studies showed that treatment of hiPSCs in the 3D culture resulting in differentiated cells with strong characteristics of IPCs including pancreatic-like cells, the expression of the islet-associated genes at the mRNA and protein levels in comparison of 2D culture group. Furthermore, the immunoassay tests showed that these differentiated cells in two groups are functional and secreted insulin in a glucose stimulation challenge. The results of our study for the first time demonstrated that the PES nanofibrous scaffolds along with the optimized differentiation protocol can enhance the differentiation of IPCs from hiPSCs. In conclusion, this study provides a new approach for future pancreatic tissue engineering and beta cell replacement therapies for T1DM.

Key word: human induced pluripotent stem cells, Insulin-Producing Cells, 3D culture, PES
Downregulation of MiR-219 by DNA Hypermethylation Is Associated with Glioblastoma Multiform

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Background: Deregulated tumor suppressor microRNAs mediated by epigenetic modifications are strongly implicated in the pathogenesis of several cancers including glioblastoma multiform (GBM). Although it has been shown that microRNA-219 (miR-219) has a tumor suppressive function in glioblastoma multiform but, the potential linkage between miR-219 epigenetic modification and its gene expression levels has not been studied.

Methods: Real-time polymerase chain reaction (real-time-PCR) and bisulfite genomic sequencing technology were used respectively, to determine gene expression and methylation levels of miR-219 in GBM (n=23) and their adjacent normal tissues (n=23).

Results: Our results showed a significant low gene expression levels of miR-219 in GBM patients relative to their adjacent normal tissues (P< 0.01). Promoter region of miR-219 gene was hyper-methylated in GBM tissues and correlation analysis revealed an association between expression and methylation levels of miR-219 gene in GBM tissues compared to normal adjacent tissues. Conclusions: These findings suggested that the hyper-methylation of miR-219 promoter may be a mechanism involved in its decreased gene expression levels and may in turn lead to the increased risk of GBM pathogenesis.

Key words: miR-219, GBM, U87 cells
Improvement of catalytic efficiency of chondroitinase ABC I by Site-directed mutagenesis method

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Background: Chondroitin sulfate proteoglycans (CSPGs) are known inhibitors of axonal growth. Chondroitinase ABC I (cABC I) eliminates glycosaminoglycan chains and facilitates axon regeneration after central nervous system injuries. However, the activity and stability of cABC I at physiological temperature is one of the current problems to its clinical application. Therefore, we investigated the effect of a new added aromatic pair (Tyr⁶²³/Tyr⁷³⁰) on the stability and catalytic efficiency of cABC I.

Methods: Bioinformatics approaches used to examine the effect of added aromatic pair at the surface of chABC I. Distance of 7 Å between γ carbons of phenyl rings were considered as maximum effective distance for creating an aromatic pair. The site-directed mutagenesis was used to produce G730Y mutant of cABC I. Then cABC I was purified by affinity chromatography that used Ni column and eluted with imidazole and phosphate buffer. The purity of the recombinant cABC I and mutant was assessed by SDS-PAGE analysis and long term stability of cABC I and the mutant were monitored at 4 °C for 3 week. Moreover, Circular dichroism (CD) technique was used for examining the secondary structure content of enzymes in solution to clarify the structural effect of the mutation.

Results: Experimental data showed that K_m value of G730Y mutant decreased relative to wild type enzyme. Also, the catalytic efficiency (k_cat/K_m) of the variant was improved 1.17 times than of wild type with C4S as substrate. Nevertheless, this variant has not significant difference on long term stability (4 °C) when compared to the wild type enzyme. Moreover, circular dichroism studies demonstrated that this mutation doesn’t significant effect on secondary structure content enzyme.

Conclusion: The data revealed that activity of chABC I can be improved by introducing appropriate aromatic pairs at the surface of the enzyme.

Key words: Chondroitinase ABC I; Circular dichroism; Enzyme activity
Predictive Value of Serum Cystatin C in Kidney Recipients

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Background: Cystatin C is a member of anti-proteinase family, produced in a constant rate by nucleated cells, freely filtered by glomerulus and entirely catabolized by the tubules. Our aim was to evaluate the predictive value of a new version serum cystatin C (Scys) for the diagnosis of early graft function in kidney recipients (KRs).

Martial and methods: KRs (n=39; KRs) were enrolled. These were divided into two groups including delayed graft function and immediate graft function according to existing protocols. Blood samples were collected at 2-, 16-, 36- and 48 hrs post-kidney transplantation (post-KT). Serum cystatin C was measured using a new micro-latex assay on a BT-autoanalyzer.

Results: Median SCys (mg/L) at 2, 16, 36 and 48hrspost-KT were 2.2, 1.3, 1.2 and 1.3, respectively. At all-time points SCys in DGF group was higher than those in none-DGF (P<0.01). At 2hrs post-KT, AUC (95% confidence interval), cut-off value sensitivity and specificity were 0.87 (0.75-0.99), 2.89, 100% and 67.7%, respectively. Outstanding sensitivity and specificity (100%) were identified at 24hrs post-KT.

Conclusion: The new Scys assay identified changes within 2hrspost-KT with high sensitivity and specificity which indicates the usefulness of the assay for early diagnosis of allograft function. If the outcome established in larger prospective studies it will have important impact in the management of early graft function.

Keywords: Transplantation, Delayed graft function, Serum cystatin C
Obi-13

Altered bone remodeling markers in Metabolically Unhealthy Obesity

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Background: Considering the importance of the obesity and related health problems, the notion of “metabolically healthy obesity (MHO)” needs more attention to prevent inevitable public health messages. Several lines of evidence suggest that obesity and bone metabolism are interrelated. It is more complicated when to extend MHO for bone health. The aim of this study is to compare three different bone markers in metabolically healthy and unhealthy obese and non-obese subjects according to different metabolic healthy criteria.

Methods:
A total of 35 subjects were enrolled in the study that included 11 healthy normal-weight and 23 obese subjects. Based on HOMA-Beta, all participants were divided into three groups; normal weight (HOMA Beta<%100, n=11), obese (HOMA-Beta <%100, n=12) and obese (HOMA-Beta >%100, n=12). The serum levels of osteocalcin, procollagen I aminoterminal propeptide (P1NP) and beta-cross Laps as bone turnover markers, as well as serum levels of 25 (OH) vitamin D3, and PTH were analyzed.

Results:
There were significant differences in body mass index (BMI), age, 25(OH)D3, fasting blood sugar (FBG), Insulin, aspartate amino transferase (AST), (ALT), triglycerides (TG), HOMA-IR, HOMA-Beta among groups. Analysis of bone markers showed the serum levels of Beta-cross Laps was significantly different among the all studied groups categorized by HOMA-Beta model. In this regard, circulating levels of Beta-cross Laps in normal weight (HOMA-Beta<%100) were significantly higher than obese group (HOMA-Beta <%100). In obese patients with HOMA-Beta <%100, Beta-cross Laps (p=0.03) levels was lower compared to obese group with HOMA-Beta >%100.

Conclusion:
Our data showed that HOMA-Beta, as an index of β-cell function, can use in part of MHO criteria and bone remodeling altered in the context of MHO.

Keywords: HOMA-beta, bone marker, metabolically healthy obesity
Study of smad2 phosphorylation induced by endothelin-1 in the presence and absence of inhibitors Rho / ROCK kinases, ETB and evaluation of CHSY-1 protein level in bovin aortic endothelial cells

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Objective

In atherosclerosis disease, hyperelongation of glycosaminoglycan chains elevated on proteoglycans, which leads to increased the binding of lipoproteins to the proteoglycans within the sub-endothelial space. Endothelin-1 (ET-1), peptide secretion from endothelial cells, as kind of the GPCR agonists, plays a important role in development of atherosclerosis. This study determined that ET-1 through transactivation of TGF-β receptor leads to increased the level of the GAG chain synthesizing enzyme (CHSY1) in bovine aortic endothelial cells (BAEC).

Method

In this study, we identified effective intermediate in TβR I transactivation with Using inhibitors suchas Rho/ROCK kinase (Y27632), ETB receptor (BQ788) and subsequently examined the role of this pathway in protein level of CHSY1 by western bloting

Key finding

ET-1-induced Phosphorylation of Smad2Cblocked in the presence of inhibitors Y27632 and BQ788. Also protein level of ET-1-increased CHSY1 in presence of Y27632, and BQ788 decreased.

Conclusion

ET-1 via the ETB receptor with utilizesof Rho kinase lead to TβR I transactivation and through this pathway, increased the level of CHSY1 enzyme.

Endothelin-1, GPCR, transactivation, CHSY1
Association of TaqI (rs731236) (C/T) and BsmI (rs1544410) (A/G) vitamin D receptor polymorphisms with nontraditional risk factors involved in vascular calcification in hemodialysis patients

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Objective: Cardiovascular disease (CVD) is the leading cause of death in patients that receive HD treatment. The main of this study was to inquire the association between serum GAS6, Fetuin-A, intact parathyroid hormone (iPTH) and Vitamin D (Vit D) levels with the presence of VDR 731236 (C/T) and VDR 1544410 (A/G) polymorphisms in HD patients. Methods: 46 HD patients and 43 age and sex matched control subjects were included in a cross-sectional study. The VDR 731236 (C/T) and VDR 1544410 (A/G) polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The serum levels of iPTH, GAS6, Fetuin-A, Vit D were determined by Elisa methods.

Findings: A highly significant association was found between the TaqI polymorphism and iPTH in hemodialysis patients. There was no association among BsmI polymorphisms of VDR gene and serum Fetuin-A, GAS6, Vit D and iPTH levels in patients with HD. There was a significant negative correlation between GAS6 and Fetuin-A levels in bb genotype in BsmI polymorphism (P = 0.02, r = -0.7) and tt genotype in TaqI polymorphism (P <0.001, r = -0.1).

Discussion: Lower Vit D and Fetuin-A levels and higher levels of iPTH, GAS6 may indicate increased susceptibility of atherosclerosis in the HD patients. Although, our study reveals that VDR gene TaqI polymorphism associated with serum iPTH level in HD group, direct roles of this polymorphism on atherosclerosis needs further studies.

Key words: GAS6, Fetuin-A, Vit D, Hemodialysis Patients, Vit D receptor polymorphisms
Obi-16

High glucose induces inflammatory responses in HepG2 cells via the oxidative stress-mediated activation of NF-κB, and MAPK pathways in HepG2 cells

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Running Title: High glucose induces inflammation in HepG2 cells

Objective: It has been suggested that in addition to immune cells, hepatocytes also produce pro-inflammatory cytokines in response to different stimuli. However, the role of high glucose (HG) condition on inflammatory responses in liver cells remains unexplored. In the present study we investigated the effects of HG on inflammation and elucidated the underlying mechanisms in HepG2 cells. The results showed that HG significantly enhanced TNF-α, IL-6 and PAI-1 expression in C2C12 cells after 6, 12 and 25h treatment. Increased expression of cytokines was accompanied by enhanced phosphorylation of JNK, P38, ERK1/2 and IKKα/IKKβ. In addition, JNK, ERK, P38 and NF-kB inhibitors could significantly attenuate HG-induced expression of TNF-α, IL-6 and PAI-1. In addition, HG could promote the generation of reactive oxygen species (ROS), while N-acetyl cysteine, a ROS scavenger, had an inhibitory effect on the expression of TNF-α, IL-6 and PAI-1 in HG-treated cells. Conclusion: Our results also indicated that HG-induced inflammation is mediated through the generation of ROS and activation of the MAPKs and NF-kB signaling pathways.

Keywords: Diabetes, Hyperglycemia, Inflammation, HepG2 cells, NF-κB, MAPKs, hepatocytes
Obi-17

Ethanol effects on histobiochemical parameters of suckling pups borned from alcoholic rat mothers

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Background: Fetus and neonate growth retardation is one of the main characteristics of fetal alcohol syndrome (FAS) disorders. Ethanol can be transferred to the fetus through the placenta and to newborns through suckling. This study was designed to investigate the effects of consuming different doses of ethanol during lactation on enzymatic and tissue changes and growth indices in suckling rat pups.

Methods: Forty-five lactating Wistar rats with nine lactating pups each were randomly allocated to three treatment groups. The two treatment groups received 2 and 4 % v/v ethanol, while the third group was the control on distilled water for 24 days. On day 25 after birth, 30 newborn rats were randomly selected from each group and serum activity of liver enzyme markers, lactate dehydrogenase, blood urea nitrogen, creatinine, and creatine phosphokinase enzymes was measured. Pathological examinations were performed on brain, liver, and kidney tissues. The obtained data was analyzed using one-way analysis of variance (ANOVA) and Dunnett’s tests.

Results: Enzymatic activity of lactate dehydrogenase and creatinine was significantly (p < 0.05) higher in the rats that received alcohol as compared to the control. In histopathological examinations, different injuries were observed in kidney, liver, and brain tissues of suckling pups exposed to 4 % v/v ethanol.

Conclusion: Consumption of alcohol in the lactating rats can cause irreparable effects on the suckling neonate.

Keywords: Enzymatic changes, Pathological changes, Growth indices, Ethanol, Lactation, Suckling rat pups.
Obi-18

Comparison of biochemical factors and liver enzymes in type 2 diabetes patients and healthy individuals

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Diabetes mellitus is one of the most common global health threats (is endocrine malfunctions) that is considered as one of the five major causes of death in all communities. About 90% of the patients suffer from type 2 diabetes. It has been demonstrated that the serum concentration of Alanine and Aspartate aminotransferase enzymes (ALT, AST) and the triglyceride (TG) and cholesterol (Chol) metabolites are increased in type 2 Diabetes. In this research, the population study was selected from 2240 patients who referred to diabetes center (Hamadan, Iran) for checking the hepatic enzymes and biochemical factors. Blood samples under fasting condition were collected. The concentrations of ALT, AST, TG and Chol in serum were measured. The results showed that diabetic patients had more increased TG concentration than healthy subjects; the increase of TG concentration was higher in the women than men (about 7%). The difference of Chol concentration between diabetic and healthy individuals was not significant. But, the results suggested that the gender of the patients affects the TG and Chol concentration, so that diabetic women showed more amount of TG and cholesterol than the diabetic men. The body mass index (BMI) investigation showed that the diabetic women had more over weighting than the diabetic men. There was direct relation between the BMI and TG density. The hepatic enzymes examination showed that the ALT concentration was significantly higher in both male and female diabetic patients in comparison with healthy individuals. Final results of the study suggested that we could use the TG and ALT as the markers for type 2 diabetes in human populations.

Key words: Diabetes mellitus, Triglyceride, cholesterol, ALT, AST, Iran
Theoretical and experimental Investigation of Bovine Liver Catalase interactions with Oxali-Platin

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In the present study, the effects of oxali-platin as an anticancer drug on bovine liver catalase activity and secondary and tertiary structures were studied using various spectroscopic methods of uv-visible, fluorescence and CD and molecular docking methods. By the kinetic data it can be concluded that oxali-platin inhibits catalase activity by competitive inhibition mechanism. Fluorescence studies indicated that oxali-platin can be quenched the fluorescence of catalase by the dynamic mechanism by one binding site through van der waals force and hydrogen bond interaction.

The results of CD spectra indicate that the secondary structure of catalase was changed in the presence of oxali-platin under our experimental conditions. The data of fluorescence and UV–vis absorption showed that the environments of Trp residue were changed. The molecular docking results in coherent with excremental data proved that oxali-platin bind to BLC by van der waals driving force at one position. Also, by docking calculation it was understood that the interaction of oxali-platin with BLC is occurred at the distance of Föster theory so in agreement with fluoresce emission intensity study quenching mechanism is dynamic.

The importance of this study is to investigate and show that the alteration in antioxidant enzyme during chemotherapy can change tumor progression and metastasis through detoxification of ROS and the decrease in catalase activity by oxali-platin can be introduced as a secondary mechanism of this anticancer drug, because high concentration of ROS (for example, H₂O₂) could be a therapeutic option for the treatment of cancer.

Keywords: catalase, reactive oxygen species, anticancer, oxaliplatin
Oxidative Stress Biomarkers in Endometrial Secretions: A Comparison Between Successful and Unsuccessful In Vitro Fertilization Cycles

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Background: A potential role of oxidative stress has been implicated in the outcome of various steps of assisted reproductive technology (ART).

Methods: In a prospective cohort study, a total of 100 patients undergoing IVF/ICSI procedure due to male factor infertility were recruited based on the inclusion criteria. In all patients, 1–2 ml of endometrial secretions was aspirated prior to embryo transfer. The oxidative stress markers in endometrial secretions, including superoxide dismutase (SOD), catalase (CAT) activities, lipid peroxidation (LPO), total thiol groups (TTG), and total antioxidant power (TAP) were investigated and compared among study groups including term pregnancy, failed IVF cycle, and miscarriage. P < 0.05 was considered statistically different.

Results: Of the 100 patients, 28 cases (28%) resulted in ongoing pregnancy (biochemical pregnancy followed by clinical pregnancy), 11 cases (11%) resulted in miscarriage, and 61 cases (61%), resulted in failed IVF cycle. SOD, LPO, CAT, and TAP levels in the endometrial secretions of the three groups were statistically different (P-value <0.01, <0.001, <0.001, and <0.001, respectively). TTG levels in endometrial secretion of three groups were not statistically different (P-value = 0.837).

Conclusion: Our results indicated that higher levels of antioxidants such as SOD, CAT, or TAP, and lower levels of oxidative stress markers such as LPO in the endometrial secretions were associated with successful IVF outcome.

Keywords: Stress Oxidative, Biomarkers, Fertilization, IVF.
Biological effects of a new anti-cancer compound of methyl-glycine 1,10-phenanthroline platinum nitrate: Two most important blood carrier proteins of human serum albumin and hemoglobin as targets

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Background: The biological effects and the structural alternation ability of a new designed Pt(II) complex, methyl-glycine 1,10-phenanthroline platinum nitrate, as an anti-cancer compound was studied at different temperatures by multi-spectroscopic methods such as fluorescence and the far-UV circular dichroism (CD) in combination with a molecular docking against two most important blood carrier proteins of human serum albumin (HSA) and hemoglobin (Hb) in drug delivery system. Alterations in intrinsic fluorescence intensity of the proteins upon binding of Pt(II) complex indicated a static quenching mechanism. The binding and thermodynamic parameters were estimated by assessing the results of quenching and those of the van’t Hoff equation for both proteins.

Results: Our experimental and theoretical results revealed that the driving force for Pt(II) complex interaction with HSA was electrostatic and with Hb was hydrophobic interactions. Fluorescence studies also revealed that there is one binding site with a negative Gibbs free energy value due to Pt(II) complex binding in both proteins. Far-UV CD data displayed that Pt(II) complex induces changes in the secondary and tertiary structures of HSA as a notable decrease in α helical content of the protein structure at both temperatures of 25 and 37 °C but no significant changes was observed in related structures of Hb.

Conclusion: By the way, the interaction between the novel synthesized drug (Pt(II) complex) and two most important blood carrier proteins of HSA and Hb included in significant changes in the structure and conformation of proteins through several alterations in secondary and tertiary structures of both proteins. Therefore, these kinds of studies on the design of newly metal anticancer complexes can provide helpful information for finding more appropriate pathway in confront with severe diseases such as different cancers via design and synthesis more benefit metal drug complexes with fewer side effects.

Keywords: Serum Albumin, Hemoglobin, blood carrier proteins, Pt(II) complex, static quenching, anticancer complex.
Introduction:
Nanoclusters fabricated from cadmium (Cd) have been shown to be of suitable fluorescence properties, which makes them as applicable tools for tumour-targeted drug delivery. Using protein scaffolds for fabrication of nanoclusters, provides valuable advantages such as lower toxicity and immune responses as well as higher solubility in blood and more biocompatibility.

Methods and Results:
In the present study, a nanocluster in size range of 2-5nm with fluorescence excitation weight and length of 350nm were synthesized and characterized with fluorimetry, circular dichoromism, FT-IR and TEM. Assessment of BSA-Cd accumulation of the nanocluster were explored in urine and blood of mice. Balb/c mice were treated with 1 mg/kg B.W of the nanocluster (i.p). After, three intervals of 1, 7, and 30 days from the injection, mice were sacrificed and accumulation of Cd were assessed in urine and blood using atomic absorption technique. The results of experiment in our study indicates that accumulation of Cd in blood of mice under BSA-Cd treatment is significantly (P < 0.05) higher than control. It is also revealed that accumulation of Cd in blood reaches to the highest in day 30 compared to day 1 and 7. However, no similar significant changes happened to be observed in the amount of urine cadmium from day 1 to 30 after BSA-Cd injection.

Conclusions:
In conclusion, our results proposed that the floroscently-active nano-product presented in the present study is a long-lasting biocompatible structure in the blood of mice, which is poorly cleared into urine and may be used in drug delivery studies.

Key words:
Cadmium nanocluster, drug delivery, Biodistribution, Safety evaluation
OBN-02

Comparison of pure and PLGA-PAA nano-encapsulated of Hydroxytyrosol effects on hTERT and CREB1 gene expression in HT-29 Colon cancer cell line

الهام احمدی

CRC is a serious contributor to cancer mortality and morbidity throughout the world [1]. CRC is the third most common cancer and the fourth most common cause of death [2]. Olive oil consumption can significantly reduce the risk of colon cancer [3]. Mediterranean diet is one of the most healthful and valuable source of antioxidants in the world in terms of preventing illnesses such as cardiovascular disease and some types of cancer. Extra Virgin Olive Oil (EVOOs) Phenolic compounds, including hydroxytyrosol, induce apoptosis and inhibit cell proliferation and modulation of cell cycle pathways and have anti-angiogenesis effects on colon cancer cells [4]. PLGA is one of the most well used polymer for the development of biocompatible nanoparticles therefore PLGA based nanoparticles has an important role in drug delivery systems for cancer therapy [5]. PLGA-PAA (Polylactide-co-glycolide-polyacrylic acid) copolymer was prepared which is the first new developed biocompatible nano-carrier, until now. We encapsulated hydrophilic hydroxytyrosol in PLGA-PAA biocompatible nanocapsules to compare the Inhibitory Effects of the PLGA-PAA Complex on the hTERT and CREB1 Gene Expression in HT-29 colon cancer Cell Line by Real Time Quantitative PCR. PLGA-PAA copolymer synthesised by ring opening polymerization method in the presence of stannous octanoate as catalyst of the reaction. MTT assay showed that PLGA-PAA nanoparticles has not cytotoxic effect on its own and it demonstrated that PLGA-PAA Hydroxytyrosol complexes has more cytotoxic effects and inhibited the growth of the HT-29 colon cancer cell line in a time and dose dependent manner compare to free Hydroxytyrosol. Our q-PCR results showed that the expression of hTERT and CREB1 genes was effectively reduced. PLGA-PAA complexes exerted cytotoxic effects on HT-29 cells through down regulation of hTERT and CREB1 expression and by enhancing hydroxytyrosol uptake by cells. Therefore PLGA-PAA could be superior carrier for this kind of hydrophilic agent.

Keywords

Colon Cancer, PLGA-PAA co-polymer, MTT assay, qPCR analysis
**OBN-03**

**Using of Gold nanoparticles as a Peptide Nucleic Acid delivery**

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**Introduction:** Gold nanoparticles (AuNPs) possess the low intrinsic toxicity and unique properties that make them as a good carrier for the drug delivery into the cell. The nucleic acid peptide (PNA), a synthetic nucleic acid, has an abnormal polyamide backbone and due to the neutral charge tends to penetrate into the cell more easily than the natural nucleic acids. PNA has more binding power to DNA or RNA than normal oligonucleic acids.

**Material and methods:** AuNPs were synthesized by reduction method. Then different concentrations of PNA were adjusted to AuNPs to gain the highest concentration of PNA which is not lead to the aggregation of AuNPs. The composite was inoculated into the MDBK cells and cytotoxicity was measured using MTT assay. Finally transmission electron microscope (TEM) imaging was used to observe the absorption of nanoparticles into the cells.

**Results:** MTT assay showed that AuNPs carrying PNA in concentrations equal or below of …. have not cytotoxicity. Images of TEM showed absorption of nanoparticles into the cells.

**Conclusion:** The AuNPs can be a suitable carrier for compounds that control the expression of the genes, including PNA.

**Key words:** Gold nanoparticle, Peptide Nucleic Acid, Cytotoxicity
OBN-04

Synthesis and Antimicrobial Activity of Chitosan-Functionalized Graphene Oxide as a Nanocarrier for Ciprofloxacin against Escherichia coli and Staphylococcus aureus

رسول منصوری
(استاد راهنما) معصومه مهدوی اورتاکند

Introduction: It is important to deal with the problem of drug resistance in order to reduce its incidence or to limit resistant microbial agents. Combined therapies are said to involve the simultaneous use of two or more biological agents with different mechanisms of action, which are more effective than traditional treatments for diseases that act only in one mechanism. The aim of this study is to synthesize graphene oxide modified with chitosan antibiotic ciprofloxacin and its antimicrobial effect on Escherichia coli and Staphylococcus aureus.

Materials and Methods: Chitosan and antibiotic ciprofloxacin from Sigma Aldrich Company. After the synthesis of graphene oxide, the surface structure was modified with chitosan (CS/GO). The approximate shape and size of GO and CS/GO nano sheets were investigated using a field diffusion electron microscope (FESEM) equipped with X-ray diffraction detector X and X-ray diffraction (XRD). Chitosan-modified graphene oxide nanoparticles were used as an adjuvant for the administration of ciprofloxacin antibiotic by adsorption. The chemical structure of the above compounds and their functional groups were determined by the FT-IR spectrometer. Finally, the antimicrobial activity of ciprofloxacin, chitosan-modified graphene oxide and CS/GO carrying antibiotic ciprofloxacin on the standard strain of E. coli and S. aureus were performed by broth microdilution.

Results: showed that modified chitoprofenic graphene oxide could be a suitable bed for loading ciprofloxacin antibiotics. Antimicrobial activity was observed in the results of antimicrobial activity that improved chitosan modified graphene oxide against E. coli and S. aureus alone. The ciprofloxacin antibiotic loaded on CS/GO also had antimicrobial activity.

Conclusion: Based on the results, CS/GO nanocomposite can be used as an adjuvant for the administration of antibiotics. Also, this nano-antibiotic can be effective in treating bacterial infections and reduce the dose of antibiotics.
OC-01

The effect of 17-AAG, capecitabine and irinotecan double combinations on MMP-9 and VEGF gene expression in HT-29 human colorectal cancer cell line

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Background: Despite great improvements in colorectal cancer chemotherapy, in some cases treatments are undesirable. According to our previous report about high cytotoxic effect of recently presented anticancer agent (17-AAG) double combination of this drug with capecitabine and irinotecan have shown high cytotoxic effect against single treatments on HT-29 human colorectal carcinoma.

Methods: With regards to the effect of 17-AAG on HSP90 and its interaction with MMP-9 and VEGF gene expression in cells treated with IC50 dose of every single drug and 0.5×IC50 double combination groups. In order to investigate the anti-angiogenic and anti-metastatic effect of our tested drugs on HT-29 cell line, real-time PCR was performed.

Results: 17-AAG/capecitabine and irinotecan/capecitabine (standard chemotherapy) double combination groups significantly decreased VEGF mRNA expression. MMP-9 mRNA down-regulation at IC50 dose of 17-AAG, capecitabine and irinotecan single treatments and 17-AAG/capecitabine and 17-AAG/irinotecan double treatments were also significant.

Conclusion: Overall our findings proposed that 17-AAG had more significant effect on angiogenesis and metastasis when used in double combination especially with Capecitabine, so it could have therapeutic value in colorectal cancer combination therapy with common chemotherapy regimen. Our results suggest prospective investigations to establish effective regimens for colorectal cancer chemotherapy.

Keyword: chemotherapy, matrix metalloproteinase 9, vascular endothelial growth factor, HT-29
N-terminal domain of fragile histidine triad can arrest human fibrosarcoma cells in G2 phase of cell cycle

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Introduction: Fragile histidine triad as a tumor suppressor protein is important in pathogenesis of human cancers and it can inhibit p53 degradation by MDM2. Prior studies have indicated the interaction of FHIT with MDM2 or p53; however, there is no exact research for inferring the functional domains of FHIT involved in the cancer inhibition. Then, determining main parts of FHIT to induce cell death is important.

Methods and Results: Based on our previous in silico screening and MTT results, truncated forms of FHIT were selected and their cell cycle inhibiting properties were assessed in HT1080 human fibrosarcoma cell line. Functional analysis of these fragments was performed by flow cytometry.

Functional analysis showed that these fragments could arrest cells in the G2 phase of the cell cycle as specified by flow cytometry.

Conclusion: The FHIT functional domains can be used as lead compounds for development of drug designs and gene transfer for cancer therapy.

Keywords: FHIT truncated forms, HT1080, flow cytometry
OC-03

Anti-inflammatory and anti-tumoreffects of α-L-guluronic acid (G2013) on cancer-related inflammation in a murine breast cancer model

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Cancer-related inflammation (CRI) is associated with the malignant progression of several cancer types. Targeting these pathways is a novel promising strategy for cancer prevention and treatment. In this present study, we evaluated the efficacy of α-L-guluronic acid (ALG), a potent anti-inflammatory agent on breast cancer-related inflammation both in vitro and in vivo conditions. Our results indicated that ALG can effectively inhibit the CRI and tumor-promoting mediators (COX-2, MMP2, MMP9, VEGF and proinflammatory cytokines) without direct toxic effects on the cells. Moreover, it was found that, ALG can effectively inhibit the tumor cell adhesion to extracellular matrix, seeding in implantation tissue, reduce accumulation of immunosuppressive and inflammatory cells in tumor-bearing mice. These findings were associated with decreased tumor growth, metastasis, angiogenesis and prolonged mice survival. In conclusion, our data provide a cellular and molecular justification for the use of nonsteroidal anti-inflammatory drugs (NSAIDs) in treating cancer and imply the potential anti-tumor activity of ALG therapy via inhibition of CRI. These findings could lead to the establishment of novel NSAID-based cancer therapy in the near future and open a new horizon for cancer treatment.

Keywords: Cancer-related inflammation, Guluronic acid, NSAIDs, breast cancer
Study of glutathione –S-transferase T1 genetics polymorphisms in Iranian population with lung cancer

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Purpose: Lung cancer is currently one of the most common malignant diseases, is responsible for the leading cause of cancer deaths worldwide. Glutathione S-transferase (GSTs) are phase II transformation enzymes involved in the detoxification of hazardous agents. The main role of GSTs is to detoxify xenobiotic thereby preventing their interaction with crucial cellular proteins, nucleic acids. In the present study, we investigated the polymorphism of GSTT1 genotypes in lung cancer patient compared to controls to determine the possible relation between polymorphisms of these enzymes, lung cancer.

Materials and Methods: In this study, selected 120 lung cancer patients tissue, 120 normal cases (as control) The GSTT1 genotypes were determined in all cases. Controls, patients were adjusted according to their age, sex, being diabetic or non-diabetic, then accepted for the study. Using PCR GSTT1 that allows identification of all genotypes were investigated for this deletion.

Results: the GSTT1 null genotype (deletions) were determined in 26 (36.1%) men with lung cancer and 14 (15.6%) controls group. GSTT1 null genotype was found to be associated with lung cancer risk (P=0.003) 16 (33.3%) women with lung cancer and 8 (26.7%) in controls. GSTT1 null genotype was found to be associated with lung cancer risk (P=0.62).

Conclusions: It was concluded that the increased frequencies of GSTT1 null based on sexual, in men with lung cancer and controls could be associated with an increase in incidence of risk lung cancer.

Keywords: Lung cancer, Glutathione S-transferees T1 and Genetic polymorphism
Promoter methylation status of SERPINA1 gene in colorectal cancer patients from Iran

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Background: Colorectal cancer (CRC) is a common type of cancer morbidity and mortality worldwide. CRC is a multistep disorder from the accumulation of genetic and epigenetic abnormalities. Serine protease inhibitor A1 (SERPINA1) expression has been correlated with CRC. Hypomethylation of the SERPINA1 gene has been associated with increased gene expression in rat models. According to the lack of information about the role of epigenetic changes on gene expression profiling of SERPINA1 in human, in this study we investigated the association between SERPINA1 gene promoter methylation with CRC incidence in Iranian population.

Methods: Surgically resected tumors and adjacent normal tissues from 86 patients with CRC were collected from patients who also received surgical treatment. Genomic DNA was extracted from tissues using a standard phenol: chloroform procedure. The status of promoter methylation of SERPINA1 gene in samples was determined by methylation specific PCR (MS-PCR).

Results: The rate of SERPINA1 promoter methylation in CRC normal tissues was significantly higher than that in CRC tumors (53.2% vs 8.1%, p < 0.0001). In the present study, the frequency of tumors with fully methylated SERPINA1 gene was higher in well differentiated tumors (p = 0.02). No other association was found between SERPINA1 methylation and the clinico-pathological parameters of CRC patients.

Conclusion: Our findings show that there is a trend for SERPINA1 gene promoter hypomethylation and in CRC tumors.

Keywords: SERPINA1, methylation, colorectal cancer
The increased level of local and circulating CSC markers in patients with Functional and Non-Functional Pituitary adenoma

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Background and aim: The cancer stem cell (CSC) hypothesis has captured the attention of many scientists. It is believed that elimination of CSCs could possibly eradicate the whole cancer. CSC surface markers provide molecular targeted therapies for various cancers, using therapeutic antibodies specific for the CSC surface markers. The cell surface proteins CD133 and CD44 are putative markers for cancer stem cell populations and can account as CSC markers. The aim of the present study was to investigate the expression level of local and circulating CD133 and CD44 (CSC markers) in peripheral blood mononuclear cells and tumor tissue of patients with Functional and Non-Functional pituitary adenomas.

Materials and Methods: 40 patients from Firouzgar Hospital in Tehran with Functional and Non-Functional pituitary adenomas have participated in this case-control study. The peripheral blood mononuclear cells of the subjects and tumor tissues were used for mRNA extraction and cDNA construction, and to determine the expression level of CD133 and CD44 genes, a Real-Time PCR-based Cyber Green method was used and data were analyzed using ΔΔCT method. Finally, statistical analysis was performed using Graph Pad Prism software version 5 and independent t-test.

Results: Measurement of CD133 and CD44 expression level in mononuclear blood cells extracted from the peripheral blood and also tumor tissues of patients with Functional and Non-Functional pituitary adenomas revealed that the level of these markers were significantly increased in patients comparing to healthy subjects and normal tissues. Also, the increased level of CD133 and CD44 were associated with elevated level of tumor grade and stage.

Conclusion: The results of the current study have shown that the CD133 and CD44 can account as a circulating also local CSC markers in patients with Functional and Non-Functional pituitary adenomas and can be noticed as a possible biomarker for controlling disease.

Key Words: CD44, CD133, gene expression, Pituitary Tumor.
Evaluation of the expression of apoptosis-related genes in the peripheral blood mononuclear cells of leukemia patients

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Background and Aim:

Apoptosis or programmed cell death is the cell’s intrinsic death program which plays a crucial role in the regulation of many normal physiological processes in the body’s tissues. In the cancer field, apoptosis induction by TRAIL is a promising strategy for the treatment of human cancers because of its limited toxicity to normal tissues. Interestingly, the majority of leukemia cells express TRAIL receptors. The aim of this study was evaluation of the expression of apoptosis-related genes in the peripheral blood mononuclear cells (PBMCs) of leukemia patients.

Methods:

Peripheral blood samples were obtained from patients with leukemia (n=20) as well as healthy individuals (n=10). PBMCs were isolated using Ficoll-Paque density gradient centrifugation protocol. After RNA extraction and cDNA synthesis, gene expression levels of molecules involved in the extrinsic (death receptor) and intrinsic (mitochondria) pathways of apoptosis were evaluated by real-time RT-PCR technique.

Results:

Gene expression analysis showed that expression levels of the initiator caspases-8 and -9 are increased in the PBMCs of leukemia patients when compared with healthy individuals. Increased gene expression level of the proapoptotic protein Bak was also detected in PBMCs of leukemia patients. In contrast, decreased expression levels of the proapoptotic protein Bax and the executioner caspase-3 were observed in the PBMCs of leukemia patients.

Conclusions:

These results suggest that both extrinsic and intrinsic signaling pathways of apoptosis may be triggered in PBMCs of leukemia patients but activation of other proapoptotic molecule and the executioner caspase-3 diminished in cancer cells. This finding indicates that resistance to apoptosis may be one of the hallmarks of leukemia cells.

Keywords: Leukemia, Apoptosis, PBMC, Gene expression evaluation
Apoptosis induction and Inhibition of human bladder cancer cell growth by Rhus verniciflua Stokes extract

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Background: Cell death can occur through several different mechanisms of which its most widely described forms are apoptosis and necrosis. Flavonoids are commonly found in most plants. Flavonoids exert a remarkable spectrum of biological activities affecting the basic cell functions, such as growth differentiation, and apoptosis. Flavonoids are known to have anticarcinogenic, anti-inflammatory, antibacterial, and antiviral activities. Formerly, a flavonoid fraction, consisting mainly of protocatechuic acid, fustin, sulforetin, and butein, herein named RCMF (RVS chloroform-methanol fraction), was prepared from a crude acetone extract of Rhus verniciflua Stokes (RVS) which is traditionally used as a food additive and an herbal medicine.

Methods: The human bladder cancer cell line (EJ138) were used for this study. The cells were grown in RPMI1640 culture medium. RVS was dissolved in DMSO and stored in a dark-colored bottle. (MTT) was used to evaluate the viability of the cells. Cellular cytotoxicity induced by the RCMF treatment was measured using a trypan blue exclusion assay.

Results: Addition of RCMF to the cultured EJ138 cells markedly inhibited the incorporation of tritium by the DNA in the cells, in a dose-dependent manner. Treatment with 100 µg/ml RCMF almost completely inhibited the tritium uptake by EJ138 cells. In addition, the RCMF mediated inhibition of the tritium uptake in EJ138 cells was time dependent. These findings suggest that RCMF inhibited the proliferation of EJ138 cells in dose- and time dependent manner. MTT assay showed that the addition of RCMF reduced the viability of EJ138 cells in a dose- and time-dependent manner. Trypan blue exclusion assay showed that the added RCMF had a substantial cytotoxic effect.

Conclusion: The available experimental evidence suggests that it is worth testing RVS as a cancer therapeutic agent because the results of this study demonstrate that RVS has the ability to reduce the viability of bladder cancer cells through induction of apoptosis.

Keywords: Rhus verniciflua Stokes, bladder cancer, apoptosis, growth inhibition
OC-09

Evaluation of the expression of CTLA-4 and Tim-3 genes in the peripheral blood mononuclear cells of leukemia patients

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Background and Aim:
Cancer cells can escape tumor immunosurveillance by multiple mechanisms such as expression of immunoinhibitory molecules which and induction of immunosuppressor cells. Expression of some cell surface molecules in the immune cells are associated with suppression of antitumor activity of cells or cell exhaustion. The aim of this study was assessment of the expression of Cytotoxic T lymphocyte antigen-4 (CTLA-4) and T cell immunoglobulin and mucin domain 3 (Tim-3), which are respectively associated with the suppressed antitumor activity and exhaustion, in the peripheral blood mononuclear cells (PBMCs) of leukemia patients.

Methods:
PBMCs were isolated from peripheral blood of 20 patients with leukemia and 10 healthy individuals. RNA was extracted from PBMCs. After cDNA synthesis from extracted RNA, expression of CTLA-4 and Tim-3 genes were evaluated by real-time RT-PCR technique.

Results:
Expression of CTLA-4 gene was at lower levels in PBMCs isolated from leukemia patients when compared with CTLA-4 expression in PBMCs of healthy donors. Nonetheless, PBMCs from leukemia patients showed significantly higher expression levels of Tim-3 than PBMCs of healthy individuals (P<0.05).

Conclusions:
Expression of high levels of Tim-3 in the PBMCs of leukemia patients suggests that PBMCs show an exhaustion phenotype in these cancer patients.

Keywords: Leukemia, PBMCs, CTLA-4, Tim-3, Gene expression
Generation of insulin producing cells from bone marrow derived mesenchymal stem cells using siRNA in vitro

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Back ground:

Bone marrow derived mesenchymal stem cells (BMSCs) are potential multipotent cells derived from adult tissue. So far, growth factors have been used for differentiation of BMSCs to insulin producing cells. In the present study, in the absence of growth factors, siRNA was used to silence targeted genes.

Method:

In this experimental study, BMSCs were derived from human bone marrow tissue. The BMSCs were then cultured in four groups; three test groups (containing culture medium with siRNA) and the control (the same culture medium used in test group without siRNA). After three weeks, differentiated cells were analyzed by using RT-PCR (expression of some pancreas-specific genes), immunocytochemistry (detection of insulin presence in cells) and ELISA (evaluation of the amount of secreted insulin to culture medium).

Results:

The RT-PCR analysis of differentiated cells on three test groups showed expression of beta cell specific markers including insulin and Pdx1. The results of immunostaining showed that the insulin protein are expressed in differentiated cell of Foxo1 siRNA group and Foxo1/Gcg-siRNA group with different amounts and finally insulin secretion assay show that differentiated cells on Foxo1 siRNA group secreted more insulin in comparison with the other groups.

Conclusion:

Our data indicate that human BMSCs differentiate into insulin producing cell using siRNA, without growth factors. Therefore, siRNA can be used as a novel approach for generating insulin producing cells from BMSCs in vitro.

Key words: Bone marrow derived mesenchymal stem cells, Insulin producing cells, siRNA
Higher diagnostic yield can be achieved by using multigene NGS panel in testing patients with Marfan-related disorders

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Background: Marfan syndrome is a life threatening condition with estimated prevalence of 1:5,000-1:10,000. This disorder may occur secondary to FBN1 gene mutations. However, there are other disorders resulting from other genetic defects with different, but occasionally overlapping, phenotypes. Next-generation sequencing (NGS), as a high throughput method can differentiate between them. Here, we aim to compare the diagnostic yield of using NGS multigene panel with FBN1-only testing.

Methods: Targeted NGS was applied to analyze 34 samples from individuals with clinical presentation of Marfan-related disorders. Twenty-one samples were referred for “Marfan Syndrome” panel (FBN1-only) and 13 samples for “Marfan, Aneurysm and Related Disorders” testing panel (including 14 genes). Target regions capture with Nimblegen chip in the genes of interest followed by Next Generation Sequencing on Illumina. The reported variants were interpreted according to ACMG-guideline for variant interpretation 2015.

Results: Of 21 samples subjected to analyze FBN1 only, in 11 samples (52%), variants with VUS, likely pathogenic or pathogenic classification were detected. In 10 samples (46%), we did not detect any suspected causative variant.

Multigene panel of 14 genes was analyzed in 13 samples. In 9 samples the causative variants were located in FBN1 gene (69%). Remaining four samples had possible causative variants in SLC2A10, MYLK, COL5A2 and TGFBR2 genes.

In total, missense variants were the most common ones. In addition, 7 novel variants were identified. Details of novel and reported variants detected in this study will be presented at the meeting.

Conclusion: This study showed that using NGS panel of genes associated with all Marfan-related disorders, gives a 31% increase in diagnostic yield of genetic testing for such patients. Nearly 30% of disease causing variants associated to Marfan-related disorders in Iranian patients have not been reported before in other populations.

Keywords: Marfan syndrome, High-Throughput Nucleotide Sequencing, FBN1 gene
OG-03

Evaluation of an improved method for detection of intron 22 inversion mutation of the factor VIII gene in patients with Hemophilia A

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Hemophilia A is an X-linked bleeding disorder in all over the world and intron 22 inversion mutation (inv22) of the factor VIII (FVIII) is a hotspot mutation causing severe hemophilia A. Our hypothesis was aimed to present an improved method for detection of inv22 of the FVIII gene in patients affected with severe Hemophilia A, using Real-time PCR. In this study, whole blood samples were drawn from 21 patients with Hemophilia A and, after DNA extraction, a classical Inverse PCR (I-PCR) was performed on the samples to detect the FVIII Inv22 mutation. Eleven samples were found to be affected with this inversion mutation and results were reconfirmed using the long distance PCR (LD-PCR) on the same samples immediately afterwards. Since the classical I-PCR is a tedious and time-consuming technique involving several stages such as enzyme digestion, enzyme ligation to make a circular template, followed by a PCR and post PCR, we have tried to simplify this method by modifying these stages using Real-time PCR. For this purpose, the confirmed samples with I-PCR technique were taken to the ligation stage, followed by a Real-Time PCR instead of LD-PCR and I-PCR. In conclusion, in this study we could present the improvement of the classical I-PCR, named Real-time I-PCR, it was taken in only one day instead of 3 days, which is more accurate and specific for detecting the FVIII inv22 in patients with severe Hemophilia A and other genetic diagnosis in well-equipped laboratories as well.

Key words: Hemophilia A, FVIII, Inv22, classical Inverse PCR, Real-time PCR
Assessment of protein prenylation pathway in multiple sclerosis patients.

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Background: Multiple sclerosis (MS) is a chronic inflammatory disorder with several genetic and environmentalfactors being implicated in its pathogenesis. Protein prenylation as one of the importantposttranslational modifications of proteins has a crucial role in immune system regulation.

Methods: In thecurrent case–control study we compared expression of five genes coding for the different subunitsof proteins implicated in protein prenylation in 50 Iranian MS patients with those of healthysubjectsby Taqman Quantitative Real-Time PCR.

Results: No significant difference has been found in FNTA and PGGT1B expressions between cases and controls. Spearman Correlation analysis between FNTA relative expression and diseaseduration showed a significant correlation in male patients (r=-0.671, P=0.024) but not female patients (r=0.253, P=0.12). FNTB expression was significantly higher in MS patients compared with healthy subjects. Spearman Correlation analysis between FNTB relative expression and disease duration showed significant correlation in male patients (r=-0.876, P=0.004) but not female patients (r=0.296, P=0.06). RABGGTA was significantly upregulated in total MS patients, total male patients, female patients aged between 30 and 40 and male patients aged >40 compared with corresponding control groups. RABGGTB was significantly down-regulated in total MS patients, total female patients and female patients aged >40 compared with corresponding control groups.

Conclusion: Totally, we demonstrated dysregulation of protein prenylation pathway in MS patients compared with healthy subjects. Future studies are needed to find the clinical implication of this pathway in MS patients.

Keywords: Multiple Sclerosis, Gene Expression, Prenylation.
OH-01

Bone marrow mesenchymal stem cells improves expansion and better maintain C-MYC gene expression of human cord blood CD34+ stem cells

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Introduction:

Umbilical cord blood (CB) has been found to be a rich source of hematopoietic Stem Cells (HSC). One factor limiting the therapeutic efficacy of CB transplantation is the low cell dose of the graft. Cell dose can be increased by ex vivo expansion. Identifying strategies to enhance expansion and maintain self-renewal of HSCs can improve engraftment. Regulation of self-renewal for sustained maintenance of HSC pool, is controlled by specific interactions with the microenvironment in the bone marrow (BM). The goal of this study was to examine BM-MSC on ex vivo expansion and C-MYC gene expression, as a self-renewal marker, in cord blood CD34+ stem cells.
Materials and methods:

In this study, human cord blood CD34+ HSC isolated by MACS, cultured in the serum-free medium (Stem line II) supplemented with cytokines (TPO, FLT3L, SCF) with/without Bone marrow mesenchymal stem cell for 7 days. Before and after of this period, Total nucleated cell count (TNC), CD34+ cells count, CFC assay and C-MYC expression by Real time PCR were evaluated. The data analyzed using the t-test and ANOVA. Value < 0.05 were considered statistically significant.

Results: At the end of 7 days of culture, in the presence of MSC, CD34+ cells gave rise to higher nucleated cell (TNC) and CD34+ cells, produced more CFUs and maintain a higher C-MYCMRNA level compared to control culture.

Conclusions: Bone Marrow MSC through cell-to-cell interactions, as well as through secretion of hematopoietic cytokines not only improves ex vivo expansion of human HSC but also contribute to the regulation of HSC self-renewal and provide a "niche-like" milieu for hematopoietic stem cells.

Keywords: Cord blood, Hematopoietic stem cell, CD34+ cells, Mesenchymal stem cell, C-MYC.
Establishment of a cell line expressing recombinant factor VII and its subsequent conversion to active form FVIIa through hepsin by genetic engineering method.

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Background
Factor VII (FVII) is a plasma glycoprotein that participates in the coagulation process leading to generation of fibrin. It is converted to factor VIIa that plays an important role in the coagulation cascade. The aim of this study was isolating and cloning the genes of human factor VII and hepsin and subsequent co-transfection of the constructs to Chinese hamster ovary (CHO) cell line to obtain rFVIIa.

Methods
Factor VII and hepsin cDNAs were isolated from HepG2 cell line and cloned into pcDNA3·1 (+) vector. The constructs were co-transfected to CHO cell line. A cell line that permanently expressed recombinant factor VII (rFVII) and hepsin was established. The expression of rFVII was confirmed by reverse transcriptase–polymerase chainreaction, enzyme-linked immunosorbent assay and Western blot analysis. Biological activity of rFVII was evaluated by prothrombin time assay.

Results
The results showed that the genes of FVII and hepsin were successfully cloned and expressed. Stable CHO cells co-transfected with pcDNA3·1-FVII and pcDNA3·1-hepsin expressed FVII and hepsin mRNA, but there was no expression in the CHO cells transfected with insert free pcDNA3·1. FVIIa protein was secrted to medium of CHO cells co-transfected with pcDNA3·1-FVII and pcDNA3·1-hepsin. The expected band of rFVII was detected in Western blot analysis. A three- to fourfold decrease in clotting time was observed when human FVII-depleted plasma was used in combination with human thromboplastin in the presence of rFVII, confirming the biological activity of rFVII.

Conclusion
As we are aware, this is the first report of establishing a cell line expressing FVIIa using genetic engineering methods.

Key words:
CHO, co-transfection, genetic engineering, FVIIa, hepsin, recombinant.
Kaempferol sensitizes Chronic myelogenous leukemia cells to TRAIL-induced apoptosis via decrease of apoptosis inhibitor genes and death receptors up-regulation

Introduction: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a new anticancer component for cure of malignancies because of its tumor selectivity without toxicity for healthy cells. Unfortunately, a common unfavorable event is malignant cells resistance to TRAIL. Kaempferol is an anti-oxidant flavonoid inducing apoptosis in tumor cells. Here, we investigated that whether cotreatment TRAIL with Kaempferol decrease resistance in leukemic cell line.

Method: To determine the IC50, we investigated the effect of Kaempferol in different concentrations on cultured leukemic cells. The viability was determined with MTT assay, then, to study of cell growth inhibition and apoptosis, the cells were treated with different concentrations of TRAIL alone, TRAIL plus Kaempferol and Kaempferol alone for 12, 24 and 48 hours. Afterwards, cell apoptosis was evaluated via flow cytometry by Annexin Vfluorescein isothiocyanate/propidium iodide staining and the expression level of apoptosis inhibitor genes and death receptors were analyzed using quantitative real time PCR.

Result: our study showed that combination Kaempferol plus TRAIL was effective than either Kaempferol or TRAIL alone in the drug resistance in CML cells. We also showed that low-toxicity Kaempferol lead to up regulation TRAIL death receptor (DR4 and DR5) expression to enhance apoptosis and we observed remarkable reduction in the expression of apoptotic inhibitors including c-FLIP, c-IAPs and NF-KB while there was no significant change in XIAP.

Conclusion: These data indicate that flavonoids like Kaempferol can be considered as plant compounds increasing TRAIL-induced apoptosis and may serve as a promising targeted therapy tool in leukemia.

Keyword: TRAIL, Kaempferol, chronic myelogenous leukemia, apoptosis
OH-04

Survey of changes in standard coagulation test results during cardiopulmonary bypass

Elham Khalf Adeli

Background and Aim: Patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) are at increased risk of bleeding due to multifactorial coagulopathies. In the present study, we aimed at investigating the changes in standard coagulation test results during cardiopulmonary bypass.

Methods: A total of 40 adult patients scheduled for cardiac surgery were included in this prospective observational study. Standard laboratory coagulation tests (PT, APTT, fibrinogen assay and D-dimer) were performed by STA Compact Analyzer (Diagnostica Stago) at two time points: before CPB and after CPB. The rate of change in each result was calculated as percentage.

Results: Before CPB, the mean value (SD) for PT, APTT, fibrinogen concentration and D-dimer were 18.7(4), 45(14), 317(82), 0.77(0.57) respectively. After CPB, values for these tests were 24.9(14), 64(24), 231(91), 1.9(1.6) respectively. Both PT (33%) and PTT (42%) significantly increased during CPB (P<0.05). Concentration of fibrinogensignificantly decreased by an average 27% after CPB (P<0.05). The increasing rate of change for D-Dimer was 146% which was statistically significant (P<0.05).

Conclusion: Post-operative blood loss is common in patients undergoing cardiac surgery. Understanding of rate of changes in standard coagulation testing during CPB can be useful in early decision for coagulopathy and bleeding management.
Mimicry of bone marrow niche by 3D co-culture of hematopoietic stem and progenitor cells and mesenchymal stem cell in microfluidic chip

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Aim: Ex vivo mimicry of bone marrow (BM) microenvironment provides a potential platform to dissect regulatory mechanism in hematopoiesis and it also can be used for expansion of hematopoietic stem and progenitor cells (HSPC). 3D cell culture in bioreactor allows mimicking the HSPCs niche. HSPCs expansion potency over stromal cells layer has been targeted in demineralized bone matrix as scaffold via 3D dynamic co-culture.

Materials and methods: We have developed a bioreactor made of PDMS. Cord blood CD34+ HSCs cultured on the stromal layer under cytokine free condition for 7 days. Flow cytometry, Colony assay and SEM morphologically assessment on the result and initiative samples were performed.

Results: At 7 days co-culture, CD34+ cell number increment difference was statistically significant between dynamic and static groups. CD34+ HSCs expansion 4.7 folds increased in perfused culture on day 7 compared to static culture at the same day.

Conclusions: The ability of HSPCs 3D culture expansion in bioreactor with stromal support over DBM scaffold could have emerged new approach for the current limitations of HSC transplantation.
Overexpression of mir-138 inhibits cell growth and induces caspase-mediated apoptosis in acute promyelocytic leukemia cell line

Background: MicroRNAs are small, single stranded, non-coding RNAs which play key role in regulation of gene expression. It has been shown that alteration of microRNAs have been resulted in cancer development. Here, we investigated the effect of overexpression of mir-138 on proliferation and apoptosis of acute promyelocytic leukemia (APL) derived cell line, NB4.

Methods: mir-138 was overexpressed in NB4 cells using GFP hsa-mir-138-expressing lentiviruses. hTERT mRNA and protein expression levels were assessed by qRT-PCR and western blot analysis. For evaluation of apoptosis Annexin-V staining and caspase activation were assessed using flow cytometry and western blot analysis, respectively.

Results: Our data demonstrate that, overexpression of mir-138 attenuated the hTERT mRNA and protein expression levels. In addition, cell growth was inhibited and malignant cells underwent caspase mediated-apoptosis in response to overexpression of mir-138.

Conclusions: These findings suggest that loss of mir-138 expression may be associated with increased telomerase activity in APL cell line NB4. Therefore, strategies for up-regulation of mir-138 may result in inhibition of malignant cell growth and provide a promising therapeutic approach for acute promyelocytic leukemia.

Keywords: Apoptosis, Caspase, hTERT, mir-138, PARP
Serial investigation of TIM-3 gene expression in renal transplant recipients for diagnosis and prognosis of acute allograft rejection

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Background: Renal transplant recipients need to periodic surveillance for immune-based complications such as rejection. Noninvasive monitoring methods instead of core needle biopsy as an invasive method are preferred. Because TIM-3 is expressed on primary T effector cells, including Th1, CTL, and Th17, which have an important role in allograft rejection, we evaluated serial changes of TIM-3 mRNA expression in blood and urine samples of renal transplant recipients to determine whether serial investigation of TIM-3 mRNA expression in human renal allograft can predict acute allograft rejection.

Materials and Methods: In this study, 30 kidney transplant recipients were studied. Blood samples at 4 times (1 day before, 1 day, 3 months, 6 months after transplantation) and urine samples at 3 times (1 day, 3 months, 6 months after transplantation) were taken. RNA extraction, cDNA synthesis and mRNA expression of TIM-3 gene by RT-PCR were performed.

Results: Blood TIM-3 mRNA expression at the month 3 and 6 after transplantation and urinary TIM-3 mRNA expression at the first day, month 3 and 6 after transplantation was significantly higher in patients who developed graft dysfunction compared with well-function graft patients (p < 0.001). We also found a high correlation between blood and urinary TIM-3 mRNA expression.

Conclusion: Our study suggests that TIM-3 mRNA quantification by Real-time PCR in PBMCs could be used as a promising tool for noninvasive diagnosis and prognosis of acute renal allografts rejection.

Keywords: TIM-3, Renal Transplant recipient, Acute renal allografts Rejection.
OI-02

Analysis for relative expression level of mir-181b-1 and its target, Cylindromatosis (CYLD), expression in breast cancer specimens

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Abstract

Background: Searching for biomarkers during tumor progression could help for defining stages, classification, improving diagnosis, prognosis and prediction of therapeutic outcome. Many microRNAs are aberrantly express in cancers and seem to influence tumor behavior and progression. Mir-181b-1 and its mRNA target, cylindromatosis (CYLD), are involved in regulating the inflammatory pathways. The current study was carried out to investigate the expression levels of mir-181b-1 and CYLD in a cohort of breast tumor tissues and normal adjacent tissues to assess their association with breast cancer stages.

Materials and Methods: A total number of 20 breast samples including cancerous and normal adjacent tissue specimens were collected. Total RNA from the tissues were extracted and expression of mir-181b-1 and CYLD were measured by qRT-PCR method.

Results: The miR-181b-1 expression level was significantly increased in breast tumor tissues compared to the controls (14.2±11.96 vs 0.81±0.44, P=0.001). This increase was associated with the disease progression (p<0.001). Conversely, CYLD expression level was decreased in tumor samples compared to normal samples, significantly (1.09±1.49 vs 0.85±0.17, P=0.24). ROC curve data add other prestigious information of miR-181b-1 and CYLD by defining cancer and healthy tissues with high specificity and sensitivity at a proposed cutoff point.

Conclusion: Here we showed increased level of miR-181b-1 and decreased level of CYLD expression in breast cancer versus normal tissues. Thus, we suggest that miR-181b-1 and CYLD might be involved in the pathogenesis of breast cancer and could be considered as two biomarkers for prediction, prognosis and diagnosis of the stages of the breast cancer.

Keyword: breast cancer, mir-181b-1, CYLD, molecular inflammation.
OI-03

CD4+ T cells Are Exhausted and Show Functional Defects in Chronic Lymphocytic Leukemia

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Abstract

Exhausted T cells are a group of lymphocytes which are developed in chronic infections and malignant tumors. In the current study, the frequency and functional properties of exhausted CD4+ T lymphocytes were investigated in patients with CLL. PBMCs were obtained from 25 untreated CLL patients and 15 healthy volunteers. The frequency of CD4+/Tim-3+/PD-1+ cells was measured by flow cytometry. For evaluation of cell proliferation and cytokine production, CD4+ T cells were isolated with MACS and stimulated with Phytohemagglutinin and PMA/ionomycin. Concentrations of cytokines were measured in the culture supernatants of stimulated cells by ELISA. The percentage of CD4+/Tim-3+/PD-1+ cells was significantly higher in CLL patients than those of normal controls. CD4+ T cells from CLL patients showed lower proliferative responses, lower production of IL-2, IFN-γ, and TNF-α, and higher production of IL-10, compared to healthy controls. Functional properties of isolated CD4+T cells from CLL patients were inversely correlated with the frequency of exhausted Tim-3+/PD-1+/CD4+ cells. Collectively, CD4+T-cells from CLL patients showed phenotypic and functional characteristics of exhaustion. Given that exhaustion phase of T cells can be reversible, targeted blocking of immune inhibitory molecules could be a promising tool to restore host immune responses against leukemic cells in CLL.

Keywords: Chronic lymphoblastic leukemia, Exhausted T cell, PD-1, Tim-3
The Effects of Silymarin and Cyclosporine A on the Proliferation and Function of Regulatory T Cells

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Background: Immunosuppressive agents are necessary to enhance allograft tolerance after transplantation and the treatment of autoimmune disorders. Regulatory T cells (T regs) play a pivotal role in improving allograft tolerance and determining the fate of transplanted organs. Therefore, the specific aim of this study was to investigate the immunomodulatory effects of Cyclosporine A (CsA) and silymarin on Treg proliferation and function.

Methods: T regs were isolated from peripheral blood mononuclear cells (PBMCs) from healthy donors and phenotypic characteristics of T regs were determined by flow cytometry. T regs were expanded and then cultured with different concentrations of CsA and silymarin. The immunomodulatory effects of CsA and silymarin on the proliferation and function of T regs were determined after 3 and 5 days of culture.

Results: Our results showed that CsA significantly decreased Treg proliferation in a dose-dependent fashion (p< 0.05-0.01). However, CsA did not have the ability to induce Treg function through the production of transforming growth factor-beta1 (TGF-β1). In contrast, silymarin significantly increased the proliferation of T regs (p< 0.05-0.01). A statistically significant increase was also observed in the function of T regs through TGF-β1 production (p< 0.05-0.01).

Conclusion: Overall, the results of this study, for the first time ever, show that silymarin, unlike CsA, possesses the ability to increase the proliferation and function of T regs and may be beneficial in the treatment of autoimmune disorders and improvement of Treg-dependent allograft tolerance after transplantation.

Keywords: Silymarin, Cyclosporine A, Regulatory T cells, TGF-β1
Modulated expression of IL-12 (P35/P40) in ginger-treated EAE mice.

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Objective:

The main function of IL-12 is differentiation of naive T cells into Th1 cells. It has been reported that the autoreactive Th1 cells were responsible for demyelination in EAE. The immunomodulatory and anti-inflammatory properties of ginger have also been reported in some studies. The aim of this study was to evaluate the effects of ginger extract on the expression of IL-12 (P35/P40) mRNA in the CNS and serum in a model of experimental autoimmune encephalomyelitis (EAE).

Materials and Methods:

EAE was induced in Female (6-8 week old) C57BL/6 mice by immunization with myelin oligodendroglial glycoprotein emulsified in complete Freund's adjuvant. The mice were administered intra-peritoneally with ginger extracts or PBS, from day +3 to +30. On day 31, mice were scarified and the expression of IL-12 (P35/P40) mRNA in the spinal cord were determined by using real time-PCR. The serum levels of cytokines were measured by ELISA.

Results:

in PBS-treated EAE mice, the expression of IL-12 P35 and IL-12 P40 mRNA in the CNS and the mean serum levels of IL-12 were significantly higher than those of healthy group (p<0.001). In ginger-treated EAE mice, the expression of IL-12 mRNA and its serum levels were significantly lower as compared to PBS-treated EAE mice.

Conclusion:

In conclusion, our results showed higher expression of IL-12 in the spinal cord and serum of EAE mice. Accordingly, the up-regulation of the expression of IL-12 may be involved in the development EAE. Moreover, treatment of EAE mice with ginger extract modulates the expression of IL-12 in CNS and serum of mice with EAE.

Key Words:

Experimental autoimmune encephalomyelitis, Ginger, IL-12, Serum
Investigation of \textit{CD18} gene expression profile and methylation of its promoter region in Systemic Sclerosis disease

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\textbf{Background}: Systemic Sclerosis (SSc), an autoimmune disease of connective tissue, is characterized by inflammation, fibrosis, and vessel endothelial damage. Products of integrin subunit beta 2 (CD18) gene participate in several functional pathways of immune system. The aim of this investigation was to survey the transcript level of \textit{CD18} gene as well as methylation status of CpG sites in promoter region of gene in PBMCs of SSc patients.

\textbf{Methods}: PBMCs were isolated from whole blood of 50 SSc patients and 30 healthy controls. Total RNA and DNA contents of PBMCs were extracted. Gene expression was analyzed by real-time PCR using the SYBR Green PCR Master Mix. To investigate the methylation status of CpG sites, DNA samples were treated by bisulfite, amplified through nested PCR, and sequenced through Sanger difficult sequencing method.

\textbf{Results}: \textit{CD18} gene in PBMCs of SSc patients was overexpressed significantly in comparison to healthy controls. Three CpG sites of 12, 13 and 14 were significantly hypomethylated in patients group, despite overall methylation status of \textit{CD18} gene promoter revealed no significant difference between study groups. There was no statistically significant correlation between methylation status of \textit{CD18} promoter and the gene expression in patients.

\textbf{Conclusions}: Regarding to lack of correlation of increased expression of \textit{CD18} with its promoter hypomethylation in SSc patients, our study suggests that upregulation of \textit{CD18} in PBMCs from SSc patients is probably due to another mechanism other than methylation alteration.

\textbf{Keywords} Systemic sclerosis, CD18, CpG site, DNA methylation

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The potential role of IL-4 and receptor activator of NF-κB ligand in the pathogenesis of giant cell granuloma

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Background: Giant cell granuloma (GCC) is a non-malignant lesion characterized by proliferation of granulation tissues with multinucleated giant cells. Although giant cell granuloma is a well-described clinical entity, little is known on its pathogenesis. Given the contribution of IL-4 and receptor activator of NF-κB ligand to the disease pathogenesis, we investigated effects caused by possible synergism between IL-4 and RANKL on the viability and apoptosis induction of monocytes derived from GCG patients.

Methods: Monocytes were isolated from peripheral blood of GCC patients and healthy subjects using monocyte isolation kit (II). The isolated monocytes were treated with 10 and 20 ng/mL concentrations of IL-4 and RANKL for five days. MTT assay and Annexin V-FITC/PI staining followed by flow cytometry were used to investigate the viability and induced apoptosis of monocytes, respectively.

Results: In monocytes isolated from both GCG patients and healthy groups, treatment with IL-4 and RANKL led to increased viability and decreased apoptosis, even though these changes were significantly more obvious in patient-derived monocytes. Also, we found that compared with RANKL, IL-4 results in significantly higher resistance to apoptosis in monocytes.

Conclusion: Based on these findings might provide evidence for the potential role of IL-4 and RANKL in the pathogenesis of GCG.

Keywords: Pathogenesis, Interleukin-4, receptor activator of NF-κB ligand, Giant cell granuloma
Increased levels of IL-23 in peripheral blood mononuclear cells of patients with chronic heart failure

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Introduction: Chronic heart failure (CHF) is a complex clinical syndrome that represents the end stage of various cardiac diseases and is characterized by the inability of the heart to meet the metabolic demands of the body. Many physiological systems involved in this disease. In particular, the activation of the immune system has received considerable interest in the last decade. Evidence from both experimental and clinical trials indicates that inflammatory mediators are of importance in the pathogenesis and progression of chronic heart failure. Excessive proinflammatory cytokines induce contractile dysfunction, hypertrophy, fibrosis and cell death in Cardiac myocyte. We examined the expression of IL-23 in PBMCs between CHF patients and healthy controls.

Methods: In this report we used real-time PCR assay to compare the relative expression of IL-23 in peripheral blood mononuclear cells (PBMC) from CHF patients with various heart diseases (n=42, EF<45%, range of New York Heart Association (NYHA) 1 to 4) and matched healthy control subjects (n=42). We also determined the IL-23 concentrations of cell culture supernatant of PBMCs with ELISA.

Result: A total of 42 patients with CHF, with 42 age and sex-matched control group subjects were enrolled in the present study. The culture supernatant levels of IL-23 in PBMC of CHF patients were significantly higher (133.95 ± 108.99 pg/mL) than in the control group (83.43 ± 76.2 pg/mL) (P < 0.05). The gene expression of IL-23 was also markedly upregulated in PBMC from CHF patients in comparison with control group but it was not statically significant.

Conclusion: These results demonstrate that in patients with CHF and especially those with severe CHF, expression of pro-inflammatory cytokines and levels of IL-23 cytokine is markedly increased in PBMC. These finding suggested that IL-23 may plays an important role in the progression of CHF among these patients.

Keyword: Chronic heart failure ; immune system; IL-23
Clinical and hematological characteristics in human Trichostrongyliasis patients
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Background: Information about the clinical aspects of human trichostrongyliasis is limited. Hereby, clinical and hematological characteristics of a large number of trichostrongyliasis patients are described.

Materials and methods: Sixty patients were identified as positive with Trichostrongylus spp. using parasitological methods in residents of Fouman district of Guilan province in northern Iran in 2015 to 2016. Among them, 55 patients had trichostrongyliasis only and data of this group were subjected to analysis of clinical manifestations.

Results: Twenty-three patients (38.3%) were male and 37 (61.7%) female. Among the only Trichostrongylus infected individuals, 9 patients (16.4%) were asymptomatic; rest of them (46; 83.6%) reported symptoms. Gastrointestinal manifestations, pulmonary symptoms and cutaneous symptoms were reported in 76.3%, 30.9% and 12.7% of patients, respectively. No statistically significant difference was found between clinical manifestations with sex and age groups. Ten patients (18.1%) illustrated blood eosinophilia and 5 (9.1%) individuals presented hypochromic microcytic anemia. There was not a statistically significant difference between eosinophilia with age groups, sex and clinical manifestations of the patients.

Conclusion: This data is useful for physicians, since patients in endemic areas may experience gastrointestinal symptoms or pulmonary allergy and cutaneous symptoms which would probably be related to trichostrongyliasis.

Keywords: Clinical manifestations, human, Trichostrongyliasis, Iran, Guilan
OP-02

The ability of Antigen B originated from G1 strain of *Echinococcus granulosus* for serodiagnosis of confirmed cases of human liver cystic echinococcosis (CE)/hydatidosis

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**Background:** Cystic echinococcosis is an important zoonotic parasitic disease worldwide. Different strains of the parasite causes human CE in different organs especially in the liver. Different antigens have been used for serodiagnosis of the disease. The native antigen B has been suggested more by the scientists. Application of strain specific antigen in serodiagnosis of human hydatidosis is important to prevent cross reactions. Strain specific AgB has not been used for serodiagnosis of CE, so far. Keeping in mind, AgB originated form G1 strain of hydatid cyst which was confirmed by molecular biology and sequencing was used for evaluating its ability in diagnosis of confirmed human liver CE.

**Methods:** Hydatid cyst fluid (HCF) was collected from sheep liver cysts. The genotype of the cysts were confirmed by PCR and sequencing of cox1 and nad1 genes. The HCF of G1 strain was used for preparation of AgB. A total of 22 sera samples from confirmed liver CE patients, 22 sera from healthy people as negative control and also 22 sera from other parasitic diseases were used for measurement of the diagnostic ability of G1 strain originated antigen B by ELISA. Sensitivity, specificity, validity, positive and negative predictive values were evaluated (95% CI).

**Result:** The results showed that 20 out of 22 confirmed human liver CE were positive by ELISA using G1 strain originated AgB. These values were 5 out of 22 sera for the other parasitic diseases and 0 out of 22 for negative controls. The sensitivity, specificity, validity, positive and negative predictive values were determined as 90.5, 88.63, 89.75, 90.5 and 88.63 respectively.

**Conclusion:** The ability of antigen B originated from G1 strain of *E. granulosus* metacestode for diagnosis of confirmed cases of human liver CE is high. However, due to the diversity of the cyst strains in Iran, application of other prevalent strains of AgB alone or in combination with other strain sources is suggested.

**Keywords:** Cystic echinococcosis, Hydatidosis, G1 strain, AgB, Diagnosis, Human
Comparison between Nested-PCR and microscopic techniques in detection of asymptomatic malaria

Introduction
Malaria as a parasitic disease is an important infection in the world in term of being harmful to social and economic enlargement, morbidity, mortality especially among the children and fast detection of a symptomatic patient is essential. So, the aim of this study was comparing two diagnostic tests, microscopy and nested-PCR in detection low parasite in malaria.

Material and methods
A cross-section study was conducted in Zahedan City, Iran from April to September 2017. A total of 300 blood samples of volunteers were collected and evaluated by two microscopy and nested-PCR technique. Completion of the questionnaires was performed by participants.

Results
Of 300 samples, 4 (1.3%) had symptomatic malaria and all of the species of a positive case was detected by Nested-PCR.

Conclusion
Nested-PCR is a sensitive and proper method and uses it is suggested to detect malaria in asymptomatic patients.

Keywords: malaria, Nested-PCR
The role of mesenchymal stem cells to improve healing of cutaneous leishmaniasis by using MTT test

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Introduction

*Leishmania* parasites is a causative agent of leishmaniasis, cutaneous leishmaniasis is the most common type of leishmaniasis. Considering the importance of mesenchymal stem cells in the treatment of cutaneous lesion, the study on the effectiveness of these cells can be useful in the treatment of *Leishmania* lesion.

Materials and Methods

For this experiment, a total number of 60 female Balb/C mice (6 to 8 weeks old) was investigated and animals were divided into test and control groups. Treatment group received MSCs(Intralesion 1x10⁶/100ul). Spleen cells were treated with soluble *leishmania* antigenas astimulus, then cell cytotoxic response was measured using a MTT test. Optical absorbance of trial measured(450nm).

Result

Reducing the size of the lesion in the mesenchymal stem cell treatment group was observed during treatment, which was not significant p˂0.05. On the 20th day, significantly the survival of the cell increased in mesenchymal stem cell group p<0.05.

Conclusion

Effectiveness of mesenchymal stem cells in the treatment of *Leishmania* lesion healing is significant via cell responses and requires further research in this field.

Key words

Cutaneous leishmaniasis, Mesenchymal stem cells, MTT test
OP-05

LB broth-lyophilized Rabbit anti-Sheep Cell Haemolysin as a simple culture medium for cultivation of Leishmania major promastigotes

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Background and aims: The protozoan parasites of the genus Leishmania are the causative agents of the various clinical diseases. Different methods of cultivation of Leishmanian parasites are available. In the present work, the efficacy of the LB broth with rabbit Lyophilized anti-Sheep RBC Haemolysin was evaluated in cultivation of promastigotes of Leishmania major.

Material and methods: Conventional LB broth medium was prepared and autoclaved for 15 min at 121 °C and thenlyophilized Rabbit anti-Sheep Cell Haemolysin was added at the 1-10% final concentrations. The efficacy of medium was evaluated by assessing the growth ability and replication pattern of the promastigotes of Leishmania major.

Results and discussions: According to our finding, this medium with 1% lyophilized Rabbit Haemolysin supported the growth of the parasites and can be used for cultivation of Leishmanian parasites with acceptable In vivo infectivity for research purpose. The ability of the parasites to survive and proliferating in the presence of lyophilized Rabbit Haemolysin indicating that this material a good nutritional source. This study opens a new way to make low-cost medium that could be used in cultivation of Leishmanian parasites.

Keywords: Leishmania major, lyophilized Rabbit anti-Sheep Cell Haemolysin, fetal calf serum.
Molecular identification between *Fasciola hepatica* and *Fasciola gigantica*: is the High Resolution Melting Analysis an appropriate method to differentiate them?

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**Background:** Fasciolosis is a shared disease between humans and livestock caused by hepatic trematodes; *Fasciola hepatica* and *Fasciola gigantica*. In human infections, the parasites can result in inflammation and damage of liver and bile ducts. Role of these parasites has been established in reduced quantity of milk and other livestock products. For prevention and management of fasciolosis, making a discrimination between the two species of *F. hepatica* and *F. gigantica* is essential. High Resolution Melting Analysis technique represents a new approach for understanding of differences between types of each infectious agent. This method applied following termination of Real-Time PCR. In Iran, this technique has not been used for identification of adult *F. hepatica* and *F. gigantica* genotypes. The aim of this study determine *Fasciola spp* genotypes using HRM technique in Ardebil and Zahedan province.

**Methods:** Seventy-Seven *Fasciola spp* samples were collected from infected slaughtered animals in different region of Iran, including north west (Ardebil province) and south east (Zahedan province) during 2016. Genomic DNA from the samples was extracted using a DNA extraction kit (Bioneer, Daejeon, Korea), according to the manufacturer's reference instructions with some modification and then after Real-Time PCR amplification, High Resolution Melting (HRM) were done.

**Results:** overall, of seventy-seven isolates were amplified using partial sequence of cytochrome oxidase subunit I (COI) gene of *Fasciola spp* and then High Resolution Melting (HRM) were performed. \( T_m \) analysis was repeated three times in each run to confirm the repeatability of the \( T_m \) assay. The result of HRM analysis showed that 49 and 27 isolates were identified as F. hepatica and F. gigantica genotypes, respectively.

**Conclusion:** HRM is powerful technique for identification between *F. hepatica* and *F. gigantica*. It should be noted that this method is very sensitive to detection any Single nucleotide (SNP) in DNA sequence.

**Key word:** HRM, *Fasciola hepatica*, *Fasciola gigantica*, COI.
Evaluation of Nitric Oxide (NO) Synthesis to *L. major* isolated from unsuccessfully antimonial therapy patients

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**Background:** Leishmaniasis is one of the most important infectious diseases worldwide. Macrophage is a primary phagocyte that plays host for *Leishmania*. Activated macrophages are able to produce strong oxidants such as superoxide ions (-O2) from the respiratory burst and NO that NO is necessary to kill and control the infection. Many studies show that Antimony compounds stimulate both of the innate immune systems by inducing NO. In this study, the immune response has been studied by evaluating of Nitric Oxide (NO) Synthesis in two untreated and Glucantime treated groups in vitro and in vivo.

**Methods:** Nested-PCR was performed to identifying and confirming patient’s lesion isolates with at least one course of failure Glucantime treatment. Cell line J774 was used for in vitro stage and were infected with the patient isolates or the standard type (MRHO/IR/75/ER). In vivo stage was done using Balb/c mice that *Leishmania* lesion were induced by injection of patient’s *L. major* isolates into the tail base and treated with one dose of Glucantime. Nitric Oxide amounts were evaluated after 24 h by Griess kitein supernatant cultured macrophages and in blood serum of Balb/c mice.

**Results:** The mean of levels of NO for patients isolates in vitro phase were 5.28 ±0.95 and 5.8±1 and for standard specie were 5.18±0.02 and 6.1±0.03 in each untreated and treated groups. Also results of 24 hours later in vivo stages showed increasing in both patient isolates and the standard specie comparing with untreated although this increasing was not significant (P>0.01).

**Conclusion:** According to the same results in the patient isolates with clinical resistance to Glucantime and standard specie can still hope to effectiveness of 5 valence antimonies and can be discussed that not well respond to antimonial therapy in these patients might be due to other causes, such as the lack of proper use of the drug and the barriers to drug and delivery to target tissues and cells.

**Keywords:** Nitric Oxide, antimonial therapy, Leishmaniasis
OP-08

P-glycoprotein A, G-glutamylcysteine synthetase1 and Aquaglyceroporin1 genes expression in antimony resistance leishmania major isolates

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Background: pentavalent antimony (SbV) compounds play the main role in leishmaniasis treatment. Increasing numbers of clinical failures of treatment have been seen, likely for the creation of parasite resistance to this compound. Overexpression of ATP-binding cassette (ABC) proteins is a mechanism of resistance and in Leishmania, P-glycoprotein A (PgpA) is a related gene for this transporter. The existent of the internal reductions like Thiols are important for neutralizing the oxidative stress and the level of TSH, one of the tiols, increase due to high expression of G-glutamylcysteine synthetase 1 (Gsh1). It is proven that Aquaglyceroporin1 (AQP1) proteins are mediated the uptake of the trivalent antimony. The propose of this study was to finding the expression level of PgpA, Gsh1 and Aqp1 genes in clinical resistance isolates against non-resistant Leishmania major (MRHO/IR/75/ER).

Methods: Samples were obtained from 10 phenotypic resistant patients and by Nested-PCR the species of them was identified. For in vitro, isolates were tested as amastigotes infecting J774 cell line and vivo was performed by infecting the Balb/c mice in the base of the tail and the values of genes expression, were obtained by triplicate quantitative RT-Real time PCR method before treating with glucantime and after that and were compared with non-resistant Leishmania major (MRHO/IR/75/ER).

Results: Nested-PCR showed all samples to be L. major. Comparison of rate of genes expression in clinical resistance samples and L.major (MRHO/IR/75/ER), didn’t show any statistically significant difference, before and after treat both in vitro and in vivo (P>0.05).

Conclusion: The results indicated that the level of gene expression didn’t correlate with the clinical picture, so suggests that there aren’t molecular resistance in the isolates, but it is noted that some things such as large-scale misuse of the drug and disregard to treatment protocol can induce untreated cases and gradually drug resistance.
Identification of gene expression of J-Binding protein 1 from Leishmania major (MRHO/IR/75/ER) exposed with glucantime

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Introduction-Leishmaniasis is caused by protozoan of Leishmania parasite. The pentavalent antimonials compounds remain the first-line treatment. J-binding protein encoded by JBP1 involves in starting synthesis of J base that is unique in kinetoplastida. In this study, we were assessed the JBP1 gene expression in exposure of different doses of glucantime.

Methods- L. major (MRHO/IR/75/ER) promastigotes were distributed in groups for exposure with glucantime with end concentrations of 5, 10, 15, and 20 mg/ml. Then, RNA was extracted and cDNA synthesis was performed. Gene expression of JBP1 was assessed using SYBR Green Real Time PCR by ΔΔCT analysis.

Results- Gene expression of all groups exposure with different concentrations of glucantime were the same but the JBP1 gene expression showed 1.4 fold decreasing in groups with promastigotes exposed with glucantime with the end concentration of 5 mg/ml.

Discussion- The base J is synthesized with JBP1. More synthesis of J base is resulted in decreasing RNA polymerase II that could affect the gene expression of other genes. In this study, we showed that in high concentrations of glucantime, the JBP1 was increased resulted in increasing in J base synthesis. Therefore, it seems that expression regulation of the genes involving in drug exposure would be in the other mechanisms.

Key words: Leishmania major (MRHO/IR/75/ER) · JBP1 · Glucantime,
Post-treatment follow up to sufficient eradication therapy for hepatic echinococcosis

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Background: Cystic echinococcosis, also known as “hydatid cyst”, is one of the commonest important zoonotic diseases of animal and human. The aim of this retrospective study was to evaluate clinical and paraclinical presentation and long-term outcome of patients treated surgically for complicated liver hydatid cysts.

Methods: A total of 145 patients who were operated on for 197 echinococcal cysts at the surgery department of Shohadaye Ashayer Hospital, Lorestan University of Medical Sciences between 2007 and 2015 were evaluated. It included 54 men and 51 women with median age 35.2 years. A computed tomography scan, ultrasound from abdominal and serological test established for diagnosis. Cysts with infection, rupture into the biliary tract and peritoneal cavity were categorized as complicated cysts.

Results: One-hundred and five (72.4%) patients underwent surgery for hepatic hydatid cysts. Most patients (81.9%) were intact and uncomplicated. In total, 49.2% of the hydatid cysts localized in the right lobe. In the liver cysts, 9 cysts were both ruptured and infected and 5 cysts were ruptured only. Four patients had giant liver hydatid cysts, which were equally and/or more than 10 cm diameter and had hepatomegaly. Wound infections developed in 6 cases and resolved with local treatment.

Conclusion: Complicated liver hydatid disease is frequent and was observed in more than half of patients operated for liver hydatid cysts at our center. Using a surgical strategy aimed at complete removal of cystic and pericystic tissue with simultaneous treatment with albendazole may be preferred owing to lower mortality and morbidity rates and hospitalized time.

Keywords: Echinococcosis; Laboratory finding; Surgical Treatment; Liver cyst
Anti-Toxoplasma gondii IgG and IgM Antibodies in Diabetic Patients with Thyroid Disorder

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Background: Evidence suggests the involvement of autoimmune mechanisms in the pathogenesis of diabetes mellitus and thyroid gland dysfunction, and also, end discussed separately. In this regard, the present study aimed to evaluate the association of endoscopy discussed separately. In this regard, the present study aimed to evaluate the association of Toxoplasma gondii infection in diabetes and autoimmune diseases has been of anti-Toxoplasma gondii antibodies in diabetic patients with thyroid gland dysfunction.

Materials and Methods: In this cross-sectional and descriptive study, the study population consisted of 582 patients, referred to Endocrine and Diabetes Clinics, were enrolled in the study by an endocrinologist. Thyroid function in all patients was evaluated by TPOAb, TSH, T4, and T3 tests, and anti-toxoplasma antibodies of IgG, IgM. The collected data were analyzed using SPSS version 20.

Results: Anti-Toxoplasma gondii IgG immunoglobulin showed the highest mean in subjects. The mean and standard deviation of anti-Toxoplasma gondii IgG and IgM in diabetic patients with and without thyroid dysfunction showed a significant difference (p < 0.05). Furthermore, the proportion of patients with acute and chronic Toxoplasma gondii infection was significantly different in diabetic patients with and without thyroid disorders (P < 0.005). The highest correlation was observed between anti-Toxoplasma gondii IgG and IgM antibodies and anti-thyroid antibody and TSH test.

Conclusion: The results of this study showed that Toxoplasma gondii infection can increase the risk of diabetes and thyroid problems; so, screening tests and preventive measures are recommended in susceptible patients.

Keywords: Toxoplasma gondii, Diabetes, Thyroid disorder
Evaluation of mummy substances on the function of fibroblast and endothelial cells in skin wound healing

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Therecent decades, the trends to use natural ingredients to regeneration skin tissue have led serious challenges in treating patients who have skin wounds. Therefore, the purpose of this study is to investigate the effect of mummy on skin regeneration. In this study, the effects of mummy on human fibroblast cells (HF2FF) and HUVEC cells in the form of 7 treatment groups (with 7 different concentrations of mummy substances 31.25, 62.5, 125, 250, 500, 1000, and 2000 μg / ml) and a control group (no mummy substance) at 24, 48 and 72 hourswere investigated and level of optical absorption of cells were recorded by ELISA. According to statistical results, in the HUVEC cells level, the viability of cells in concentrations of 500 to 2000 μg / ml in 24-hour incubation, decreased significantly P <0.01 and P <0.001 respectively. Also, the percentage of viable cells increased than the control group in concentrations of 31.25 to 1000 μg / ml and 500 μg / ml at 48 h (P <0.01 and P <0.001) and 72 (P <0.001) hours, respectively. In the HF2FF cells level, the survival of the cells in concentrations of 250 to 2000 μg / ml of mummy material at all three incubation time (24, 48 and 72 hours) had a significant decrease compared to control, which indicates the toxicity of the mummy substance in these concentrations. The findings from our statistical result show that mummy in different concentrations of endothelial cells over the periods of 48 and 72 hours not only caused the cell cytotoxicity, but also increased the proliferation of endothelial cells, except in the concentration Top. In fibroblast cells, the mummy substances can stimulate the growth and survival of fibroblastic cells at low doses, which will ultimately facilitate and accelerate skin regeneration. It has a high effect on the cells in high concentrations and it is likely toxic.

Keywords: Mummy substance · Wound healing · Fibroblast cells · HUVEC cells
Synergistic inhibitory effect of 17-AAG in combination with routine chemotherapy agents (capecitabine-irinotecan) on human colorectal cancer cell line HT-29

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Background: Chemotherapy has a special place in colorectal cancer treatment but in some cases treatments are insufficient. Standard chemotherapeutic regimens in this cancer treatment include 5-FU, oxaliplatin and irinotecan (FOLFOX and FOLFIRI).

Methods: In this study we try to investigate the inhibitory effect of recently presented anticancer agent, 17-allylamino-17-demethoxygeldanamycin (17-AAG), a heat shock protein 90(HSP90) inhibitor, in combination with irinotecan or capecitabine on human colorectal carcinoma cell line HT-29. Anti-proliferative effect of 17-AAG, irinotecan and capecitabine alone on HT-29 cells were assessed by WST-1 viability test 24 hours after treatment with various concentration of each drug. After IC50 (50% inhibitory concentration) calculation with Compusyn software, 17-AAG, irinotecan and capecitabine were used in pair with IC50 values and 0.5×IC50 values in constant ratio.

Results: The IC50 for 17-AAG, irinotecan and capecitabine were 60 nM, 6.9 µM and 3.4 µM, respectively. We observed significant differences between single treatments and double treatment (p value < 0.05). Besides 17-AAG in double combination with irinotecan and capecitabine showed synergistic effect (Combination Index<1).

Conclusion: We demonstrated that our tested agents double combination when used with 0.5×IC50 are more effective than single treatments with IC50 values. According to our result 17-AAG could be a suitable candidate to combine with standard chemotherapy regimen for colorectal cancer patients. Our data offer these new combinations for future clinical trial setting for colorectal cancer patients.

Keywords: chemotherapy, 17-AAG, HT-29, WST-1 viability assay
Astaxanthin Effects on Sensory-Motor function in a clip compression Model of Spinal Cord Injury

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**Background:** Spinal cord injury (SCI) often leads to constant neurological deficits and long-term unalterable disability. Ongoing research are to find a new and potent drug to improve sensory-motor function following SCI is heavily dependent on animal testing. Astaxanthin (AST) as a potent lipid-soluble and red-orange keto-carotenoid has promising applications in human health with excellent safety and tolerability. Thus, we aimed to investigate the effect of AST on sensory-motor function following SCI in male rats.

**Method:** Thirty rats were randomly divided into three experimental groups consisted of 10 rats per each group for behavioral assessments. In the first group (Sham), animals received laminectomy surgery without compression lesion. In the second group (Injury), rats were subjected to laminectomy and compression injury then treated with vehicle. The third group (Treatment) of rats received laminectomy and compression injury and 30 min after injury were treated with administration of AST. Animals were subjected to behavioral tests and the assessments was done preinjury and on postinjury days 0, 7, 14, 21, 28 of the chronic phase of injury. Twenty four rats were assigned to measure their blood glucose on days 7, 14, 21 and 28 after surgery, six rats per group.

**Results:** Rats in AST groups showed significant differences in von frey, BBB, open field, inclined plane test, hot plate, cold drop test, weight change, body temperature and blood glucose. These findings revealed that systemic single-dose administration of AST can attenuate sensory-motor function induced by SCI in rats.

**Conclusion:** This study is the first to report that AST reduces NP and improves functional recovery with different behavioral tests, after SCI. The observed prominent effects, introduces AST as a promising therapy for SCI.

**Key words:** Astaxanthin, Spinal Cord Injury, Sensory-motor function
Simvastatin: a potential neuroprotective agent in Parkinson's disease.

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Abstract

Background: Parkinson's disease (PD) is a neurodegenerative disease clinically characterized as a movement disorder. The motor symptoms in PD arise due to degeneration of dopaminergic nigrostriatal neurons in the midbrain thereby depleting the dopamine levels in the striatum. The present study was undertaken to investigate the neuroprotective effects of simvastatin on 6-hydroxydopamine (6-OHDA)-induced Parkinson's disease in rats. 6-OHDA-induced Parkinson's disease rat model involves chronic inflammation, mitochondrial dysfunction, and oxidative stress, and the loss of the dopaminergic neurons in the midbrain is the predominant lesion. Simvastatin has been shown to have anti-inflammatory actions, and thus was tested for its beneficial effects using 6-OHDA-induced Parkinson's disease rat model.

Methods: Adult wistar rats were unilaterally injected with 6-OHDA (12.5 microgram/5 microliter) into the left striatum, and the striatum damage was assessed by rotational test, histology, and molecular alterations. Simvastatin (5, 10 and 20 mg/kg) was then given orally to Parkinson's disease rats, daily for 10 weeks to examine the protective effects.

Results: Rotational test showed that simvastatin significantly attenuated apomorphine-induced turns of rats in 6-OHDA-injured Parkinson's disease rat model as early as four weeks of administration. Furthermore, simvastatin treatment also significantly decreased the levels of COX-2 mRNA in the substantianigra as detected by real-time RT-PCR. These results demonstrate that simvastatin exerts a neuroprotective effect on 6-OHDA-induced Parkinson's disease rat model, and this protection is related to the reduced inflammatory reaction.

Keywords: Simvastatin, Parkinson’s disease, 6-OHDA
OV-02

Frequency of Xenotropic Murine leukemia virus-related virus (XMRV) nucleic acid in HIV RNA positive Patients in Tehran, Iran.

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Introduction: Despite of isolation of Xenotropic murine leukaemia virus-related virus (XMRV) from the patients acquired prostate cancer in ٦٠٠٢ и patients with chronic fatigue syndrome in ٩٠٠٢، there have been controversial findings about its potential role in human diseases and frequencies in different population groups. In the present study, we aimed to determine the frequency of XMRV genome in Iranian HIV-infected patients for the first time.

Material and methods: We performed a cross-sectional study on the prevalence of XMRV nucleic acid among ٠٥١ patients diagnosed with HIV infection from Tehran’s hospitals. After extracting the viral RNA from plasma samples, specimen's XMRV nucleic acid was amplified by Real-Time PCR, also HIV viral load testing was performed for all of the patients.

Results: Out of ٠٥١ patients, ٣٢١٢٤١ of them were negative for XMRV nucleic acid. XMRV RNA was found in ١٣٥١٤١ patient's specimens, including ٧٩ males (٩٦١٢٪) and ٤١ females (٦٨٪). Average HIV viral load was ٢٩٠٧٦ and ٦٦٠٩١ copies/ml in positive and negative XMRV patients, respectively.

Conclusion: Our results has shown a presence of XMRV infection in HIV-infected patients. But no other significant association was observed between XMRV with gender, age and HIV viral load of the patients. However, more studies are needed to demonstrate the actual prevalence of XMRV infection by geographical regions and different populations.

Keywords: XMRV, HIV, Iran, Real-Time PCR
OV-03

Oncolytic Reovirus-Infected Mesenchymal Stem Cells (MSCs) as an Emerging Treatment Strategy for TC1 Tumor Model

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Background: Oncolytic virus (OV) therapy is an emerging immunotherapeutic modality for cancer treatment. Reovirus as an oncolytic virus induce apoptosis in vitro. One of the problem in oncolytic virotherapy is delivery of naked viruses to the target site due to neutralizing antibodies. For bypassing this issue, cell carrier is a promising approach and able to conceal the therapeutic virus from the host immune defense and enhance the delivery and quality of treatment. The ability of mesenchymal stem cells (MSCs) as a permissive host and protect oncolytic reovirus (Reo-OV) from circulating antibodies makes it as a potential carrier. Here, we examined the effects of oncolytic reovirus-infected MSCs (derived from C57BL/6 mice adipose) in cancerous TC1 cell culture model.

Methods: MSCs isolated from C57BL/6 mice adipose tissue using collagenase enzymatic digestion protocol. MSCs were infected with reovirus and virus growth were optimized. Next, the ability of these cells to deliver the virus to TC1 cell lineand the effects of reovirus infected-MSCs on cell death were measured via flow cytometry. Furthermore, the mechanisms involved in the cytotoxic effects of the reovirus-infected MSCs were examined.

Results: Reovirus lead to induction of apoptosis both in MSCs and TC1 cell line. MSCs loaded with reovirus delivered the virus to co-cultured TC1 cells and result in apoptosis activation. The virus cytotoxic effect was observable in MSCs, which are susceptible to reovirus infection and supported the virus replication.

Conclusion: Reovirus-infected Adipose-derived MSCs may provide a novel effective therapeutic approach for targeting TC1 tumor model cells. These results suggest the suitability of MSCs as an efficient carrier for wild-type Reo-OV to target the cancer cells.

Keywords: Oncolytic Reovirus, Mesenchymal Stem Cells, TC1 Tumor Model Cell, Cell Carrier, Apoptosis
OV-04

LncRNA-ATB plasma level as a potential biomarker for chronic HBV infection

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Abstract

Hepatitis B virus (HBV) currently infects about 2 billion individuals in the world, including around 350 million people with chronic HBV infection. Progression of chronic HBV infection may lead to liver fibrosis, cirrhosis and also hepatocellular carcinoma (HCC). As, HBV infection is a worldwide health concern, it is important to find novel early screening and diagnostic methods.

Long non-coding RNAs (lncRNAs) are a new class of non-coding RNAs with more than 200 nucleotides. LncRNAs have roles in many cellular processes. Compared with other RNAs, lncRNAs are found in body fluids and often form highly stable secondary structures that making it possible to quantitatively detect free lncRNAs. These features make lncRNAs as potent biomarkers in the early diagnosis and also prognosis of diseases progression. Recently, some studies suggested that lncRNAs can mediate immune function in infectious diseases. Among these RNAs, LncRNA-ATB is a long non-coding RNA that has a significant role in TGF-β signaling pathway.

Here, we hypothesized that the plasma levels of LncRNA-ATB may have significant association with chronic HBV infection.

Material and methods

In this case-control study, we studied on 20 chronically HBV infected patients who were referred to Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences in comparison with 20 healthy controls. After total RNA extraction and cDNA synthesis, we used quantitative Real-time PCR to detect the plasma levels of LncRNA-ATB.

Results

The result shows that plasma level of LncRNA-ATB in chronic HBV infected patients is up-regulated in comparison to control group by a mean factor of 4.393. Also plasma level of LncRNA-ATB is significantly different to control group P value=0.024.

Discussion

According to our results, plasma levels of LncRNA-ATB may serve as a potent non-invasive biomarker for diagnosis of chronic HBV infection.

Keywords: Long non-coding RNA, Hepatitis B virus, plasma, biomarker
OV-05

The role of microRNAs in respiratory viral infection: friend or foe?

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Background: MicroRNAs (miRNAs) have emerged as a class of regulatory RNAs in host-pathogen interactions. Aberrant miRNA expression seems to play a central role in the pathology of several respiratory viruses, promoting development and progression of infection. We present a comprehensive review of recent findings related to the role of miRNAs in different respiratory viral infections and discuss possible therapeutic opportunities aiming to attenuate the burden of viral infections.

Methods: A systematic review was undertaken for articles published due to Dec 31, 2017 across PubMed and Google Scholar related to interaction between respiratory viruses and miRNAs.

Results: miRNAs modulate a variety of cellular processes by regulating multiple targets, promoting or inhibiting the development of respiratory viral infection. There are several reports demonstrating that some viruses take advantage of cellular miRNAs by enhancing or inhibiting the expression of specific cellular miRNAs. Respiratory viruses can induce certain cellular miRNAs that affect the virus life cycle positively and inhibit those that affect the virus life cycle negatively.

Conclusion: Our review supports the emerging concept that cellular and viral-encoded miRNA...
OV-08

Design, construction and expression of recombinant vector containing the rabies virus nucleoprotein in prokaryotic expression system

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Background: Rabies infection, as one of the dangerous neurological fatal zoonotic disease, continues to give a great burden on public health and the global economy. Development of new rabies vaccines is critical for controlling of the disease; however, a safe, cheap and effective vaccine against the disease remains unaffordable in developing countries. The nucleoprotein (NP), the major internal protein of the virus, is highly conserved and can activate the cellular immuneresponses against rabies. Thus, nucleoprotein can be an alternative candidate antigen for development of rabies vaccines. The aim of this study was to develop a repeatable, convenient and low-cost system that can express the N at high scale.

Methods: The nucleoprotein gene of rabies virus (PV), was amplified and subcloned into the pET28a between NheI and BamHI restriction sites and expressed in Escherichia coli, Rosetta strain. The expression of the NP was identified by SDS-PAGE and western blot after induction with 1mM IPTG. NP was purified by Anti His Tag Column chromatography. Finally, amount of purified proteins was evaluated.

Results: Restriction enzyme digestion and sequencing showed that the N gene had been successfully subcloned into the pET28a expression vector. The expression construct was further characterized by SDS-PAGE and western blots.

Conclusion: This study showed that the N protein of rabies virus can be expressed in a reproducible prokaryotic expression system using the pET28a expression vector. In this study, N protein produced in large scale, therefore, it can be used in diagnosis and research fields.

Keywords: Prokaryotic expression system, Nucleoprotein, Rabies virus
Expression of the rabies virus matrix protein gene in prokaryotic system at high-levels: an efficacious production method

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Background: Rabies virus (RV) is the one the oldest deadly infectious disease agent among mammals, including humans. The use of VLP-based vaccines as a new approach is used to develop new generation rabies vaccines. VLPs are nonpathogenic and no replicating particles, therefore, they are one of the most attractive goals for production of vaccines. Furthermore, VLPs can induce immune responses at lower doses of antigen, which significantly reduces the cost of the vaccine. Due to dangerous nature of the whole virus and essential need for vaccine production, expression of matrix protein (MP) and its spontaneous assembly feature to the glycoprotein, can be the applicable approach in developing the rabies virus VLP particles. In this study, our aim was to achieve the repeatable, convenient and low-cost system that can express the MP at high scale for research and vaccine purposes.

Methods: Extraction of RNA was performed from infected BSR cells to CVS and followed by cDNA synthesis. It was amplified by a pair of designed primers and was cloned into pET28a between BamHI and HindIII restriction sites and then expressed in Escherichia coli, Rosetta strain after induction with 1mM IPTG. The expression of the MP was identified by SDS-PAGE and western blot. MP was purified by Anti His Tag Column chromatography. Finally, amount of purified proteins was evaluated.

Results: Restriction enzyme digestion and sequencing showed that the M gene had been successfully cloned into the pET28a expression vector. The expression construct was further characterized by SDS-PAGE and western blots.

Conclusion: This study showed that the M protein of rabies virus subtype CVS can be expressed in a reproducible prokaryotic expression system using the pET28a expression vector. M protein as a nonglycosylated protein produced in prokaryotic expression system developed in this study, can be used in vaccine production and research fields.

Keywords: Prokaryotic expression system, Matrix protein, Rabies virus
Corelation between gingival expression of STAT1 and Wnt5a with Periodontal disease.

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Abstract

Background and aim: Periodontal disease is a most common inflammatory disease of periodontium. Several transcription factors that involved in inflammatory signaling pathway play important roles in the pathogenesis of the disease. So, the aim of this study was to evaluate the corelation between gingival expression of STAT1 and Wnt5a with Periodontal disease.

Methods: Gingival tissue samples were collected from 20 individuales with clinically healthy gingiva, 25 patients with chronic periodontitis and 25 patines with aggressive periodontitis. The expression of STAT1 and Wnt5a were evaluated using Real-time PCR.

Results: higher expression of STAT1 was found in chronic periodontitis in comparison with healthy control (P<0.05). However, lower expression of STAT1 was found in aggressive periodontitis in comparison with chronic periodontitis and healthy control (P<0.05). In addition, a significant correlation was found between gingival expression of STAT1 with clinical attachment loss and probing depth (P<0.05). Higher expression of Wnt5a was found in chronic periodontitis in comparison with aggressive periodontitis and healthy control (P<0.05).

Conclusion: The findings of this study concluded that expression of STAT1 was associated with severity and progression of periodontal disease. Therefore, STAT1 may consider as an important target for future therapies.

Keywords: chronic periodontitis, aggressive periodontitis, STAT1, Wnt5a
Urinary cell free fetal DNA as a new source in evaluation of prenatal diagnosis and urological cancer: Advantages and disadvantages

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Background:

Circulating cell-free DNA (cf-DNA) has been found in the plasma of human subjects. It has been extensively studied over the past few decades. Supported by theory and observation, three major sources of cf-DNA have been postulated: first, fragmented DNA released as a consequence of cell death (apoptosis-sis/necrosis of blood and tissue cells) and, second, active metabolic secretion of DNA from cells, third, immunological reaction between feta-maternal.

Now a day, urine sample as a non-invasive source of biomarkers are used in clinic. Cell-free fetal DNA in the maternal urine in relation to gestational age as a potential source for non-invasive diagnosis. Also, UcfDNA has been mostly evaluated for urological cancers. UcfDNA appears to be a promising source of early diagnostic markers. In most of the published data regarding urine focus on biomarkers from exfoliated cells, and very little is known about the role of UcfDNA.

Since, glomerular filtration acts as a “dimensional selection”: only small DNA fragments from circulation (about 100 bp) can penetrate through the pores of the glomerular barrier, appearing in urine. As a consequence, UcfDNA could provide important information on specific alterations of circulating cfDNA and genomic DNA coming from cells shedding into urine, thus being useful for identifying cancers and others clinical status evaluation.

Methods:

This systematic review was conducted to outline comprehensive studies published in science direct, PubMed, Elsevier and google scholar data bases. From 2010 to November 2017. Approximately 46 articles initially fund and then 18 articles to the aim of study were identified and reviewed.

Results:

Recent studies have revealed that the important role of UcfDNA as a non-invasive method with advantages over plasma samples and other diagnostic methods, in the diagnosis, evaluation and monitoring of urological cancers (prostate and bladder cancer and renal
transplant injury) and prenatal diagnosis such as Non–Invasive prenatal diagnosis, Sex determination, X-linked disorders, determining aneuploidies and single gene disorders. The studies have been reported 80% detection after 20 weeks in pregnant women for prenatal diagnosis. Also other studies have been shown 95% sensitivity and specificity in early prostate cancer diagnosis, 90% Positive predictive value in bladder cancer and 94% specificity, known as sensitivity marker of early renal transplant injury.

Conclusion:

Urinary cfDNA as a liquid biopsy holds potential for a more sensitive alternative to blood biopsies and urine sediment-based test for clinical use in prenatal diagnosis, urological and non-urological cancers. UcfDNA offer advantages including the truly non-invasive method, potential for more frequent testing, monitoring, home use, safety and no infection risk, No refrigeration required and lower cost.

Keywords:

UcfDNA, Prenatal diagnosis, Pregnancy, Cancer, Clinical diagnosis, Immunological reaction
Pre-gestational parental stress influences anxiety behaviors of offspring

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Introduction: Anxiety disorders are among the most common mental disorders, with up to 15% of all people suffering from an anxiety disorder during their lifetime. Prenatal stress is associated with later-life symptoms of anxiety, helplessness, attentional deficits and social withdrawal in both humans and animal models. It has been shown that both male and female prenatally stressed offspring display significantly enhanced HPA axis responses to acute stressors in later life, reflected by greater stress-induced ACTH and corticosterone secretion and increased levels of vasopressin. Considering that most previous studies have examined the effect of stress during gestational period on the offspring behavior, in the present study, the effect of predatory stress as a chronic stressor in pre-gestational period of parent rats in anxiety behaviors of their offspring was investigated.

Method: Adult rats were divided randomly into two groups [control group (8 male and 8 female) and predatory stressed group (8 male and 8 female)]. The predatory stress involved 1-h sessions twice daily (8-9 AM and 3-4PM) in a cage placed within the visual range of a caged cat (50 day for males and 15 day for females, according to their spermatogenesis and oogenesis period), then rats were paired. The female stressed rats were paired with male stressed rats (parent stressed group) and the male non-stressed rats were paired with non-stressed female (control group). After parturition their pups were investigated in two groups: control pups and parent stressed group. On postnatal 30-31 day, the Elevated Plus Maze (EPM) test was performed between 10-11 a.m on pups of each groups by the same person. This test is commonly used to assess anxiety behavior in rodents. The EPM test is based on the conflict induced in subjects by the presence of safe parts of the apparatus that are closed, shadowed and protected, and open ones, bright, aversive and unprotected. The pups was placed at the center of the apparatus composed of an elevated cross (50 cm above the floor) with two open arms (16 lx) and two enclosed arms (8 lx). A video camera was placed above the apparatus and was connected to a computer. Each rat was placed in the same orientation and all trajectories, including time, distance, and entries in each compartment, were analysed during a 5 min session. For the analysis, we used the mean of time that spent in open arm (OAT) for each group.

Results: According to our data, open arm time of offspring were significantly decreased from 194.80 sec in control female pups to 111.80 sec in parent stressed female pups and from
155.20 sec in control male pups to 91.80 sec in parent stressed male pups \((p<0.05)\). No significant changes in anxiety behaviors were observed between female and male pups.

**Conclusion:** Pre-gestational stress can increase anxiety behaviors of offspring similar to which occurred in prenatally exposed stress pups. This indicates that stress experienced by parents may alter gene expression in their gametes and embryo potential to stress. Pre-gestational stress also alters HPA axis activity in offspring, which influenced anxiety behaviors. However, further investigation is required to clarify the underlying mechanism.

**Key words:** predator stress Anxiety behaviors rat
Pre-gestational period
ElevatedPlus Maze (EPM)
OKP-04

Simple method for Isolation and characterization of single chain fragment variable (scFv) antibodies

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Background: Phage antibody display is an effective in vitro technique for the selection of tumor-specific recombinant human antibodies. Single-chain variable fragment (scFv) antibodies can be usefully employed in the imaging, tumor penetration and tumor specific retention because of their smaller size compared to their IgG counter parts (~28kDa). Moreover, Antibody fragments such as Fabs and scFvs generated by phage display technology can be useful to overcome some limitations of whole immunoglobulins such as cost intensive and immunogenicity. In the current study, we employed phage display technology to isolate scFv antibodies against specific oligopeptide from extracellular domain of target using a naïve human phage library (Tomlinson I + J).

Methods: Human single chain scFv libraries I + J (Tomlinson I + J), were amplified and used for selection of scFvs against specific peptide. After five rounds of affinity selection, by counting the colony-forming units (CFU) of the infected E. coli TG1 was estimated the phage titer. Briefly, five rounds of panning were performed, and selected scFvs were characterized using polyclonal and monoclonal ELISA, sequencing and Western Blotting.

Results: The results showed that the number of eluted phages was increased from 103 to 107 cfu/ml following five rounds of panning. Outputs from rounds 4 and 5 that giving the strongest signal in polyclonal and monoclonal phage ELISA were obtained. DNA from the selected clones was amplified with LMB3 and pHEN primers, and PCR products were analyzed in agarose gel to confirm the presence of the desired band corresponding to the scFv gene (VH and Vk insert, approximately 935bp). Western Blotting results demonstrate successful isolation and characterization of specific scFvs.

Conclusion: Phage display is an efficient alternative to hybridoma technology for the production of therapeutically relevant antibodies. We envision that the produced scFvs using Phage display technique can be used for diagnosis, targeting and antibody-based therapy strategies.

Keywords: single chain fragment variable, antibody, scFv, phage display technique
Medical Laboratory Director Competencies: State of the Basic Medical Sciences and Global Perspective

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Background: There has been a long-lasting debate among medical laboratory-associated specialists about required competencies and educational background to qualify for directing medical laboratories in Iran. The aim of this study was to provide a comprehensive review regarding required training and competencies for becoming a medical laboratory director in Iran and all around the world.

Methods: A thorough search of the literature was carried out in scientific databases including Web of Science, Science Direct, Springer Link, Wiley Online, PubMed, Scopus, SID and web-based search engines such as Google and Google Scholar.

Results: The results revealed detailed required competencies for directing medical laboratories in the United States of America, Canada, European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and its many affiliated European countries.

Conclusion: Our results indicate that in all assessed countries, specialists of basic medical sciences and also physicians, after passing certifying examinations, are equally qualified to direct medical laboratories. Indeed, Iran is the only country within the evaluated ones, in which basic medical sciences specialists (which have their own specific curriculum and are educated as clinical majors) have been eliminated from directing medical laboratory and the position has almost exclusively been available for graduates of pathology and it is important to revise this approach.

Keywords: Basic Medical Sciences, Medical Laboratory, Medical Laboratory Director, Medical Laboratory Specialty, Medical Laboratory Personnel
Genotyping of Clinical *Candida parapsilosis* isolates from Iran Using Microsatellites

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**Introduction:** *Candida parapsilosis* is one of the most common non-*albicans* *Candida* species which can caused noscomial infection particularly in hospitalized patients. Despite an increasing number of infections due to this species, the molecular epidemiology of clinical strains has not been well studied.

**Materials and Methods:** A total of eighty one clinical *C. parapsilosis* isolates were analyzed. A panel of six STR markers was used for typing all clinical isolates of *C. parapsilosis* in this study. Generally three trinucleotide repeat markers and three hexanucleotide markers were amplified in a multiplex PCR. Similarities between genotypes were determined using BioNumerics, version 6.0.

**Results:** The STR typing of 81 *C. parapsilosis* isolates demonstrated 68 separate genotypes. Among all genotypes, 57 genotypes were observed once and the remaining 11 genotypes were identified multiple times. The panel of all six markers combined yielded a diversity index of 0.995187. The heterogeneity was observed among Iranian and the Netherlands clinical *C. parapsilosis* isolates.

**Conclusion:** The high genetic diversity of Iranian *C. parapsilosis* isolates was observed. The molecular epidemiology could be useful for screening during outbreak investigation where this species is involved.

**Key words:** *Candida parapsilosis*, Microsatellite, Genotyping, Iran
The Cytotoxic Effects of Aflatoxin B1 on mice T Lymphocytes

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Introduction: Aflatoxins are natural carcinogenic metabolites which are produced by Aspergillus fungi. These products have destructive effect on body organs and are responsible for establishment or development of infectious diseases and cancer. According to potential effect of aflatoxin B1 and G1 on immune cells, this study was designed to investigate the cytotoxicity effects of this toxin on mouse T lymphocyte.

Materials and methods: Four different standard and feed derived strains of A. parasiticus and A. flavus were cultured in appropriate condition and the levels of G1 and B1 aflatoxins were determined by TLC and HPLC methods. For evaluation of cytotoxity effect, the different concentration of aflatoxins B1 was mixed by mice T cell line (EL4) on different time and the percentage of cell death were been evaluated by MTT assay and flowcytometry (FCM) method.

Results: The results indicated that among different strains, the standard A. parasiticus has highest levels of B1 (707 ppb). However, feed derived A. flavus has 0.850 ppb of B1. The most cytotoxity effect was seen on 1:10 concentration of A. parasiticus extracted aflatoxins.

Discussion: These results shown feed derived aspergillus have high levels of B1 aflatoxins which have considerable cytotoxicity effect on T lymphocytes. This confirmed the importance of cattle feed on human health.

Keywords: Aflatoxin B1, T Lymphocytes, cytotoxicity, MTT assay, flowcytmetry