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P-10-112-1
Peptidyl arginine deiminase-4 (PADI-4) gene polymorphism in Iranian rheumatoid arthritis (RA) patients

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Rheumatoid Arthritis (RA) is a systemic, autoimmune disease that causes chronic inflammation and joint destruction. Its etiology is unknown, but immunologic and genetic factors have a main role in its pathogenesis. Individuals with RA frequently have autoantibodies to citrullinated peptides (anti-CCP), suggesting the involvement of the Peptidyl Arginine Deiminase enzymes (encoded by PADI genes) in the pathogenesis of RA. An association between RA and a PADI4 haplotypes has been reported by some researchers. In this study, we intended to study the association between different haplotypes of PADI4 and susceptibility to Rheumatoid arthritis in Iranian patients. This study was done on 50 Iranian RA patients at Imam Reza hospital and 50 sex and age-matched healthy subjects. After DNA extraction from peripheral blood by salting out method, the genotype of PADI4 was determined by using sequence specific primers and SSP-PCR method. Data were analyzed using chi-square and fisher exact test. 58% of the patients had genotype 1, 2; 38% genotype 1, 4; 2% genotype 1b, 2 and 2% genotype 2, 4, but in the control group, 72% had genotype 1, 2; 22% genotype 1, 4 and 6% genotype 1b, 2. Totally, there was not any significant association between the presence of PADI4 genotypes and the disease. But, there was a significant association between the presence of genotype 1, 4 and the susceptibility to RA only in females and there were significant differences between female patients and female controls in the frequency of haplotypes 2 and 4. So, in the Iranian population, PADI4 gene polymorphism is only associated with RA in females. Further studies with larger sample size should be designed to determine the exact relationship between different genotypes of PADI4 and RA.

Keywords: rheumatoid arthritis, polymorphism, peptidylarginine deiminase 4, Iranian patients

P-11-467-1
Association of the sumo4 polymorphism M55V variant in type 2 diabetes

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SUMO4 is recently found to be mainly expressed in kidney. A single nucleotide polymorphism was detected in sumo4 substituting a highly conserved methionine with a valine (M55V) residue. This variant of sumo4 may induce a higher nuclear factor –KB (NFKB) activity. Because NF-KB is known to mediate the development of diabetic nephropathy, and the sumo4 gene encoding small ubiquitin-like modifier4 is a posttranslational modifier which has recently been identified as a novel member of the sumo family and is suggested to modify immune response through the substrate, inhibitor of NFKB, a suppressor of nuclear factor KB (NFKB), we examined the association between sumo4 M55V variant and the incidence of type II diabetic diseases. We recruited a total of 50 patients with type 2 diabetes and 50 healthy subjects. The M55V polymorphism of sumo4 was genotyped by PCR and restriction enzyme digestion. The serum glucose concentration was measured by enzymatic method. The frequency of sumo4 AA, GA and GG was 20%, 44% and 36% in patient group and 26%, 50% and 24% in control group respectively. The association of this polymorphism with diabetic disease was also evaluated which according to the results, the distribution of Sumo4 genotype was not significantly different comparing with the incidence of diabetic disease. There results suggested that the sumo4 M55Vvariant is not associated with type 2 diabetes mellitus susceptibility, suggesting that it may not be involved in the pathogenesis of type 2 diabetic.

Keywords: diabetes, polymorphism, mutation and restriction enzyme

O-10-180-4
K-ras, p53 genes mutations and microsatellite instability among Iranian sporadic colorectal cancer

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Colorectal cancer (CRC) can progress through two different pathways of genomic instability or microsatellite and chromosomal instability (MSI, and CIN). Microsatellite instability (MSI) is a hallmark of defects

in mismatch repair genes. A common finding in the classic adenoma-carcinoma pathway for CRC progression is the mutation of p53 tumor suppressor gene and K-ras oncogene. Only a few studies have addressed the spectrum of the above mentioned changes among Iranian CRC patients. We analyzed 151 tumor samples from Fars province in Southern Iran for K-ras mutations at codon 12, 13 and 61, and p53 mutations at the exon 5, 7 by PCR–SSCP technique. The specimens were also examined for MSI by PCR amplification and subsequent denaturing PAGE analysis of 5 selected microsatellite markers (Bat-25, Bat-26, D2S123, D5S346, and D17S250) from tumors and their corresponding adjacent normal tissues. K-ras and p53 mutations were observed in 30.5% and 41.1% of tumors, respectively. K-ras, and p53 mutations were significantly more frequent in distal than proximal CRC tumors. We also observed high microsatellite instability (MSI-H) in 23.8% of cases, a higher rate than those reported in western countries. There was an inverse relationship between p53, K-ras mutations and MSI-H incidence in tumors. Our study shows that the proximal and distal colorectal tumors might progress through different molecular pathways.

Keywords: K-ras, p53, colorectal cancer, microsatellite instability

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O-10-163-1

Amino acid substitutions in the nonstructural protein 3B of foot and mouth disease virus new subtype (A-05-Iran)

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Foot-and-mouth disease (FMD) is a clinically severe vesicular disease of cloven hoofed animals. FMD has a great potential for causing economic losses in the farming industry tremendously in countries which are naturally FMD-free. The present study was planned to investigate on the amino acid sequence variations resulting from single-nucleotide polymorphisms (SNPs) in new FMDV Subtype based on an alignment of three copies of the 3B gene region. After specimen collection, viruses were passaged in BHK cell monolayer cultures to provide RNA for analysis. Total RNA was extracted from infected cell culture supernatants by using the total RNA isolation kit according to the manufacturer's instructions. After that, the 3B coding region was reverse transcribed into cDNA and the identified DNA fragment of 213 bp was cloned into the PNTZ57T vector (Fermentas, Germany). DNA of clones obtained by this procedure was sequenced using T7 promoter. The amino acid sequence of 3B gene obtained was aligned with that of other type A isolates deposited in the GenBank database. The study results demonstrated that 3B1 gene copy was conserved in terms of amino acid substitution among the FMDV-A subtypes which have been previously reported. Sequence alignment comparisons revealed that two other 3B copies (3B2, 3B3) contain amino acid substitutions in their protein structure. Because of multiple mutations that had been detected in the 3B gene nucleotide sequence, amino acid replacement has occurred. Mutation G10→A in the 3B2 nucleotide sequence led to the amino acid replacement A→T at position 4. Surprisingly, this substitution has taken place exclusively in A-05-Iran isolate in comparison to other considered subtypes. It has also been shown that point mutations C17→T and G18→T in the 3B3 nucleotide sequence caused the amino acid substitution P→ L at position 6.

Keywords: amino acid, FMD, substitution, 3B

P-10-180-1

Distinct genes methylation profile in colorectal cancer: A population based study

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Colorectal cancer (CRC) is one of the most common cancers in the world. Recent studies have indicated that both genetic and epigenetic processes are important in CRC progression. In a comparative study, we analyzed promoter hypermethylation of 13 cancer candidate (CAN) genes in CRC tumors from two different populations, Iranians (as a Caucasian part of Asian ethnicities) and African Americans (AAs). DNA was extracted from tumor samples obtained from surgical resections of 51 AAs and 51 age and sex- matched Iranian CRC patients. We determined the methylation status of CAN genes (SYNE1, GPNMB, APC2, EVL, PTPRD, CDH5, LGR6, STARD8, CD109, RNF, ICAM-5, MMP-2 and RET) promoter by methylation specific PCR. We found a distinct level of genes promoter methylation for GPNMB, ICAM5 and CHD5 genes among Iranians compared to those in African-Americans. Our study revealed a statistically significant higher methylation level for promoter of 3 above-mentioned CAN genes in African Americans compared to Iranians. The frequency of GPNMB, ICAM-5 and CHD-5 genes promoter hypermethylation among AA samples were 100%, 40%, and 78%, respectively, and those for Iranians were 89%, 8%, and 47%. The high frequency of GPNMB, ICAM-5 and CHD5 genes methylation in AAs might explain, to a certain extent, the aggressiveness and high incidence of colon cancer in African Americans.

Keywords: candidate cancer gene, promoter methylation, colorectal cancer

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O-10-306-1

PRKAA2 variants have a key role to susceptibility to type II diabetes

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AMP-activated protein kinase (AMPK) is a key molecular regulator of cellular metabolism, and its activity is induced by both metformin and thiazolidinedione antidiabetic medications. It has therefore been proposed both as a putative agent in the pathophysiology of type 2 diabetes and as a valid target for therapeutic intervention. The gene encoding the alpha2 isoform of the catalytic subunit of AMPK (PRKAA2) is located at one of the Japanese type 2 diabetes loci mapped previously. PRKAA2 is, therefore, a good candidate gene for insulin resistance and type 2 diabetes. We therefore set out to test for the association of common variants and common haplotype in the PRKAA2 gene with type 2 diabetes and related phenotypes. We undertook an extensive case-control association study using a total of 911 unrelated Japanese T2D patients and 876 control subjects at 6 single nucleotide polymorphisms in the PRKAA2 gene. We observed associations of

nominal significance with two intronic SNP in the PRKAA2 (rs932447; OR 0.62, 95% CI 0.40-0.96, p=0.033 and, rs1418442; OR 0.62, 95% CI 0.40-0.96, p = 0.030, both under a dominant model). However, we were unable to observe the association between the PRKAA2 haplotype and type II diabetes. Our results indicated that the PRKAA2 gene variants have an important impact on T2D susceptibility in Japanese.

Keywords: association study, polymorphism, type 2 diabetes, PRKAA2, haplotype analysis

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P-10-203-1

The association of matrix metalloproteinase-3 gene -1612 5A/6A polymorphism with susceptibility to coronary artery stenosis in Iranian population

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Matrix metalloproteinases (MMPs) have been increasingly implicated in connective tissue remodeling during atherogenesis and have a role in early atherosclerosis, plaque rupture and myocardial infarction. MMP3 (Stromelysin-1) is key member of the MMP family, and is known to be present in coronary atherosclerotic. Several studies have demonstrated that MMP-3 5A/6A polymorphism modify each transcriptional activity in allele specific manner. We hypothesized that this polymorphism may play a role as a risk factor for development of coronary artery stenosis. The aim of our study was to estimate possible effect of the MMP-3 (5A/6A) gene polymorphism on interindividual variability in risk for coronary artery stenosis in an Iranian population. DNA was extracted from white blood cells and genotypes were obtained from coronary artery stenosis cases (n=95) and controls (n=100) through PCR (polymerase chain reaction) with RFLP (primers and restriction-fragment length polymorphism) techniques. Significant differences between cases and controls were observed for MMP3 genotype frequencies (X2=143, p<0.0001); These data imply the involvement of MMP3 gene -1612 5A/6A polymorphism in CAD, and suggest that probably the 5A/6A MMP-3 genotype is a genetic susceptibility factor for coronary stenosis.

Keywords: coronary artery stenosis, matrix metalloproteinase 3, Polymorphism

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P-10-476-1

Prevalence of factor V Leiden, prothrombin G20210A and MTHFR C677T mutations in deep venous thrombosis patients from Western Iran

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Inherited disorders related to hemostatic system are known as risk factors for thromboembolic events (TE) such as myocardial infarction, stroke, pulmonary embolism, pregnancy complications, and especially recurrent deep vein thrombosis (DVT). The present study was aimed to investigate the prevalence of factor V Leiden G1691A, prothrombin G20210A and methylene tetrahydrofolate reductase (MTHFR) C677T in DVT patients and their possible association with DVT in Western Iran. Seventy two DVT patients with the mean age of 40.4±13.6 years including 37 females and 35 males and 100 age and sex matched healthy individuals from Kermanshah Province of Iran with ethnic background of Kurd were studied for factor V Leiden, prothrombin and MTHFR C677T by PCR-RFLP method using Mnl I, Hind III and Hinf I restriction enzymes, respectively. Prevalence of factor V Leiden was 12.5% in patients and 2% in control group. A significant association was found between factor V Leiden mutation and DVT with odds ratios (OR) of 7.0 (95% confidence intervals [CI=1.46-33.46, P=0.018]). The prevalence of prothrombin G20210A variant in patients (4.16%) was insignificantly higher than control individuals (1.0%) [OR 4.3 CI 0.43-42.24 P=0.2]. The prevalence of MTHFR C677T was found to be 38.9% and 44% in patients and control groups, respectively. Venous thrombosis in legs was the most frequent clinical manifestation (n=67), corresponding to 93.1% of the TE, followed by pulmonary thromboembolism (6.9%). Our study has determined the prevalence of inherited thrombophilia in a homogenous ethnic group of DVT patients and suggests that factor V Leiden, and not the prothrombin gene mutation is a risk factor for DVT in Western Iran.

Keywords: deep vein thrombosis, factor V Leiden, prothrombin, methylene tetrahydrofolate reductase

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P-10-324-1

Evaluation of biofilm production among typical and atypical Enteroaggregative Escherichia coli (EAEC) isolates from diarrheal patients

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Enteroaggregative Escherichia coli (EAEC) are an increasingly recognized enteric pathogen. It is a cause of both acute and persistent diarrhea among children, adults and HIV-infected persons, in both developing and developed countries. In this study, we determined biofilm formation in 114 EAEC isolates (as defined by multiplex PCR and HEp-2 adherence) from diarrheal patients. The presence of putative EAEC virulence genes; i.e., aap, AA and aggR was determined by a multiplex PCR and divided the strains into two groups of typical EAEC strains showing presence of all three genes together, and atypical EAEC strains showing presence of two or one of the three genes. Biofilm formation is considered as virulence factor for EAEC strains. Therefore, in the present study the biofilm production was measured by using a microtiter plate assay with the crystal violet staining method among EAEC isolates. The biofilm production was analyzed by using two different culturing medium, namely, Mueller Hinton broth (MHB) supplemented with 0.45% glucose and M9 minimal essential medium supplemented with 0.45% glucose. The results obtained revealed that in typical EAEC isolates around 60% of isolates on MHB produced biofilm with OD at 570 nm OD≤ 0.2 and 40% of isolates OD≥ 0.2. However in M9 medium, 55% of isolates showed OD ≤ 0.2 and 45% OD≥ 0.2. In case of atypical EAEC isolates only around 25% of isolates exhibited OD≤ 0.2 and 75% OD≥ 0.2, but in M9 medium 45% showed OD≤ 0.2 and 55% OD≥ 0.2. Overall, it could be concluded that the biofilm production is a common feature of typical and atypical EAEC isolates and the biofilm production varies with

different media and its formation could be influenced by external factors.

Keywords: EAEC, biofilm, Hep-2, Multiplex PCR, Diarrhea

O-10-180-2

The association between DNA methyltransferase 3b C-149T genotype, and promoter methylation of PTEN, and APC tumor suppressor genes among Iranian colorectal cancer patients

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Several studies have shown that colorectal cancer (CRC) incidence is rising in Iran. Loss of APC and PTEN tumor suppressor genes expression by mutation or promoter hypermethylation are often involved in colorectal carcinogenesis. DNMT3b is one of the 3 genes involved in DNA methylation in humans. The DNMT3b gene contains a C-to-T polymorphism at position -149 that is associated with increased promoter activity and higher risk for several cancers. We studied the influence of the DNMT3b C-149T transition on the risk of sporadic CRC incidence and tumor methylation among Iranian population. We analyzed the DNMT3b C-149T genotype in 110 cases and 241 controls by PCR-RFLP. APC and PTEN genes promoter methylation status in tumors were determined by MSP-PCR. The most frequently methylated locus was PTEN (65 %; 60 of 110), followed by APC (11.8 %; 13 of 110). Eight of 110 (7.2 %) tumors showed hypermethylation in both APC and PTEN promoters. Younger males (<60) with TT genotype were at a higher risk for CRC incidence (OR 3.3, 95% CI=1.6-6.9, p=0.001). The DNAM3b T allele was also strongly associated with tumor methylation in the entire group of patients (OR 3.9, 95% CI=1.7-9.4) as well as in both males and females (p=0.001 and p=0.02, respectively). Our study provides evidence that the DNMT3b C-149T variant genotype is strongly associated with CRC risk and tumor methylation among Iranian patients. Determining tumors methylation status has implication for choosing the best drugs for cancer chemotherapy.

Keywords: DNA methyltransferase 3b, promoter methylation, colorectal cancer, tumor suppressor genes

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P-10-180-3

Inhibition of carcinoembryonic antigen release from colorectal cancer cells to prevent CRC-Liver metastasis

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Elevated carcinoembryonic antigen (CEA) blood levels are found in a wide variety of epithelial neoplasms. Recent studies show that CEA might have an instrumental role in cancer progression and human colon cancer liver metastasis. The latter function has been suggested to be facilitated by soluble CEA through induction of cytokine production by kupffer cells. We hypothesized that blocking CEA release from cancer cells might prevent CRC-liver metastasis. A duplicate aliquot of LS180 cells was seeded into four well plates (200,000 cells/well in 250µl medium) and allowed to grow to sub-confluence. Then they were incubated in fresh medium with or without FBS containing either crude or purified GPI-PLD inhibitors (CI, and PI; 10-100µM). The medium of controls were replenished with medium without inhibitors. The cell culture media from the duplicate set of wells was collected after every 2 h of incubation. The effect of inhibitors on the secretion of CEA was measured using a commercial ELISA kit. The levels of another GPI-anchored protein, alkaline phosphatase (ALP), and a TM-anchored CA19-9 tumor marker were also measured in the cell culture medium as controls. LS180 treatment with 20-100 µM concentrations of inhibitors reduced the release of GPI-anchored CEA and ALP, but not TM-anchored CA 19-9, from cancer cells ($p<0.05$). No measurable alteration was observed at 10 µM inhibitor concentration. CEA release is inhibited efficiently by micromolar concentration of GPI-PLD inhibitors. Inhibition of CEA release from cancer cells may have therapeutic application to prevent CRC-liver metastasis.

Keywords: CEA, GPI-PLD inhibitor, metastasis

P-10-352-1

Increased risk of head and neck squamous cell carcinoma metastasis in carriers of 5A allele of MMP3 gene promoter

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Matrix metalloproteinases (MMPs) comprise a family of enzyme that is able to degrade components of extra cellular matrix (ECM). Degradation of basement membrane and extra cellular matrix is important for tumor progression and metastasis. MMPs are known as key- players for tumor growth and metastasis. MMP3, also known as stromelysin-1, is up-regulated in a variety of tumors and has been shown to influence tumor initiation and progression. It also induces synthesis of other MMPs such as MMP1 and MMP9. The MMP3 gene is located on chromosome 11q22. An adenosine insertion/deletion polymorphism (5A/6A) at position -1171 of the MMP3 gene promoter influences transcription factor binding and MMP3 promoter activity. Two alleles have different activities. The MMP3 single nucleotide polymorphism (SNP) has been reported to be associated with both susceptibility to and the invasiveness of various cancers. In this study, the role of 5A/6A polymorphism for head and neck squamous cell carcinoma was evaluated. We genotyped 150 patients and 100 controls using PCR-RFLP. The HNSCC patients were divided into a group without metastasis (M-) and a group with developed metastasis (M+). The 5A allele was more prevalent in the M+ group than in control. The difference between M- patients and controls was not significant statistically. Therefore, the 5A allele carriers of HNSCC patients are susceptible to metastasis.

Keywords: extracellular matrix, head and neck squamous cell carcinoma, matrix metalloproteinase, restriction fragment length polymorphism, single nucleotide polymorphism

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O-10-476-5

A study of FV Leiden G1691A, prothrombin G20210A and MTHFR C677T genotypes polymorphisms in patients with CVST in Western Iran

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The present study was aimed to investigate the association between FV Leiden G1691A, prothrombin G20210A and MTHFR C677T with CVST in Western Iran. Twenty four CVST patients with the mean age of 37.1±11.7 years and 100 age matched healthy individuals from Kermanshah Province of Iran were studied for FV Leiden G1691A, prothrombin G20210A and MTHFR C677T by PCR-RFLP method using Mnl I, Hind III and Hinf I restriction enzymes, respectively. A significant association was found between FV Leiden mutation and CVST with an odds ratios (OR) of 9.8 (95% confidence intervals [CI] 1.68-57.2, P=0.01). No prothrombin G20210A was found among patients. In patients, MTHFR C677T tended to be higher (58.3%) compared to control (44%); odds ratios (OR) of 1.8 (95% CI 0.72-4.4, P=0.2). Our study indicates that CVST is not rare among population of Kermanshah and FV G1691A is the most important genetic thrombophilic risk factor associated with CVST in Western Iran, and its presence must be investigated in any patient presenting with this disease.

Keywords: factor V Leiden, prothrombin G20210A, MTHFR C677T, CVST, Western Iran

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O-10-504-1

The evaluation of epigenetic changes in GSTP1, TIMP3, P16, E-cadherin and BRCA1 genes in breast cancer tumors and it's relation with grade of tumor

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Epigenetic changes in breast cancer often occur and mostly happen in promoter's CpG islands that affects the expression state of genes, in this study we performed the evaluation of these alterations in GSTP1, P16, BRCA1, E-cadherin and TIMP3 genes for methylation pattern and it's relation with grade of tumor. A total of 100 samples prepared from 50 patients and 50 control samples obtained from the same patient and breast were selected following to pathological diagnosis. We performed bisulfite sequencing, immunohistochemistry and western blotting for evaluation of promoter methylation pattern and gene expression status respectively. Data analyses was performed using cox-regression statistics with ($p<0.05$) criteria. The results showed significant difference between methylation pattern in promoter regions and expression state between normal and tumorous tissues. A direct relation was observed with higher grade of tumors and degree of epigenetic changes with above mentioned genes. No significant variation was observed regarding the kind of tumor and alterations in the pattern of methylation in this study. These results indicate that methylation pattern in promoter region May cause changes in gene expression and might be used for prognosis in breast cancer.

Keywords: epigenetic, methylation pattern, promoter, bisulfate sequencing, breast cancer

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P-10-489-1

Study of type III NRG1 transcripts expression of NRG1 gene in M.S patients

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The complex pathogenesis of multiple sclerosis includes inflammation and potentially disabling focal lesions that are associated with heterogeneous, often destructive pathologic changes disseminated throughout the white matter of the central nervous system. Remyelination is the phenomenon by which new myelin sheaths are generated around axons in the adult central nervous system (CNS). This follows the pathological loss of myelin in diseases like multiple sclerosis (MS). Remyelination can restore conduction properties to axons (thereby restoring neurological function) and is increasingly believed to exert a neuroprotective role on axons. Remyelination occurs in many MS lesions but becomes increasingly incomplete/inadequate and eventually fails in the majority of lesions. It is necessary to understand the causes for this failure of regeneration. The specific signals that promote oligodendrocyte differentiation and myelin sheath formation in the CNS are not well understood. A potential candidate is the NRG1 family of growth factors. At least 15 different secreted or transmembrane isoforms has been known for this gene. They can be grouped into three major types: type I, type II, and type III. Recent studies have shown that a threshold level of NRG1 III regulates myelin sheath thickness in the forebrain and PNS. In this study we compare the expression of NRG1, type III transcripts in peripheral blood of M.S patients and normal controls in order to investigate the possible role of these transcripts in remyelination of lesions. The results of this study will be presented in this meeting.

Keywords: multiple sclerosis, remyelination, type III NRG1, central nervous system

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P-10-410-1

The relevance of angiotensin converting enzyme insertion/deletion gene polymorphism and endothelial nitric oxide synthase Glu298Asp gene polymorphism for susceptibility to coronary artery disease in Iranian population

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There are several genetic and environmental factors which affect cardiovascular disease, among them, angiotensin converting enzyme (ACE) and endothelial nitric oxide (eNOS) gene polymorphisms are mostly interested to study in the case of genetic factors. In this study we compared the relevance of ACE insertion/deletion (I/D) gene polymorphism and eNOS Glu298Asp gene polymorphism for susceptibility to coronary artery disease (CAD) in Iranian population. 487 individuals including 224 patients with >50% angiographically established coronary stenosis from Shahid Rajaee heart hospital and 263 healthy subjects were genotyped by a standard method. The systolic and diastolic blood pressure, serum cholesterol and LDL-C were significantly increased in CAD patients. The genotype frequencies of Glu298Asp polymorphism for Glu/Glu, Glu/Asp and Asp/Asp were 61.3, 32.2 and 6.5 percent in control subjects, and 46.5, 42.7 and 10.8 in CAD patients, respectively. The genotype frequencies differed

significantly between the two groups (P=0.003). The frequencies of the Asp alleles were 32.2 and 22.6 percent for CAD patients and control subjects, respectively, and was different significantly between two groups (p= 0.001, odds ratio= 1.6). Our results showed that there is no increased risk of CAD in association with DD genotype in Iranian population. Allele frequencies were also similar in both groups. Therefore, DD genotype does not increase the CAD susceptibility in the studied population. These results suggest that CAD is associated with Glu298Asp polymorphism of endothelial nitric oxide synthase gene in our population as an independent risk factor of CAD, but ACE I/D polymorphism does not affect the susceptibility to CAD in Iranian population.

Keywords: ACE, coronary artery disease, eNOS, polymorphism

O-10-536-1

β-Thalassemia mutations in Iranian Kurdish population and West Azerbaijan province

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It is well known that β-Thalassemia (β-thal) is a common inherited disorder in the Iranian population, which is a mixture of different ethnic groups with a great variety of mutations. Therefore, it is necessary to determine the frequency and distribution of these mutations in the various regions of the country. In this study, sixty unrelated patients with known β-thal major and intermedia registered at Kurdish β-thal clinics in different cities of Kurdistan and west Azerbaijan provinces were studied. Molecular analysis was based on polymerase chain reaction amplification refractory mutation system (PCR-ARMS) and samples negative on PCR-ARMS were analyzed by direct sequencing. We found fifteen β-Thalassemia mutations; the most prevalent allele (35%) was the IVS-II-1, the second FSC 8/9 (15.7%). Other mutations were IVS-I-1(G>A) 8%, FSC8 (-AA) 6.75%, FSC 5(-CT) 6.7%, IVS-I-110 (G>A) 6%, FSC 36/37 (-T) 4.2%, FSC 44 (-C) 3.4%, IVS-I-5 (G>C) 3.4%, IVS-I-6 (T>C) 3.4%, Codon 39 (C>T) 1.7, Codon 15 (TGG>TAG) 1.7%, IVS-I-128 (T>G) 1.7%, +22 3'UTR (G>A) 0.8%, and Codon 127 (CAG>CGG) 0.8%. This report is the first comprehensive study investigating the prevalence and spectrum of β-Thalassemia mutations in these populations, and the results of this report will be useful in identifying the β-Thalassemia mutations, essential for genetic counseling and for organizing a prenatal diagnostic service to improve the health in this deprived population.

Keywords: β-thalassemia, mutation, Kurd

P-10-556-1

Screening of Y chromosome microdeletions in Iranian infertile men

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It has been hypothesized that microdeletions of Yq may account for a significant proportion of men with infertility. Three non-overlapping regions, referred to as "azoospermia factors" have been defined as spermatogenesis loci, and deletions in these regions have been shown to be pathogenically involved in male infertility. 50 infertile men were selected. Semen analysis was done, and all patients were categorized into two groups as, azoospermia, and oligozoospermia. Genomic DNA was extracted, and amplified by sequence tagged sites-polymerase chain reaction method to evaluate micro deletions in AZF locus. 34 STS primers including 2 controls were selected to identify micro deletions of Y chromosome on each subject. 26/50 cases (52%) showed deletion of at least one of the STS Marker. 17 cases (34%) had deletion in one STS site. Four oligospermia cases (8%) had deletion in 2 STS site. Three azoospermia cases (6%) had again deletion in 2 STS site, but in different STSs. One case had three deletions in three STS site and one individual had seven deletions in AZF locus. The overall frequency of Y chromosome microdeletions observed in the present study was found to be 26/50 (52%). These data shows that the incidence of Yq microdeletions in Iranian population is much higher than international frequency, especially for microdeletions of AZFa.

Keywords: infertility, Y-chromosome, microdeletions, azoospermia, oligospermia

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O-10-728-1

Increased telomerase expression and activity by androstenedione and its suppression by phosphatidylinositol 3-kinase inhibitor in ovarian cancer

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Telomerase is a cellular reverse transcriptase that catalyses the synthesis and extension of telomeric DNA. This enzyme is usually inactive in normal somatic cells but is highly activated in most malignant tumors including epithelial ovarian cancer which is one of the most frequent causes of cancer death in women. The aim of this study was to investigate the effect of androstenedione, the main androgen of the ovaries, on telomerase activity and the expression of its catalytic subunit (hTERT). Human ovarian adenocarcinoma cell line (OVCAR-3) was cultured and incubated with various concentrations of androstenedione with or without PI3K/AKT inhibitor. Total cellular RNA extraction, cDNA synthesis and semi-quantitative real-time PCR were performed to examine the expression of hTERT. The activity of telomerase was measured by a telomeric repeat amplification protocol based method (TRAP) using PCR-ELISA. Androstenedione significantly increased both the expression and activity of telomerase after 48 hour. On the other hand, PI3K/AKT inhibitor suppressed the effect of androstenedione on telomerase. In conclusion our results showed that androstenedione may have a role in ovarian carcinogenesis.

Keywords: telomerase, androstenedione, ovarian cancer, phosphatidylinositol 3-kinase

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P-10-586-1

Molecular genetic of A2 proteins analysis from Iranian Leishmania species

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Leishmaniasis is a disease of human beings and animals caused by protozoan parasite of the genus Leishmania. Dogs usually develop the systemic (visceral) form of infection, with a highly variable clinical appearance. Canine leishmaniasis may be difficult to diagnose and frustrating to treat. In Iran, VL is endemic in some areas of northwest and south; the main causative agent is L. infantum and domestic dogs are the major reservoir for humans specially children. There are some stage specific antigens in Leishmania parasites that play a role in the pathology and protection mechanisms of leishmaniasis. The A2 genes within a multi-gene family encode amastigote-specific A2 proteins as virulent factors for the survival of leishmania parasite in the mammalian host. Immunoblot analysis of L. infantum isolates revealed for the first time that A2 proteins are detected in extracted amastigotes of L. infantum isolates from humans and dogs of Meshkinshahr as endemic area in northwestern Iran. In the present study we amplified by PCR and sequenced the A2 genes from genomic DNA of some old world CL species comparatively to L. infantum visceral parasites.

Keywords: Leishmania infantum, A2 protein, Iran, Meshkinshahr, visceral leishmaniasis

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O-11-591-1

Analysis of reversible interaction between beta-casein chaperone and partially unfolded state of target protein (ADH)

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In general, protein misfolding and aggregation are prevented by the machinery of molecular chaperones. The chaperone-like activity of bovine beta-casein has been documented recently. The estimation of the chaperone-like activity of beta-CN based on suppression of aggregation is of importance for being understood mechanistically. In this study chemometric analysis of UV-absorption spectra of alcohol dehydrogenase (ADH) under thermal stress, led to the existence of three different molecular species including native (N), aggregation-prone intermediate (I) and final aggregate (A) species. In the presence of beta-CN, however, a new complex between I and beta-CN as I-beta-CN was formed. In the absence of beta-CN chaperone, I-state was subsequently converted to the final aggregate species. In the presence of beta-CN, this molecular species could be converted to the final aggregate state and/or form the I-beta-CN complex. Our study also displayed that interaction between beta-CN and I-species takes places in a temperature-dependent manner and leads to a reversible I-beta-CN complex. Our study also revealed that the intrinsic ability of beta-CN molecule to expose its hydrophobic patches is a significant functional property in the chaperoning mechanism of this protein.

Keywords: beta-casein, chaperone, aggregation, alcohol dehydrogenase, chemometric analysis

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P-10-139-1

Combination of XRCC1 (codons 399 & 194), GSTT1 and GSTM1 genetic polymorphisms and susceptibility to breast cancer

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Breast cancer is the prevailing malignancy and the second cause of cancer death among women. Inherited difference in the capacity of xenobiotic metabolizing enzymes and DNA repair protein might be important factors of genetic susceptibility to breast cancer. Glutathione S-transferases (GSTs) are phase II enzymes involved in the detoxification of a broad range of toxic and carcinogenetic compounds. X-ray repair cross-complementing group 1 (XRCC1) is a base excision repair protein that plays a central role in repair of DNA strand breaks and base damage. In the present study, we investigated whether combination of common genetic variants in the XRCC1 gene (exon 10, Arg399Gln and exon 6, Arg194Trp) and loss-of-function deletion polymorphisms in GSTT1 and GSTM1 were associated with an altered risk of breast cancer. Blood sample from 197 patients with breast cancer and 202 age (±5 years)- and sex-matched healthy persons were collected. The XRCC1, GSTM1 and GSTT1 genotypes were determined using PCR-based method. The null genotypes of GSTM1, GSTT1 and 399Gln, 194Trp alleles of XRCC1 assumed as high-risk genotypes and alleles. Statistical analysis showed that the $\chi 2$ for linear trend for 0, 1, 2, 3 and 4 putative high-risk genotypes is equal to 8.09 (P=0.004).

Keywords: genetic polymorphism, breast cancer, XRCC1, GSTM1, GSTT1

P-10-622-1

SDS-PAGE analysis of the sarcosine-insoluble outer membrane fraction of the clinical isolates of Helicobacter pylori

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The gastric pathogen Helicobacter pylori show marked heterogeneity among various strains. Little is known about outer membrane proteins (OMPs) diversity among strains. The purpose of this work was to compare OMPs profile of various strains of H. pylori isolated from children. Thirty isolates of H. pylori isolated from twenty children during 1997-2008, also 26695 ATCC strain were cultured on Modified-Campy-blood agar plates. OMPs were extracted from envelopes of sonicated cells by a sarcosyl method. Sodium dodecyl surface-polyacrylamide electrophoresis (SDS-PAGE) was performed by the method of Laemmli, and analyzed by Lab-work soft ware. Analysis of the results revealed that various strains produced OMPs with different protein profile. About ten bands were considered the major OMPs on the whole profiles of various strains however, some of them were missing or had reduced expression in the isolates submitted to more than five laboratory subcultures. These profiles corresponded to the strains isolated during 1998-2000 and conserved in -70°C with or without more than five subcultures. Repeated in-vitro subcultures or

long conservation at -70°C, may results in the loss of H. pylori surface proteins or changes in their expression. Therefore, the virulence properties of the laboratory strains, following repeated subcultures, may be quite different from the original H. pylori strain.

Keywords: Helicobacter pylori, outer membrane proteins, expression, subculture

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P-10-203-2

Promoter polymorphism of the matrix metalloproteinase 3 gene in diabetes mellitus patients and risk of coronary artery disease

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Matrix metalloproteinase-3 (MMP3) -1612 5A/6A gene polymorphism has an effect on MMP3 expression which leads to low promoter activity 6A6A, intermediate promoter activity 5A6A and high promoter activity 5A5A genotypes and have been shown to be associated with coronary artery diseases (CAD) (e.g., coronary stenosis, myocardial infarction, coronary artery calcification, and etc…). The aim of our study was to estimate the functional polymorphism in MMP3 gene in diabetes mellitus patients and test the hypothesis that this polymorphism in diabetes mellitus patients might be a predictor of susceptibility to coronary artery disease. Ninety-five CAD patients and one hundred three healthy control subjects were included in this study. MMP3 genotypes were determined by PCR and restriction fragment length polymorphism. Significant differences between cases and controls were observed for MMP3 genotype frequencies ($X2=14.606$, $p\leq0.001$). We for the first time indicated that MMP3 -1612 5A/6A polymorphism is involved in diabetes mellitus and is a genetic susceptibility factor for CAD in diabetes mellitus.

Keywords: coronary artery disease, diabetes mellitus, matrix metalloproteinase-3, polymorphism

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P-10-109-1

Molecular basis of peroxisomal biogenesis disorder

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Peroxisomal biogenesis disorders (PBDs) are a genetically heterogeneous group of diseases with impairment in one or more functions of peroxisomes as Zellweger syndrome. PBDs comprise 13 complementation groups and their responsible genes have been identified recently. The responsible genes are nominated PEX genes, which encode peroxins, proteins necessary for peroxisome biogenesis and the import of the peroxisomal matrix and membrane proteins. Peroxins are categorized into different groups including those are belong to the import complex machinery, and membrane assembly. Thus mechanisms of peroxisome assembly, including peroxisomal import of newly synthesized proteins, are one of the major foci in this field to unravel the molecular mechanisms of pathogenesis of these disorders. Here we are going to express our experience for cloning of several PEX genes and molecular events required for membrane assembly and protein sorting into the peroxisomes. Moreover clinical aspects of these disorders will be described.

Keywords: peroxisome, peroxin, PEX gene, genetic disorders

P-10-689-1

DNA interaction with Pt(II) complex, PtCl2(NN) (NN=4,7-Diphenyl-1,10-Phenanthroline)

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Studies of small molecules, which bind at specific sites along a DNA strand as reactive models for protein–nucleic acid interaction, provide routes toward rational drug design as well as means to develop sensitive chemical probes for DNA. Binding interactions of the Pt(II) complex, PtCl2(NN) (NN=chelating dinitrogen ligand: 4,7-Diphenyl-1,10 phenanthroline) with calf thymus (CT) DNA has been investidated by cyclic voltammetry, fluorescence spectroscopic, Circular dichroism spectropolarimetery, melting temperature measurement. Steady state fluorescence spectroscopic experiments of DNA interaction with Pt complex in Tris-HCl buffer were carried out at 4.0, 15.0, 25.0 and 37.0°C. Fluorescence quenching data was fitted to Stern–Volmer equation: F0/F=1–Ksv [DNA]. This study shows that the Ksv is increased by rising the temperature, indicating that DNA quenches the fluorescence of Pt complex in a dynamic way. Upon addition of the complex important changes were observed in the characteristic CV experiments showed that both the anodic and cathodic currents of the mentioned complex decreased with increasing additions of DNA. Also the anodic peak potential (Epa), cathodic peak potential (Epc), and (E1/2) all showed positive shifts, increase in melting temperature and the changes in the CD peak of DNA are indicative of structural changes in A-DNA. The experimental results show that the mode of binding of the complex to DNA is classical intercalation.

Keywords: Pt complex, Circular dichroism, (CT) DNA, melting temperature, intercalation

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P-10-686-1

The biggest founder effect of Kashan haemophilia B population in Iran

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Hemophilia B is an X-linked bleeding disorder caused by the functional deficiency of blood coagulation factor IX. Genomic DNA of 19 patients (referred from kashan) was extracted according to standard protocols. PCR amplification and SSCP were performed on each sample for eight exons separately. The result of SSCP for each sample was compared to normal ones as control and sequencings were performed for those with different migration patterns. The analysis of sequencing data representing nucleotide substitution (G6472A) in 19 patients suggested existence of local founder effect. In addition, in all of cases, the A192G polymorphism was found. Presence of this A192G polymorphism probably confirms the existence of a founder effect in this population. This conclusion is supported by haplotype analysis that was performed to determine the DdeI, TaqI, HhaI and MnlI restriction fragment length

polymorphisms (RFLPs). In addition, in this study, we designed DraI restriction fragment length polymorphism (RFLP) that was performed for confirmation and detection of the carriers. As an alternative to multiple mutations, inheritance of the same mutation might be due to a common prolific distant ancestor by silent passage through carrier women, the family histories could be positive.

Keywords: hemophilia B, SSCP, founder effect, haplotype analysis

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O-10-345-1

UBE2Q2, a novel human gene, with a putative ubiquitin-conjugating enzyme activity, is upregulated in breast cancer tissues

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The ubiquitin-proteasome pathway facilitates the degradation of damaged proteins and regulates growth and stress response. The activation of this pathway in various cancers and malignancies including breast cancer is established. According to our previous report, the novel human gene, UBE2Q2, with a putative ubiquitin-conjugating enzyme activity, is located on chromosome 15, and over expressed in tumor mass and invasive epithelium in head and neck squamous cell carcinoma. Here, we have used real time PCR to investigate the expression levels of UBE2Q2 gene in a collection of 25 breast cancer patient samples matched with normal adjacent tissues. Increased expression of UBE2Q2 gene (8.8±2.83 folds) was observed in 76% of tumor samples when compared with normal tissues. Immunohistochemistry was also performed on formalin-fixed paraffin-embedded tissue sections using a rabbit polyclonal antibody that we generated against an amino acid sequence predicted from the DNA sequence of UBE2Q2 gene. Preliminary data showed a higher level of immunoreactivity for UBE2Q2 protein in invasive epithelium of cancerous tissues when compared with that in the normal epithelium. This finding is consistent with our real time PCR data. Our data suggest that the novel human gene UBE2Q2 may have implications in pathogenesis of breast cancer and could be used in molecular diagnosis purposes in the future.

Keywords: breast cancer, ubiquitin, ubiquitin conjugating enzyme, proteasome

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P-10-719-2

Restoration of correct splicing in IVSI-110 mutation with antisense oligonucleotides: Implications and applications in functional assay development

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The use of AOs to restore normal splicing by blocking the recognition of aberrant splice sites by the spliceosome represents an innovative means of potentially controlling certain inherited disorders affected by aberrant splicing. Here we systematically tested a number of AOs with a 2′-O-methyl oligoribonucleotide (2′-O-Me) backbone in cellular splicing assay system. K562 cells were transfected transiently with a recombinant plasmid (pEGFP/I-110) carrying the EGFP gene interrupted by a mutated human b-globin intron 1 (IVSI-110). The mutation in the intron causes aberrant splicing of EGFP pre-mRNA, preventing translation of EGFP. However, treatment of the cells with a 2′-O-methyl-oligoribonucleotide targeted to the aberrant splice site induces correct splicing, restoring EGFP activity. The effects are sequence-specific and depend on the concentration of the oligonucleotide. Thus, the cell line provides, among others, a novel functional assay system superior to other procedures that are based on protein down-regulation. In particular, the system would be ideal in assessing the cellular delivery efficiency of antisense oligonucleotides.

Keywords: b-globin, IVSI-110 mutation, Splicing, antisense oligonucleotide therapy

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O-10-479-1

Induction of immunity against some gram negative bacteria with Ferric Enterobactin protein (FepA)

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Escherichia coli O157:H7 is a gram-negative rod-shaped bacterium. E coli O157:H7 is an enterohemorrhagic (EHEC) strain of the bacterium Escherichia coli and a cause of foodborne illness. Infection often leads to bloody diarrhea by producing a toxin called Shiga toxin, which damages the intestines, and occasionally leads to kidney failure, especially in young children and elderly people. Because of its essential metabolic role, iron is one of the most important nutrients of bacteria. Micro organisms employ a number of different strategies to utilize iron, which is a vital element for most organisms but is not always readily available from the environment. Many of gram-negative bacteria produce and excrete siderophores, which form high affinity iron complex in the environment. The ferric siderophore complexes are transported across the outer membrane by receptor proteins commonly known as FepA. In the present work we amplified the genomic, FepA gene by PCR. The PCR product was ligated to pET28a. The recombinant protein was then expressed in E. coli BL21DE3. SDS-PAGE analysis was carried out and the recombinant protein was purified by Ni-NTA affinity chromatography. The purified recombinant protein was injected to BALB/C mice in order to induce immunity. ELISA observation demonstrated high immunogenicity of the recombinant protein. The findings are discussed.

Keywords: siderophore, iron, FepA, immunity

P-10-153-1

Genetic analysis and clinical evaluation of vacA gene and cagA gene in Iranian Helicobacter pylori isolates from peptic ulcer patients

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Almost half of the world's populations suffer from the Helicobacter pylori infection, but only some individuals develop gastric diseases with clinical symptoms. One reason for the phenomenon may be the different pathogenicity of infected H. pylori strains. The presence of cytotoxin-associated gene A (cagA) and expression of vacuolating

cytotoxin activity encoded by vacuolating cytotoxin gene A (vacA) are considered the two major virulent markers of H. pylori. The objective of this study was to correlate vacA genotype and cagA status with gastroduodenal pathology. Seventy-seven Iranian H. pylori strains from dyspeptic patients were studied for differences in the vacA and cagA genes and their relationship to the clinical outcome. Fifty-five of 77 strains (71%) had the vacA signal sequence genotype s1 and only 22 (29%) had the type s2. After primer modification the vacA middle-region types m1 and m2 were detected in 31 (40%) and 46 (60%) strains, respectively. The combination of s1-m2 (24 [31%]) and s1-m1 (31 [40%]) and s2-m2 (22 [29%]) had similar percentage. No strain with the combination s2-m1 was found. Thirty-eight (79%) of 48 patients with peptic ulcer harbored type s1 strains, in contrast to 16 (55%) of 29 patients with gastritis. Thus the presence of the vacA s1 genotype was associated with peptic ulcer disease (P=0.04). The vacA genotype s1 was significantly associated with the presence of cagA (P=0.0001). From our study, we conclude that there is a significant association of the cagA gene and vacA s1 signal sequence with gastroduodenal ulcer disease. The relationship of the various other vacA genotypes to gastroduodenal ulcer disease is less clear.

Keywords: Helicobacter pylori, peptic ulcer, vacA, cagA

P-10-666-1

Assessment of microsatellite instability in sporadic colorectal cancer: a case series study in the North-Eastern Iran

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Colorectal cancer (CRC) is the fourth leading cause of cancer-related deaths in Iran. Age Standardized Rates are 8.2 and 7.0 cases per 100,000 in males and females respectively. Defects in DNA mismatch repair systems result in microsatellite instability (MSI) that plays an important role in the carcinogenesis of both sporadic and hereditary colon cancers. MSI was studied in Iranian CRC patients using 5 microsatellite markers for the first time in Iran. We analyzed 51 consecutive patients, histologically confirmed as CRC. DNA extracted from paraffin-embedded tissue was assayed with a panel of five pairs of microsatellite primers (BAT-25, BAT-26, D2S123, D5S346, and D17S250) proposed for analysis of MSI in CRC. The mean age for the CRC was 58.1 and 63.2% of the patients were male. With regard to stage of tumor, 51.8% were in stage B and 48.2% were in stage C. The frequency of microsatellite instability-High (MSI-H), which is defined by detection of instability in at least two markers, was 23.5% and MSI-Low (MSI-L) was 13.7%. Moreover, eight samples showed loss of heterozygosity. MSI in D17S250 was more frequent in greater size of tumors (p=0.016). Most of tumors with MSI were located in right colon (p=0.024). We conclude that the frequency of MSI in CRC in this region appears to be higher than the world average. Additional investigation in a larger patient population is required for strong conclusion.

Keywords: colorectal cancer, microsatellite instability, mismatch repair

P-10-666-2

Glutathione-S-Transferase M1, P1 and T1 polymorphism analysis in esophageal squamous cell carcinoma, first report from Iran

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Esophageal squamous cell carcinoma (ESCC) is the second most frequent malignancy in Iran (Age standardized incidence rate: 17.6 in males and 14.4 in females per 100,000). Exposure to environmental carcinogens has been associated with the increased risk of ESCC. Phase II enzymes including Glutathione-S-Transferases (GSTs) are xenobiotic-metabolizing enzymes involved in detoxifying many carcinogenic electrophiles by attaching reduced glutathione to electrophilic groups in a wide variety of toxic compounds such as Polycyclic Aromatic Hydrocarbons. We assayed genetic polymorphisms in GSTM1, GSTT1 and GSTP1 and their correlation with ESCC for the first time in Iranian population. Included were 135 ESCC patients as cases and 129 healthy controls. Polymorphism was analyzed on DNA extracted from blood using Polymerase Chain Reaction (PCR) - multiplex to detect deletions in GSTM1 and GSTT1 and PCR Restriction Fragment Length Polymorphism (RFLP) followed by digestion with Alw261 enzyme to detect Ile105Val substitution in GSTP1. The null-genotype frequencies of GSTM1 and GSTT1, and Isoleucine/Valine and Valine/Valine genotypes of GSTP1 were 41.5%, 24.4%, 34.8%, 9.6% respectively. GSTT1, GSTM1, GSTP1 genes were not significantly associated to ESCC independently, however concomitant GSTM1 wild-type and GSTP1 Valine/Valine genotypes was more frequent in ESCC patients (p=0.036, OR=8.0). With regard to increased risk of cancer, these polymorphisms were not associated to demographic features including age, sex and ethnicity, and smoking as an important environmental factor. We conclude that GST genes were polymorphic in the studied population. Interaction between wild-type GSTM and GSTP (valine/valine) genotypes increases susceptibility to ESCC.

Keywords: esophageal squamous cell cancer, Glutathione-S-Transferases, RFLP, polymorphism

P-10-696-2

Neuronal apoptosis induced by cerebrospinal fluid from Alzheimer patients

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Neuronal apoptosis has been implicated in Alzheimer's disease (AD). Neuronal damage seems to be a major source of disability in Alzheimer' patients. There is now a body of evidence that points towards a critical role of CSF in brain development and physiology. Cerebrospinal fluid (CSF) contains growth factors and cytokines secreted from the choroid plexuses which show changes associated

with neurological disorders and disorders of development. Increasing lines of evidence suggest a role of inflammatory cytokines in the neurodegeneration associated with Alzheimer's disease. The aim of our study was to ascertain whether soluble mediators present in the cerebrospinal fluid (CSF) of patients with Alzheimer's could induce neuron apoptosis in culture. Samples of CSF from normal (n=20) and AD patients (n=28) were collected by lumbar puncture. Neuron cultures from cerebral cortex from rat fetuses were used to assay the CSF from AD patients. Primary neuron cultures were prepared from 16- to 17-day-old fetuses. Neuronal viability was evaluated by quantification of living, metabolically active cells, as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The fragmented DNA of apoptotic cells was measured by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-fluorescein nick-end labeling (TUNEL). Neuronal apoptosis was measured after treatment of neuronal cultures with individual CSF samples from AD patients and control patients. The mean neuronal apoptosis induced by CSF from AD patients was significantly different from that induced by CSF from control ones. Apoptotic cell death of neurons was induced in cultures exposed to cerebrospinal fluid (CSF) from AD patients.

Keywords: Alzheimer's disease, cerebrospinal fluid, apoptosis

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P-10-666-3

Microsatellite instability in tissue and serum of patients with Gastric cancer: First report from Iran

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Gastric cancer is the most frequent malignancy in Iran. Age standardized incidence rate (ASR) is 26.1 per 105 in males, and 11.1 per 105 in females. Various genetic factors implicated, including DNA mismatch repair (MMR) deficiency manifested as tumor microsatellite instability (MSI). We analyzed DNA alterations in normal tissue, tumoral tissue and serum of 26 gastric cancer patients using a panel of 6 markers (BAT-25, BAT-26, BAT-40, D2S123, D5S346, and D17S250) to detect microsatellite instability. Surgically resected gastric tumors were histologically confirmed as adenocarcinoma. In order to achieve the most precise results, all the cases were included prior to any therapeutic intervention. Moreover, with the application of a microdissection method on paraffin-embedded tissues, we obtained tissue sections containing more than 70% of tumoral cells for extracting the DNA. Two out of twenty six (7.7%) tumoral DNA showed MSI in 3 markers (BAT-25, BAT-40, D2S123). Moreover, the corresponding sera were also assessed for instability but no changes were observed. We concluded that microsatellite instability is not a suitable clinical diagnostic tool for gastric cancer in Iranian population.

Keywords: Microsatellite instability, Gastric cancer, Mismatch repair

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P-10-513-1

Association between adiponectin gene polymorphism and coronary artery disease in Iranian population

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Adiponectin is an adipose tissue-derived protein with important metabolic effects. Adiponectin levels are reduced in subjects with obesity, insulin resistance, type 2 diabetes, and inversely associated with several traditional cardiovascular risk factors. Genetic polymorphisms of adiponectin gene in Iranian population have not been studied yet. Also there is very little information about the genetic susceptibility to Coronary Artery Diseases (CADs) in Iranian population. In this study, we attempted to answer whether there is an association between +45T>G single nucleotide polymorphism (SNP) in the adiponectin gene with risk of CADs in Iranian population. Patients were recruited from among individuals aged 18 to 75 years, who underwent coronary angiography due to chest pain at Ghaem hospital, in Mashhad. Genotyping were done for 45T>G SNP in the adiponectin gene by PCR-RFLP method. The presence of CAD was defined as a >50% reduction of coronary artery diameter. Patients were classified according to the number of significantly stenotic vessels into angiographycally-normal (n=135), 1-vessel (n=88), 2-vessel (n=111) and 3-vessel (n=132) diseased groups. Healthy control subjects (n=108) were randomly selected from among individuals, who visited affiliated hospitals or clinics and did not have any history of heart diseases. Patients and controls were matched for age and sex. There was a significant difference in the frequency of adiponectin +45T>G SNP frequencies between healthy controls and normal CAD patients (p=0.001), SVD (p=0.002), 2VD (p=0.0004), 3VD (p=0.002). Our study supports the hypothesis that functional SNP in the adiponectin play an important role in the development of IHD in Iranian population.

Keywords: adiponectin, coronary artery disease (CAD), gene polymorphism, SNP

P-10-759-1

Serum butyrylcholinesterase activity and phenotype associations with lipid profile in stroke patients

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Butyrylcholinesterase (BuChE) catalyzes the hydrolysis of acetylcholine and other choline esters and is also involved in lipid metabolism. The purpose of this study was to investigate any association between BuChE serum phenotype and activity and lipid profile of ischemic stroke patients. We determined serum BuChE activities and phenotypes, and levels of total cholesterol (TC), LDL-C, HDL-C and triacylglyerol (TG) in 33 patients with acute ischemic stroke within 12 h of the onset of the attack and 29 controls. The mean (±SD) serum BuChE activity and the BuChE of U/A phenotype in the stroke individuals were significantly lower and higher than that of the control (315 (±124) IU/L vs. 384 (±99) IU/L, p=0.02, t=−2.4 and 21.2% vs.3.4%, p=0.026, respectively). Our results showed a negative correlation between BuChE activity with TC level; in addition, the frequency of BuChE phenotypes with low activity is high in stroke patients, who have high levels of cholesterol, and may have increased susceptibility to stroke.

Keywords: butyrylcholinesterase, acetylcholinesterase, acetylcholine, stroke, lipids

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P-10-759-2

Association between enzymatic and non-enzymatic antioxidant defense

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There are evidence suggesting that APOE-ε 4 allele plays an important role in the pathogenesis of Alzheimer's disease (AD) by reducing peripheral levels and activities of a broad spectrum of nonenzymatic and enzymatic antioxidant systems. However, the link between APOE genotype, oxidative stress, and AD has yet to be established. In this study we examined whether antioxidant defense mechanism exacerbates the risk of AD in individual carrying APOE-ε 4 allele in a population from Tehran, Iran. We determined the enzymatic activities of the erythrocyte Cu–Zn superoxide dismutase (Cu–Zn SOD), glutathione peroxidase (GSHPx), catalase (CAT) and serum level of total antioxidant status(TAS) in various APOE genotypes in 91 patients with AD and 91 healthy subjects as control group (age and sex-matched). The results showed that the TAS level and the activities of enzymatic antioxidants CAT and GSH-Px were significantly lower and the SOD activity was significantly higher in AD patients compared to controls. The AD patients with APOE-ε 4 allele genotype had significantly lower serum TAS concentration and lower erythrocytes GSH-Px and CAT activities (p=0.001) but significantly higher erythrocytes Cu–Zn SOD activity (p=0.001) than the non-APOE-ε 4 carrier AD and the control group. In addition, the association observed between the factors involved in an antioxidant defense mechanism and APOE-ε 4 allele in AD increased with age of the subjects. These data indicate that the reduced serum level of TAS and activity of CAT, GSH-Px and increased SOD exacerbate the risk of AD in individuals carrying APOE-ε 4 allele. The reduced antioxidants defense in APOE–ε 4 allele carrier may contribute to beta-amyloidosis. This effect, however, is more pronounced in the AD patients older than 75 years of age. This suggests that a therapeutic modality should be considered for these subjects.

Keywords: Alzheimer's disease, antioxidants, oxidative stress, superoxide dismutase, glutathione peroxidase, catalase, total antioxidant status, APOE genotype

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P-11-765-1

Molecular diagnosis of trichomoniasis in apparently negative samples

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Trichomoniasis is an extremely common sexually transmitted infection (STI) worldwide and is associated with important public health problems, including amplification of HIV transmission. This disease is found to be in forms of symptomatic and asymptomatic in women and may depend on host as well as parasite variables. Most of the studies reported from females are based on examination of vaginal secretions and urine samples by direct smear and cultured in modified Diamond's media. Reports show that the sensitivity of culture media for detection of parasite is approximately 87-95% compared with direct smear that is 65-75%. Thus the aim of this study was checking the apparently negative samples, by direct smear and culture, with polymerase chain reaction (PCR) technique. In this study the urine samples and vaginal discharge of 100 patients attending gynecology clinics of Mazandaran province with different symptoms were checked for Trichomonas vaginalis by PCR technique using primers targeting a conserved region of the beta-tubulin genes of the parasite. Results showed that, out of 100 negative samples by direct smear and culture, four samples (4%) were positive by PCR technique. Three out of four infected women complained of discharge and or itching/burning sensation. Speculum examination showed that one of positive subjects had normal appearance of vagina and cervix. T. vaginalis infection is commonly associated with other STIs and a marker of high-risk sexual behavior. Thus diagnosis of trichomoniasis by PCR is a sensitive and specific method that could play important role to help the physicians for properly treatment and control of infection.

Keywords: diagnosis, PCR, Trichomonas vaginalis

P-10-769-1

Detailed analysis of heterochromatin polymorphism and chromosomes abnormalities in drug addicted individuals

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Polymorphisms of the size of heterochromatin regions and abnormalities of chromosomes have been well documented in human genome and it consists of DNA sequences that are not transcribed. The prime aim of the present study was to evaluate the heterochromatin polymorphism and numerical and structural abnormalities associated with chromosomes in drug addiction individuals. No data exists in Iran regarding the cytogenetic characteristics of drug addiction lymphocytes. Therefore, cytogenetic investigations were performed in 88 drug addiction lymphocytes and 34 normal controls. This randomized collected study was conducted on 88 consecutive drug addiction individuals and 34 healthy individuals in Loghman and private hospitals Tehran, Iran between years 2007-2009. By applying Barium Hydroxide saline Giemsa (BSC) method, the variant heterochromatin polymorphism of chromosomes 1, 9 and 16 on lymphocyte cultures were evaluated. Cytogenetic analysis performed at diagnosis is considered to be the most valuable prognostic factor in drug addiction lymphocytes and is a heterogeneous disease. Constitutive heterochromatin polymorphism of chromosomes 1 in drug addiction revealed statistical significant difference when compared with chromosome of healthy controls. The differences were not significant for chromosome 9, it was P=0.280% in drug addiction and 0% in the control group (P=0.196). The differences were not significant for chromosome 16, it was P=0.755 % in drug addiction. Also the frequency of partial and complete inversion did not show any significant differences between the drug addiction and the control group. Our constitutive heterochromatin polymorphism blocks and chromosomal abnormalities in drug addiction chromosome may provide an opportunity to serve as a marker for the detection and characterization of the chromosomes in drug addictions.

Keywords: addiction, chromosome, abnormalities, heterochromatin, polymorphism

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P-10-875-1

Apolipoprotein A5-1131T/C polymorphism is associated with lipid profile in Babol population

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The apolipoprotein A5 is one of the genes, which plays important role in regulation of lipid profile such as triglyceride. We evaluated the effect of a common polymorphism of this locus in Babol population to determine its association with plasma lipid levels. We selected subjects from Babolian Population for the case-control study and assessed their lipid profile levels. The segment of mentioned gene was amplified by polymerase chain reaction and the polymorphism was revealed by restriction fragments length polymorphism using MseI restriction enzyme. The frequency of T and C allele were 76.6% and 23.4%, respectively in this population. For subjects with low and high TG, we find significant association in lipid profile ($P<0.05$). The genotype and allele frequencies of T/C polymorphism in the Babolian population indicates that there is relationship between this polymorphism and biochemical parameters, which this finding confirms reports of some foreign researchers.

Keywords: apolipoprotein A5, Babol, polymorphism

P-10-591-2

Effect of position polarity of cysteine substitution on the antigenic response of mutant forms of beta-casein

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Cow's milk allergy (CMA) is the most common food allergy in young children. Beta-casein is one of the allergenic protein components of cow's milk with major sequential antigenic determinants (epitopes). The specific IgE binding capacity of native- and different recombinant forms of beta-casein was investigated using sera from well-characterized patients with confirmed CMA. This study was carried out in order to investigate effect of position polarity of cysteine substitution on the antigenic response of mutant forms of beta-casein. The obtained results on cysteine insertion in beta-casein may suggest this amino acid as a critical residue in defining protein allergenicities and that their contributions to the immune reactivity depend on their location and/or microenvironments in polypeptide chain. Additionally, the effect of beta-casein dimerization and formation of palindromic dimers with radically different arrangement of hydrophobic/hydrophilic domains on the recognition by specific IgE was studied. This study also confirm significant role of C-terminal hydrophobic domain in immune reactivity profile of native beta-casein.

Keywords: beta-casein, immune reactivity, epitopes, cow's milk allergy

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O-11-800-1

Role of glutathione S-transferase M1 5'UTR methylation in male infertility

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In about 50% of infertile couples a male factor can be found to be implicated either alone or in addition to other female factors. A new molecular mechanism recently revealed to be involved in infertility is epigenetic pattern modification like as aberrant methylation which can change or even silence gene expression. GSTM1 is shown to be important in transport of hormones and sexual steroids. Recent studies are in favor of implication of GSTM1 in male infertility. Based on the role of this gene in spermatogenesis, studies proving its epigenetic modification in male infertility and its implication in other diseases we explored its implication in Iranian men with infertility. This study was performed on 50 peripheral blood samples of 50 fertile and 50 infertile men as well as 32 testicular tissue samples of infertile men with non

obstructive azoospermia and 5 infertile men with obstructive azoospermia. Methylation detection method used in this study is Methylation Specific PCR. In both cases (infertile men with non obstructive azoospermia) and normal control (fertile men) blood samples both methylated and unmethylated alleles existed. In tissue samples, 2 out of 32 testicular tissues showed only methylated and other samples showed both methylated and unmethylated alleles. This result shows a trend to methylation in Iranian men with infertility. However, statistically this difference is not significant. This study, in contrast to previous report in another population, does not confirm the implication of methylation modification of GSTM1 5'UTR region in Iranian patients with infertility.

Keywords: azoospermia, infertility, male factor, GSTM1

P-10-799-1

Mitogen activated protein kinases in human peripheral nerve protein phosphorylation: Monoclonal antibody mediated inhibition assay

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The amount of biochemicals in the brain decreases with age. Neurodegenerative disorders are the result of massively accelerated rates of synaptic degeneration and neural cell loss. Some of these disorders are common and are thought to be caused by environmental factors and genetic susceptibility along with a small percentage due to inherited forms of the disease (e.g., Alzheimer disease). The MAP kinases and phosphatidylinositol-3kinase (PI3K) are serine/threonine protein kinases that play critical role in neuronal growth, differentiation and survival. In general, activation of the extra cellular signal-regulated kinase members of MAP kinase family (ERK or p42/p44 MAP kinases) and PI3Kt signaling pathway promote cell survival, while members of MAP kinase family known as the stress activated protein kinases (JNK) and the p38 MAP kinase, promote cell death. Present study evaluated the effect of blocking specific proteins/kinases of this pathway and observing the phosphorylation of substrate proteins. We studied this effect on a well characterized system, the peripheral nervous system. Protein phosphorylation was carried out with or without the presence of specific monoclonal antibodies. The reaction mixtures were subjected to SDS PAGE and autoradiography. The result showed that blocking of JNK and p38 MAP kinases did not significantly effect the overall phosphorylation; but, ERK MAP kinase blocking resulted in significant decrease in the phosphorylation of major peripheral nerve protein P0. Diseases caused by MAPK protein alteration are Huntington's disease, Parkinson's disease, cancer etc. Our observation bears significance in designing molecules to regulate, substitute or bypass these defects.

Keywords: MAP kinase, monoclonal antibody, protein P0

P-10-404-1

Relationship between PON1 gene polymorphisms and high ApoB/ApoA-I ratios

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Elevated Apolipoprotein B–Apolipoprotein A-I ratio is a risk factor for predicting coronary artery disease (CAD). Paraoxonase-1 (PON1) is a high-density-lipoprotein (HDL)-associated serum enzyme. PON1 protects low-density-lipoproteins (LDLs) from oxidative modifications and thus has a protective effect against CAD promotion. There are two common polymorphisms, Q192R and L55M, in PON1 gene. There may be a relationship between these polymorphisms and elevated ApoB/ApoA-I ratio. Therefore, we decided to evaluate effect of these polymorphisms on individuals with high and normal ApoB/ApoA-I ratios. To evaluate Q192R and L55M polymorphisms in Iranian case group (n=75) with high ApoB/ApoA-I ratio, and control group (n=75) with normal ApoB/ApoA-I ratio, we did PCR using specific primers. Then, we digested PCR products by RFLP. ApoB and ApoA-I levels were determined by immunoturbidimetry method. Genotype frequencies for Q192R were determined: 49.3%QQ, 44%QR, 6.7%RR in case group, and 53.3%QQ, 33.3%QR, 13.4%RR in controls (P = 0.236). Genotype frequencies for L55M were determined: 21.3%LL, 68%LM, 10.7%MM in case group, and 42.7%LL, 52%LM, 5.3%MM in controls (P = 0.016). A significant relationship between L55M polymorphism and familial history of cardiovascular disease was found (P = 0.011). PON1L55M polymorphism was associated with high ApoB/ApoA-I ratios in case group. We didn't find this relationship for Q192R. Thus, L55M polymorphism may be an independent risk factor for cardiovascular disease. Since L55M polymorphism was associated with familial history of cardiovascular disease, it is better to evaluate L55M polymorphism in younger ages even in the absence of high ApoB/ApoA-I ratios.

Keywords: apolipoprotein B, apolipoprotein A-I, paraoxonase-1 (PON1) gene

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O-10-820-1

Study of apoptosis inducing activity of calprotectin on fibroblast cell

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One of prominent types of connective tissue cells is fibroblast that synthesizes and maintains the extracellular matrix of many animal tissues. Previous studies illustrated that calprotectin has different cytotoxicity effects on fibroblast cells. Calprotectin is an abundant protein in the neutrophil cytosol; it has growth-inhibitory and apoptosis-inducing activities against various cell types such as tumor cells. The present study tries to introduce mechanism of growth inhibitory effect of calprotectin on human foreskin fibroblast cells (HFFF) and compare to etoposide (chemotherapy agent as control).

Calprotectin was purified from human neutrophil by chromatography methods. HFFF cell lines were used. These cells were maintained in RPMI 1640 medium supplemented with 10% FCS in a humidified incubator (37˚C & 5% CO2). The HFFF cells were exposed to the different concentrations of calprotectin and etoposide for 24, 48 and 72 hours. Cell proliferation was assessed using dimethylthiazol diphenyl tetrazolium bromide assay. For evaluation of cytotoxic mechanism in calprotectin on HFFF cells, flow cytometric analysis was performed. Our results revealed that calprotectin and etoposide induce growth inhibition of HFFF in a dose- and time-dependent manner. Sensitivity of HFFF cells to cytotoxic effect of human calprotectin was highly remarkable. In addition, growth inhibitory effect of this cytotoxic agent mostly was governed through induction of apoptosis in the HFFF cells. Taken together, calprotectin not only has more potent anticancer activity in comparison to the etoposide but it also is an apoptosis inducer that acts on the proliferation of normal cell like fibroblast.

Keywords: human calprotectin, etoposide, apoptosis activity, flow cytometry, fibroblast

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O-10-780-1

Introducing human calprotectin as a potent anticancer with minimal side effect

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Chemotherapy by using agents such as etoposide is a common way for inhibition of tumors. This treatment is accompanied by many undesirable side effects. Calprotectin is an abundant protein in the neutrophil cytosol; it has growth-inhibitory and apoptosis-inducing activities against various cell types such as tumor cells. In this study to introduce calprotectin as a suitable substitute anticancer, its growth inhibitory effect on human gastric adenocarcinoma cell line (AGS) and human foreskin fibroblast cells (HFFF) is compared to etoposide effect on these two cell lines. Calprotectin was purified from human neutrophil by chromatography methods. AGS and HFFF cell lines were used. These cells were maintained in RPMI 1640 medium supplemented with 10% FCS in a humidified incubator (37˚C & 5% CO2). AGS cells (10000 cells per well) were exposed to different concentrations of calprotectin and etoposide for 24 and 48 h. MTT assay was used for evaluation of cytotoxicity. Results indicate that calprotectin has more potent anticancer activity in comparison to the etoposide but it has nearly similar inhibitory effect on the proliferation of fibroblast cells. Since calprotectin effect was about 20 times more than etoposide on cancer cells, without any additional side effect, it can be concluded that it is a suitable candidate to be studied as anticancer drug.

Keywords: human calprotectin, etoposide, anticancer, chemotherapy

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P-10-786-1

Copper induced oxidation profile of serum low density lipoprotein in rabbits with mild hyperhomocysteinemia

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Low density lipoproteine (LDL) oxidation is a critical step in development of atherosclerosis and cardiovascular disease. Hyperhomocysteinemia as a novel cardiovascular disease risk factor may enhance the oxidation of serum LDL. The aim of present study was to evaluate the effects of mild hyperhomocysteinemia on LDL oxidizability in animal model. Twelve white newzland rabbits were randomely divided in experimental and control groups. Experimental group received methionine rich diet and control group fed with normal diet for 12 weeks. Fasting blood samples were obtained and sera separated from all animals after intervention. Serum LDL was purified by sequentional sedimentation ulteracentrifugtion followed by gel filtration chromatography. LDL oxidizability was evaluated by monitoring the change of light absorbance at 234 nm after addition of Cu^{+2}. A number of quantitative parameters including lag-time for initiation of oxidation, maximal rate of oxidation (V-max), and maximal amount of lipid peroxide products (OD-max) were evaluated. Statistical analysis showed that after intervention lag time was lower in experimental than control group (25.5±6.31 VS. 37.20±11.36, P=0.059), while V-max and OD-max were significantly higher in experimental than control group (128x10-5±24x10-5 VS. 46X10-5±11X10-5, P= 0.0001; 0.159±0.027 VS. 0.074±0.008, P=0.0001, respectively). Plasma levels of homocysteine were significantly correlated with OD –max, V-max and lag-time (r=0.669, p=0.024; r=0.845, p=0.001 and r=-0.696, p=0.017, respectively). These results indicated that mild hyperhomocysteinemia in rabbits increased susceptibility of LDL to copper induced oxidation. It is suggested that hyperhomocysteinemia increased the risk of cardiovascular disease partly through increased LDL oxidation.

Keywords: hyperhomocysteinemia, low density lipoproteine, oxidizability, rabbit

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P-10-113-1

Association of I405V and -629C/A polymorphisms of cholesteryl ester transfer protein gene with high-density lipoprotein cholesterol levels in coronary artery disease

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Cholesteryl ester transfer protein (CETP) exchanges neutral lipids between lipoproteins. CETP gene is known to have many single nucleotide polymorphisms which have been associated with plasma high density lipoprotein cholesterol (HDL-C) concentrations. The role of CETP in the atherogenic process is still not fully clarified. We studied the association of -629C/A and I405V polymorphism in CETP gene with coronary artery disease (CAD). We undertook a cross- sectional analysis on two polymorphism of CETP gene among 323 consecutive patients who underwent coronary angiography. Association was

analyzed among plasma lipid and lipoproteins, its gene polymorphisms and the finding in coronary angiography. Polymorphisms were determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). The allele frequencies of -629C/A: A allele was 0.66; and that of I405V: V allele was 0.35. Study of association between I405Vpolymorphisms and plasma lipid and lipoprotein concentrations revealed no significant differences between three genotypes. A genotype based subgroup analysis revealed a significant increase in HDL-C in non-CAD patients with AA and CA genotype. There was significance difference with A allele frequency in CAD and non-CAD group. In the present study there was no change in HDL-C between the I405V genotypes and CAD prevalence was not significantly different among in these three genotypes. AA genotype of -629 C□A polymorphism in promoter of CETP gene increased HDL but this genotype maybe associated with higher risk of CAD than CC genotype.

Keywords: CETP gene, I405V, HDL cholesterol,-629C/A

P-10-786-2

Evaluation the effect of diet induced hyperhomocysteinemia on oxidative stress in rabbits

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Elevated plasma levels of homocysteine considered as a new cardiovascular disease risk factor but underline mechanism is poorly understood. Oxidative stress is a condition that participated in some pathologic processes including atherosclerosis and cardiovascular disease. In this case control and experimental study we evaluated the effects of diet induced hyperhomocysteinemia on the plasma level of malondialdehyde (MDA) as an index of oxidative stress in rabbits. For induction of hyperhomocysteinemia, 6 Newzland rabbits fed with methionine rich diet for 12 weeks, while 6 rabbits fed normal as control. Fasting serum levels of lipids and MDA determined before and after intervention. Serum lipids were determined by routine lab kits, and MDA analysis was performed by high performance liquid chromatography. There were not found any significance differences in serum MDA and lipids levels between control and experimental animals before intervention, but after intervention MDA was significantly higher in experimental animals than controls (0.711±0.04 VS. 0.45±0.05 µmol/l, p=0.0001). A significant correlation was also found between homocysteine and MDA, before and after intervention (r=0.839, p=0.001; r=0.823, p=0.002, respectively). Our results indicated in rabbits that hyperhomocysteinemia induced oxidative stress and increased lipid peroxidation. This may be one possible role for high cardiovascular risk of subjects with mild hyperhomocysteinemia.

Keywords: yperhomocysteinemia, malondialdehyde, oxidative stress, rabbit

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P-10-786-3

Evaluation of the effects of diet induced hyperhomocysteinemia on serum lipids oxidizability in rabbit

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Serum lipid oxidation is a key step in initiation of atherosclerosis. Hyperhomocysteinemia is associated with thrombotic cardiovascular disease in humans but there is some controversy on its mechanism. The aim of this study was to evaluate the effects of mild hyperhomocysteinemia on sensitivity of serum lipids to in-vitro copper induced oxidation in an animal model. Mild hyperhomocysteinemia was induced in 6 Newzland rabbits by diet supplemented with methionine for 12 weeks, while 6 rabbits were fed normal diet in the same condition as control group. Fasting blood serum prepared before and after intervention in all animals for determination of serum lipids levels and evaluation of serum lipids oxidizability. Serum lipids were determined by routine lab kits and serum lipid oxidizability was followed by monitoring of the change of conjugated dienes in diluted serum after addition of Cu+2. A number of quantitative parameters including lag-time, maximal rate of oxidation (V-max), and maximal amount of lipid peroxide products (OD-max) were evaluated. There were not found any significant difference on lipids levels and serum lipid oxidation parameters before diet supplemented. After intervention, the lag time was lower, while V-max and OD-max were significantly higher in experimental animals than controls ($131x10^{-5}$± $26x10^{-5}$ VS. $47X10^{-5}$±$17X10^{-5}$, P=0.0001; 0.153±0.023 VS. 0.075± 0.006, P=0.0001, respectively). Results indicated that mild hyperhomocysteinemia is associated with increased susceptibility of serum lipid to oxidative modification.

Keywords: hyperhomocysteinemia, oxidizability, rabbit, serum lipids

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P-10-719-3

Sodium butyrate as a splicing modifier for the IVSI-110 β-thalassaemia splicing mutation

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β-thalassaemia is a common autosomal recessive disorder in humans caused by a defect in beta-globin chain synthesis. Over two hundred different mutations have been found to cause beta-thalassaemia, with the most common being splicing mutations. Most of these mutations activate aberrant cryptic splicing of 5′ or 3′ splice sites without completely abolishing normal splicing. Some mutations allow a significant level of normal splicing (e.g. IVSI-6), leading to thalassaemia intermedia, while others reduce normal splicing to low (e.g. IVSI-110) or very low levels (e.g. IVSI-5 and IVS2-654) and lead to blood transfusion dependency in the homozygote forms. In most of these cases, the normal splicing sites are not mutated, resulting in competition between the normal and aberrant splice sites and the production of variable amounts of normal mRNA. Modulation of splicing can be achieved by activation or suppression of transacting factors such as SR proteins and hnRNPs through drugs. Here, we demonstrate

restoration of IVSI-110 mutation obtained by sodium butyrate, a histone deacetylase inhibitor, known to upregulate the expression of splicing factors. These results highlight the therapeutic potential of splicing modulation for genetic diseases caused by splicing mutations

Keywords: beta-thalassaemia, splicing, IVSI-110 mutation, sodium butyrate

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O-10-719-1

A novel cryptic splice site in IVSI-110 β-thalassaemia

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The IVSI-110 β-thalassaemia mutation is the result of a G to A substitution at position 110 of intron 1 in the β-globin gene. This mutation creates an aberrant 3' acceptor splice site, which is preferentially recognised by the spliceosome over the natural 3' acceptor splice site. We recently reported the creation of a 'humanized' mouse model containing the IVSI-110 human β-globin locus. Upon further analysis we noted the presence of an unexpectedly larger reverse transcribed product. Sequence analysis revealed the existence of a previously unreported cryptic splice site in the IVSI-110 pre-mRNA. Interestingly, the novel cryptic splice site identified in mice was further confirmed in several IVSI-110 β-thalassaemia patients. Moreover, we observed quantitative differences in the levels of aberrantly spliced β-globin mRNA among patients. We conclude, that the identification of novel cryptic splice site indicates that IVSI-110 splicing is more complex than previously reported, and is worthy of further investigation.

Keywords: β-thalassaemia, IVSI-110 mutation, splice site, RT-PCR

P-10-335-3

Association between GSTT1, M1 and P1 polymorphisms with coronary artery disease

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DNA damage which occurred by the effect of oxidant and mutant agents has an essential role in development of the atherosclerosis and coronary artery disease (CAD). The essential role of glutathione S-transferases (GSTs) in cells as antioxidant enzymes has been known and polymorphism of these enzymes has been investigated in different diseases. To find out the possible association between GSTs polymorphism with coronary artery disease (CAD), we investigated the frequency of GSTT1, M1 and P1 genotypes in patients with coronary artery disease compared to controls. The genotypes of GSTT1, M1 and P1 were determined in 133 angiographically documented CAD patients and 74 normal coronary artery cases (as controls). In patients with CAD compared to controls, the frequencies of GSTT1-Null was decreased (p=0.009, OR=4.00, CI=1.312-12.194). However, GSTM1 and GSTP1 genotypes were not significant in two groups (p=0.078 and p=0.772, respectively). Overall, in patients compared to controls, GSTT1-Null genotype was decreased. However, the patients and controls did not indicate any significant difference in the frequencies of GSTP1 and GSTM1 genotypes. It was concluded that a reduction in the frequency of GSTT1-Null genotypes might be involved in the pathogenesis of CAD.

Keywords: glutathione S-transferase, polymorphism, coronary artery disease

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P-10-779-1

Investigation of matrix metaloproteinase 1 levels as effective factor in myocardial infarction

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Myocardial infarction results in myocardium remodeling and its change of structure and function. The role of Matrix Metaloproteinase (MMP) family during myocardium remodeling is an important factor regards to detection of Acute Myocardial Infarction (AMI). In this study, we investigated the gene expression and presence of MMP1 in patients with myocardial infarction compared to control cases. In this study, immunoblotting analysis was carried out in 50 patients and 50 control cases for detection of MMP1. Gene expression of MMP1 analyzed with RT-PCR. The results showed that the level of MMP1 increased in patients with MI and metabolites are released into the blood. This protein was detected in serum of patients with MI with MMP1 monoclonal antibody. MMP1 was not detected in control cases with any family background of MI and only little amount of MMP1 were detected in controls with mentioned background. RT-PCR results showed that the expression of MMP1 gene has increased. To our knowledge, the present study is the first of its kind to examine the presence of MMP1 protein and analyze its gene in Iranian race. The results of this study can help early detection of MI and finally treatment of it.

Keywords: myocardial infarction, matrix metalloproteinase, immunoblotting, MMP1, RT-PCR

O-10-855-1

A common polymorphism in the scavenger receptor class B type I gene and HDL-C metabolism in Tehran population

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The scavenger receptor class B type I (SR-BI), is a receptor for the high-density lipoproteins (HDL), facilitates cholesterol delivery to liver for excretion. Some reported polymorphism of this receptor affects lipid profile. The aim of this study is to investigate the relationship between G/A polymorphism on SRB1 and HDL metabolism in Tehran population. This study examined randomly 956 subjects (423 men and 533 women) referred to Tehran Lipid and Glucose Study (TLGS) project. Serum FBS, TG, total cholesterol, LDL-C, HDL-C and its subfractions levels, A1 and B apolipoproteins, BMI and blood pressure were measured. Common G/A polymorphism identified in samples using RFLP- PCR and association between this polymorphism and lipid profiles were also determined. The allele frequency of G and A variants in this population were 84.3% and 8.2 % respectively. The genotype frequencies of this polymorphism (G/A) are also 0.6%, 15.1% and 84.3% for AA, (GA, AG) and GG, respectively. The relationships between this polymorphism and age, sex, blood pressure, BMI, FBS, TG, total cholesterol, and HDL-C were not significant but there was only a significant association between this polymorphism and Apo A1, ApoB levels in all samples ($P<0.05$). The genotype and allele frequencies of G/A polymorphism of this receptor in Tehran population indicate that there is no major relationship between G/A polymorphism and total cholesterol, which is consistent to the Caucasian population.

Keywords: SRB1, HDL-C, Iran, ApoA1, Tehran, TLGS

P-10-673-1

Genetic study of beta-thalassemia in Sistan province

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Beta-thalassemia is the most prevalent autosomal recessive disorder in Iran. This disorder has a high frequency in northern and southern provinces. It is estimated that about 3% of Iranian population are carriers of a beta-thalassemia mutations. Some 30000 affected individuals live in the country. It is necessary to determine the frequency of mutations in every region of the country. For this purpose, we studied 20 Sistan unrelated family including parents and at least one of affected children with ARMS/PCR for 25 beta-thalassemia mutations. We have detected that the IVS I-5(G̲C) with 80% mutation was the most common. Fr8/9 (+G) (7.5%), IVS II-1(G̲A) (2.5%), IVSII-745(G̲C) (2.5%) were the other most common mutations. The pattern of beta-thalassemia in Sistan region is similar to other eastern provinces (Khorasan and Kerman). Also this frequency pattern of mutations follows the south Asian pattern.

Keywords: beta-thalassemia, Sistan

O-10-335-4

The study of GSTP1 gene promoter in colorectal cancer patients

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Transcriptional silencing of tumor suppressor genes and tumor related genes like GSTP1 by aberrant methylation of promoter region CpG island is believed to be an important mechanism of tumorigenesis. The GSTP1 gene encodes an enzyme glutathione S-transferase Pi (GSTPi) that defends cells against oxidative damage and electrophilic carcinogens. In this study, to investigate the role of epigenetic silencing of GSTP1 in colorectal cancer, we study the methylation of this gene in blood (n=37), tumoral tissue and adjacent normal tissue (n=29) from colorectal cancer patients, and blood of control subjects (n=42) by PCR after using methylation-sensitive restriction endonuclease ACCI. Methylation of GSTP was detected in 9.52%, 10.81%, 17.24% and 62.1% in blood of controls and blood, adjacent normal tissues and tumoral tissues of patients, respectively. Methylation in adjacent normal tissues was accompanied with methylation in the corresponding tumor samples. Statistically significant association of GSTP1 methylation in tumor tissues of patients was observed compare to adjacent normal tissues as a control (OR=7.86; 95%, CI=2.316-26.633), but there is no significant difference between methylation of GSTP1 in bloods of patient and control groups (OR = 1.121; 95% CI=0.26-4.84). Our findings suggest that GSTP1 promoter methylation can be detected in tumor tissues and it may serve as a new molecular diagnosis test to aid the colorectal cancer detection and treatment in future.

Keywords: tumor suppressor gene, aberrant methylation, CpG island, glutathione S-transferase Pi, colorectal cancer

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P-10-858-1

Association between adiponectin gene 276 G/T polymorphism and obesity in Tehran population

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Serum adiponectin levels have been positively associated with insulin sensitivity and obesity. Association between the 276G/T single nucleotide polymorphisms of adiponectin gene in Tehran population was studied. Blood samples from 327 subjects referred to the Tehran Lipid and Glucose Study (TLGS) project were obtained and genotype and allele frequencies of 276 G/T polymorphism of Adiponectin gene were examined using PCR-RFLP technique. Serum adiponectin levels were measured by ELISA method. Subjects were divided in 2 group of obese and nonobese on the basis of Body Mass Index (BMI). Results from the PCR-RFLP of this polymorphism demonstrated heterogeneity on the 276G/T polymorphism in obese and nonobese samples and an association was also observed between adiponectin levels and some of

samples. Our study is now continuing and we could conclude well when data will be prefect.

Keywords: adiponectin, BMI, obesity, polymorphism

P-10-841-5

Association between prostate adenocarcinoma and TGF-β1 gene polymorphism at codon 10

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Transforming growth factor-beta1 (TGF-β1) has regulatory role on prostatic cells and a T to C transition at codon 10 is altering its activity. Tissue samples of fifty patients (prostate adenocarcinoma) and 50 controls (benign prostatic hyperplasia) were collected. DNA was extracted using proteinase K digestion. The association between T (Leu) to C (Pro) polymorphism at codon10 of the TGFB1 gene with the increased the risk of CaP was studied by the amplification refractory mutation system (ARMS-PCR) method. The presence of 241 bp fragment containing the polymorphic site in codon10 of TGFB1 was determined by 2% agarose-gel electrophoresis of PCR products followed by ethidiumbromide staining. Genotype distribution between CaP patients and BPH controls was 30% and 40% for CC; 40% and 40% for CT; and 30% and 20% for TT. Results showed that there was a significant difference between CaP and controls in the CC genotype compared to TC and TT. So, codon10 polymorphism in TGFB1 has a significant influence on the development of prostate adenocarcinoma. This increase might contribute to longterm suppression of prostate epithelial proliferation and decrease in the risk of CaP.

Keywords: prostate adeocarcinoma, TGF-β1 gene, ARMS-PCR

P-10-495-2

Serotonin transporter gene polymorphism and myocardial infarction in the Iranian population

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The function of serotonin has been implicated in the pathophysiology of myocardial infarction (MI).Serotonin transporter (5-HTT) transports 5-HT into platelets. A polymorphic region has been identified in the promoter region of 5-HTT that differentially regulates 5-HTT expression and serotonin uptake. The aim of this study was to evaluate the association between a serotonin (5-HT) transporter gene polymorphism and myocardial infarction in south Iranian population. 5HTT polymorphism has been investigated in 106 patients with myocardial infarction and 90 healthy control subjects in order to determine the association between a polymorphism of the 5-HTT gene-linked polymorphic region and myocardial infarction. Using PCR techniques, the products of the 484-base pair (bp) fragments were denoted as being short alleles (s) and those of 528 bp as being long (l). The genotype distribution of the healthy controls was s/s (17.5%), s/l (39.5%), and l/l (43.0%) and that of the patients with MI was 6.5%, 51.0%, and 42.5%, respectively. The s/s genotype was present at a significantly lower frequency in MI patients compared with control group (P= 0.019; odds ratio=0.34, 95% CI 0.13-0.86).According to these data, s/s genotype would seem to have a protective role against MI in our population. This could be attributable to the lower 5HT concentration in platelets that are linked to s/s genotype.

Keywords: serotonin transporter, DNA polymorphism, myocardial infarction

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P-10-876-1

Role of chemokine receptors in late-onset Alzheimer's disease

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Chemokines participate in the regulation of immune and inflammatory responses by interacting with their receptors, which are primarily expressed on immune and inflammatory cells such as B- and T-lymphocytes and antigen-presenting cells. Chemokines and their receptors are therefore considered to mediate inflammation and tissue damage in autoimmune disorders. Chemokine receptor (CCR) genotypes were recently identified, and the importance of their genetic polymorphisms in some autoimmune and infectious disorders has been demonstrated. To define the roles of the polymorphism of the CCR2 gene at codon 64 (CCR2-64I) and the 32-bp deletion in the coding region of CCR5 (CCR5Δ32) in Iranian patients with Late-onset Alzheimer's disease (LOAD), we compared these genotypes in LOAD cases and healthy controls and investigated the clinical features associated with these genotypes. A total of 150 LOAD, and 150 healthy controls from northwest of Iran (Eastern Azerbaijan) were genotyped for the two polymorphisms and genotype frequencies statistically. The CCR2-64I and CCR5Δ32 polymorphisms were determined by the PCR-RFLP method. Further studies should focus on identification of proinflammatory genetic variants involved in AD pathogenesis.

Keywords: Alzheimer's disease, CCR2, CCR5

P-10-876-2

Tumour necrosis factor-a gene promoter variation in Alzheimer's disease

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Alterations in cytokine levels in patients with Alzheimer's disease (AD) have been documented. Recent findings suggest that production of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-a), is increased in the brains of people with AD. Tumor necrosis factor-a (TNF-a) is a pro-inflammatory cytokine that plays an important role in the inflammatory process that can be observed in AD brain. Different functional promoter polymorphisms within genes modulating inflammation have been demonstrated to elevate the AD risk. Among the best studied, the –238 and –308 polymorphisms have been associated with an increased transcriptional activity and TNF-a release, whereas the –863 polymorphism have been associated with a reduced

transcriptional activity. The distribution of –308 G/A variation was investigated in total of 150 AD, and 150 healthy controls from northwest of Iran (Eastern Azerbaijan). It is, however, apparent that further expression and genetic association work is necessary to try and clarify the mixed findings that have been reported until now.

Keywords: Alzheimer's disease, TNF-a, cytokines

P-10-876-3

CALHM1, a novel gene to blame in Alzheimer's disease

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Sporadic Alzheimer's disease (AD) is a common disabling disease of complex aetiology for which there are limited therapeutic options. We sought to investigate the role of the calcium homeostasis modulator 1 gene (CALHM1; MIM# 612234) in influencing risk of AD in Iranian population. CALHM1 encodes a multipass transmembrane glycoprotein that controls cytosolic Ca2+ concentrations and amyloid β levels. CALHM1 homomultimerizes, shares strong sequence similarities with the selectivity filter of the NMDA receptor, and generates a large Ca2+ conductance across the plasma membrane. This candidate gene is located under a linkage peak on chromosome 10, and the induction of its expression was shown to trigger a decrease in amyloid β levels and an increase in secreted amyloid precursor protein α levels via a Ca2+-dependent mechanism. A polymorphism in the CALHM1 has recently been associated with risk of late-onset Alzheimer disease. We examined this variant (rs2986017) in total of 150 AD, and 150 healthy controls from Northwest of Iran (Eastern Azerbaijan) and they were genotyped for this polymorphism and genotype frequencies were statistically analyzed.

Keywords: Alzheimer's disease, CALHM1, amyloid β

P-10-878-1

Visfatin genotype may modify the insulin resistance and lipid profile in type 2 diabetes patients

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We investigated the role of the -4689G/T promoter variant of the visfatin gene on serum visfatin concentration and biochemical markers in T2DM patient. In a cross-sectional study we recruited 93 patients with type 2 diabetes. Laboratory and anthropometric measurements were included FBS, OGTT, HbA1C, lipid Profile, fasting serum Visfatin and fasting serum insulin, Weight, Height, BMI and WHR. Genotyping for visfatin gene was performed by using the PCR- RFLP method. Our finding showed significant differences in levels of LDL Cholesterol, total cholesterol, HDL cholesterol and fasting serum Insulin among various types of visfatin genotype (TT, GG, and GT). This study showed a significant correlation between circulating levels of visfatin and weight, BMI, hs-CRP and fasting insulin in TT genotype. But regarding GG genotype, only fasting insulin had significant correlation with circulating visfatin. Visfatin genotypes may account for insulin resistance and levels of lipid profile that may cause by different visfatin expression between genotypes.

Keywords: visfatin genotype, type 2 diabetes, lipid profile

P-10-869-1

Fatty acids alter mRNA levels of ACAT1 and PPAR alpha in THP-1 macrophage derived foam cells

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One of the most important factors in the initiation and progression of atherosclerosis is the macrophage cholesterol homeostasis. Many genes and transcription factors such as Peroxisome proliferators activated receptors (PPARs) and Acyl Coenzyme A: Cholesterol Acyltransferase 1(ACAT 1) are involved in cholesterol homeostasis. One important group their of ligands are fatty acids .These compounds affect expression of these genes and so atherosclerosis.This study examined effects of linolenic acid (LA), and conjugated fatty acid(CLA), on the PPARalpha and ACAT 1 genes expression by using Real time PCR and in macrophages derived foam cells. Incubation of LA, and CLA increased mRNA levels of ACAT1 by 1.48 fold but CLA decreased its levels (by 0.84 folds). LA and CLA increased mRNA levels of PPAR alpha 1.16 and 1.42 folds in THP-1 foam cells. Further analysis of cholesterol metabolism showed that different fatty acids have different effects on gene expression in THP-1 derived foam cells.

Keywords: linolenic acid (LA), conjugated fatty acid (CLA), ACAT1, PPAR alpha, gene expression

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P-11-891-1

TNF-α-308G/A promoter variants as a risk factor in patients with gall stone

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Gallstone is a common biliary disorder with several risk factors. Immune responses and inflammatory cytokines are important in this disease and cytokines has been detected in bile fluid. TNF-α is an inflammatory cytokine and in this patients and could be used as a diagnostic factor. Polymorphism inTNF-α-308G/A promoter can affect cytokine production and probably as a risk factor of bile stone formation. For characterize the -308G/A polymorphism in 60 patients with gallstone diagnosis ARMS-PCR analysis was used. As a control group 62 normal subjects without any diagnosed disorder were matched for ethnicities. There were a significant difference in distribution of TNF-α-308G/A polymorphism between patient group and normal control group (P = 0.03). This study shows that TNF-α-308G/A variants could be risk factor for gallstone formation. TNF-α by affecting several factor such as mucin could facilitate gallstone formation. GA allele variant of TNF-α, as a high producer allele, is more frequent in gallstone patients. Probably TNF-α-308G/A polymorphisms by alerting

the TNF-α production and as fallow other factors affect gallstone formation.

Keywords: bile stone, TNF-α, polymorphism

P-10-893-1
Relationship between polymorphism of interleukin-1 receptor antagonist genes and susceptibility to Diabetes type I in Khorasan population

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To investigate the relationships between polymorphisms of interleukin-1 receptor antagonist genes and susceptibility to diabetes type I in Khorasan population. Genomic DNA was extracted from the Whole blood of 83 Diabetes patients who referred to Ghaem hospital in Mashhad and 120 Healthy controls were genotyped for IL-1 receptor antagonist genes using PCR-VNTR and association between this polymorphism and status of the disease was analyzed by Stata 6 software. The 10 kinds of polymorphism were found in this study. The frequencies of 1/1, 1/2, 2/2, 1/3, 4/4, 2/3 and 3/3 were 35%, 48.2%, 7.2%, 6%, 1.2%, 1.2% and 1.2% in patients and 1/1, 1/2, 2/2, 1/5, 2/5, 1/4, 2/3, 4/4 were 48.3%, 40%, 3.3%, 2.5% 0.8% 3.3%, 0.8% and 0.8% in healthy control. IL-1 RN allele 2 was significant between patients and healthy control (P=0.007). Our results suggested that the carriage of allele 2 of IL-1RN may play a significant role in the development of illness.

Keywords: interleukin-1 receptor antagonist gene, polymorphism, Diabetes

P-10-895-2
Distribution of XmnI and Q232Q alleles at the phenylalanine hydroxylase gene in Iranian patients

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Phenylketonuria (PKU) is an autosomal recessive inherited disorder arising from the deficiency of phenylalanine hydroxylase (PAH) which catalyzes the essential conversion of phenylalanine to tyrosine. In the majority of cases, PKU is caused by mutations in the PAH gene, and it presents with different phenotypes which are classified according to phe tolerance. More than 520 different mutations have been reported. To determine XmnI and Q232Q alleles frequency in Iranian patients, genomic DNA was extracted and examined by standard processor from 150 patients with classical PKU. Q232Q and XmnI polymorphisms were analyzed by PCR-RFLP technique with DdeI and XmnI restriction enzymes respectively. The level of observed heterozygosity in Iranian patients (XmnI (0.13), Q232Q (0.16)) are significantly different from European populations (0.47 and 0.54). Frequencies of normal alleles in Iranian population (XmnI(0.16), Q232Q(0.21)) are significantly different from European population XmnI (0.618), Q232Q (0.407) too. The XmnI and Q232Q alleles in our population had not significant difference in distribution among normal and mutant chromosomes. Then, these polymorphisms do not provide a tool for molecular diagnosis of these diseases and carrier status.

Keywords: phenylalanine hydroxylase, PCR-RFLP, PAH

P-10-891-2
TNF-α-308G/A gene polymorphism and laboratory markers in Acute Appendicitis

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Appendicitis is a common inflammatory disorder that etiology may be multifactorial in some cases. Infection is important in appendicitis. TNF-α is an inflammatory cytokine and in appendicitis could be used as an inflammatory marker or it could affect immune responses to infection. Polymorphism in TNF-α-308G/A promoter can affect cytokine production. For characterization of the -308G/A polymorphism in healthy controls and appendectomized patients that underwent appendicitis diagnosis ARMS-PCR analysis was used. CRP, ESR, WBC count and percent of neutrophils tests were done by automated methods. According to histology evaluation, patients were classified to three appendicitis subgroups. There were not any significant difference in distribution of TNF-α-308G/A polymorphism in normal control group and patient group and subgroups ($p \geq 0.05$). Laboratory markers in patient group and 3 sub groups were significantly higher than control group ($p \leq 0.05$). CRP, WBC count and neutrophilia percent of pus positive appendicitis subjects were higher than pus negative sub groups ($p \leq 0.05$). This study shows that TNF-α-308G/A variants could not be used as a predictive marker or risk factor of appendicitis infection. It is may be that CRP, WBC count and neutrophilia percent increase in pus positive appendicitis subjects as a result of infection.

Keywords: Appendicitis, TNF-α, polymorphism, WBC, CRP

O-10-899-1
Analysis of vitamin D receptor polymorphism and type I diabetes in the Persian population

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Several polymorphisms in the vitamin D receptor (VDR) gene have been reported to be associated with the risk of developing type 1 diabetes, but findings have been conflicting. The aim of this study was to test for association between common VDR polymorphisms and the genetic susceptibility to type I diabetes in the Persian population. We genotyped 87 patients with type 1 diabetes and 100 controls for the FokI and BsmI single nucleotide polymorphisms by polymerase chain reaction and restriction fragment length polymorphism analysis. The distribution of VDR genotype, allele, and haplotype frequencies did not differ significantly between patients and controls in FokI polymorphism and BsmI polymorphism and ApaI polymorphism. These data suggest that the single nucleotide polymorphisms of the VDR gene are unlikely to contribute significantly to type1 diabetes susceptibility in the Persian population.

Keywords: type I diabetes mellitus, VDR gene, polymorphism

P-10-900-1

The role of epigenetics in breast cancer

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Methylation pattern in promoter region of many genes are proved to be modified in cancers. 5′ UTR of estrogen receptor alpha and beta has been shown to be differentially methylated in patients with breast cancer and are believed to be markers in early diagnosis, prognosis and treatment. Our objective was to explore the role of epigenetic modification in ERα and ERβ 5′ flanking region in Iranian patients with breast cancer. Primers were selected to be specific for two CpG islands in 5′UTR of ERα either for methylated or unmethylated status, and a pair of primers located in the CpG Island within the promoter and its downstream untranslated exon 0N region of the ERβ. Methylation Specific PCR on sodium bisulfate treated DNAs of peripheral blood and tissue samples from 35 patients were performed. ERα: We found that in 53.85% of primary breast cancers CpG Island 1 is methylated and in 80.77% CpG Island 2 is methylated. The methylation status of these CpG islands in blood was 100% and 87.88%, respectively. ERβ: We found that the CpG Island chosen was methylated in 42.30% of primary breast cancers. All the blood samples were unmethylated. Though small in number of samples, about 82% of the methylated tissues had an invasive ductal carcinoma histopathological diagnosis. We found a trend to methylation in Iranian breast cancer patients. Our results are in concordance to the studies which showed a role of ERα and ERβ methylation in pathogenesis of breast cancer.

Keywords: breast cancer, epigenetics, estrogen receptors, methylation specific PCR

P-10-907-1

Analysis of TAP2 polymorphisms in Persian individuals with type I diabetes

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Type I diabetes mellitus is an immune-mediated disease that is known to be associated and linked with genes in the human leukocyte antigen (HLA) region on chromosome 6. Functionally, HLA class I antigen presentation may be deranged in type I diabetes. The TAP2 transporter, which mediate the translocation of antigenic peptides into the endoplasmic reticulum and whose genes are located in the HLA class II region, are potential candidates for conferrring predisposition to type I diabetes. Polymorphisms known coding region variants (codons 565 and 665) of TAP2, were typed in a cohort of 87 well-characterized Persian individuals with type I diabetes and 100 control subjects. The distribution of TAP2 genotype, allele, and haplotype frequencies did not differ significantly between patients and controls.
Keywords: type I diabetes mellitus, TAP2 gene, polymorphisms, HLA class II

P-10-785-1

Molecular analysis of a novel mutation in an Iranian hemophilia B patient

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Hemophilia B, Christmas disease, is an X-linked bleeding disorder caused by the functional deficiency of blood coagulation factor IX. The disease is due to heterogeneous mutations in the factor IX gene (F9), located at Xq27.1. It spans about 34 kb of genomic DNA and contains 8 exons. The aim of this study was molecular analysis and genotype-phenotype correlation of hemophilia B patients in south khorasan province. After obtaining informed consent, genomic DNA was extracted from the peripheral blood of patients and their family members referred from birjand hemophilia center, by standard methods. PCR amplification, SSCP techniques were performed for scanning of the all functional-important regions of the F9 gene. DNA sequencing was performed for those with different migration patterns in SSCP by chain termination method. In addition, haplotype were constructed using four the DdeI, TaqI, HhaI and MnlI restriction fragment length polymorphisms (RFLPs) markers. For one of the patient affected with severe form of the disease, a novel mutation was found, which had not been reported in the database previously. The mutation was TG 31306-7 AT in exon 8 (codon 395, 396) and causes a pre mature stop codon at codon 395. The patient's mother was heterozygous for this mutation. This mutation occurs in serine protease domain of the mature peptide and it is expected to disrupt active site of the enzyme. The information obtained from this study could be used to diagnose potential female carriers in families with hemophilia B patients and prenatal diagnosis.

Keywords: hemophilia B, SSCP, sequencing

P-10-549-1

Study of P53 mutations in gastric cancer by PCR-SSCP in Chaharmahal Va Bakhtiari province

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Gastric cancer is the most common cause of cancer death world wide after lung cancer. Genetic factors including oncogenes and tumor suppressor genes are always involved in progression of this cancer. The P53 tumor suppressor gene is believed to have a broad role in the cell such as programmed cell death and stop cell replicating damaged

DNA which has been summarized on the guardian of the genome. This study describes the mutation analysis of paraffin embedded gastric samples from 38 patients in Chaharmahal va Bakhtiari province. We have investigated the frequency of P53 gene mutation in promoter and coding region using PCR-SSCP procedure and subsequent PCR-RFLP to detected alteration in two common hot spots in codon 248 and 282. No genetic variant was detected in samples examined by PCR-SSCP procedure. We determined also no mutation in P53 gene hot spots in codon 248 and 282. We conclude that association of P53 gene mutations with gastric cancer is very low in Chaharmahal Va Bakhtiari province. However we have examined only 38 gastric samples and more samples need to be investigated to reveal the contribution of P53 gene mutation in causing gastric cancer in this province.

Keywords: P53 gene, PCR- SSCP, Gastric cancer

O-10-756-1

Amorphous aggregation and fibrillation are both accelerated by neutralization of lysine residues in HEWL

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Proteins may undergo misfolding and assemble into different aggregated forms, including off-pathway oligomers, ordered fibrils and amorphous structures. Ordered fibrils called amyloids are pathological hallmarks of several late-onset neurodegenerative disorders including Alzheimer's and Parkinson's diseases. We studied the influence of charge neutralization of lysine residues by chemical modification on two distinct aggregation processes, namely fibrillation and amorphous aggregate formation. HEWL may be converted to fibrils at low pH (pH 2.5) but at higher pH values (6-7), it predominately displays amorphous structures (as verified by various methods such as TEM, ThT fluorescence, Congo red, far-CD and turbidity). Both types of aggregation were found to possess a rate limiting nucleation step. It was observed that neutralization of lysine residues may result in the absence of the nucleation phase related to formation of both forms of the aggregates. It appears that positive charge of lysine residues may play critical roles in diminishing the time taken for the occurrence of the first steps of the aggregation process. Using a different chemical modifier, positive charge of lysine residues was changed to negative, which resulted in an increase in the nucleation time, thereby reducing the extent of amorphous aggregation and fibrillation of the protein. Based on these observations, a determining role for charge-charge interactions is suggested. Studies involving activity measurements, Tm, secondary and tertiary structural determinations have indicated no considerable difference between the modified and native forms of HEWL. It appears therefore that these structural modifications may influence colloidal parameters rather than affecting protein stabilization. These data may be found useful for exploring the mechanisms of auto-aggregation of proteins and the design of some homo peptides having inhibitory effects on protein aggregation.

Keywords: chemical modification, amyloid fibril, amorphous aggregation, Hen egg white lysozyme, neurodegenerative disorders, protein misfolding

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P-10-961-1

Spectroscopic investigation of TFE-induced α-chymotrypsin and α-crystallin fibrillogenesis: Surface hydrophobicity triggers early stages of protein aggregation

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The misfolding of specific proteins is often associated with their assembly into fibrillar aggregates, commonly termed amyloid fibrils. Despite the many efforts expended to characterize amyloid formation in vitro, there is no deep knowledge about the environment (in which aggregation occurs) as well as mechanism of this type of protein aggregation. Alpha-chymotrypsin as well as α-crystallin was recently driven toward amyloid aggregation by the addition of intermediate concentrations of trifluoroethanol. In the present study, successful approaches such as turbidimetric, thermodynamic, intrinsic fluorescence and quenching studies (in addition to chemical modification) have been used to elucidate the underlying role of hydrophobic interactions (involved in early stages of amyloid formation) in α-chymotrypsin-based experimental system. We will discuss the importance of our observations.

Keywords: fluorescence, α-chymotrypsin, α-crystallin, aggregation, TFE

P-10-987-1

Analysis of FLT3 mutations in infant acute leukemia

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91 infant with Acute Myeloid Leukemia (AML) and Acute Lymphoid Leukemia (ALL) were investigate for FLT3-gene mutations in acute leukemia (ALL, AML) and ITD mutation (Internal Tandem Duplication) that codes juxamembrane region in FLT3 receptor and also the point mutation that is coded by exon 17 in that is FLT3 receptor kinase region. We also studied; these mutations cause leukemic cells to proliferate uncontrollabely and leads to a poor prognosis. The aim of this study was to explore appropriate diagnostic molecular tests and to screen mutations that occur in patients with acute leukemia. ITD mutation in FLT3 receptor was analyzed by PCR (Polymerase Chain Reaction) in 11,12 exons and 11 intron, using designed primers. After analyzing bands of the band resulted from PCR in the 8%-acrilamid gel, the prescence of mutation of this gene was studied. For analysis of point mutation of Exon 17 in FLT3 receptor gene, the genomic DNA of patient was amplified using the PCR. Resulted PCR products were studied by ECOR V enzyme and Restriction Length Polymorphism (RFLP) in cases of positive ITD, the Sequencing Method was applied. ITD mutation was observed in 7 cases of 91 studied acute leukemia patients. The investigation the sequence of PCR products in the mutation samples showed that different insertions of 's' are seen in JM region. Also, 2 of 91 patients, studied had point mutation in which their (D835) distributions were not identical in FAB subtypes. Studying history of these patients, it was clear that there was not a significant relation between chromosome variations and induction of mutation and decision can be made about the treatment by molecular diagnosis of

this mutation independent of FAB classification and before the treatment get started.

Keywords: acute leukemia, FLT3 gene, ITD mutation, D835 Point mutation

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O-10-901-1

Resistin gene variation and atherogenesis: Is it through insulin resistance?

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Resistin is an adipokine that has been linked to insuline resistance, inflammation, and coronary heart disease. The aim of this study was to investigate association between -420C/G polymorphism of the resistin gene and severity of coronary involvement. This study consisted of 39 subjects, of whom 23 were male and 16 were female, who underwent coronary artery bypass. For all patients angiography was performed. Blood sample was obtained from these patients and after DNA extraction, the SNP analysis was performed by PCR-RFLP method using BbsI restriction enzyme.Among the individuals studied for SNP -420, 41% were diabetic and 51% were non-diabetic. The frequency of GG, CC and GC genotype was 12.82 %(95% CI; 4.3% to 27.43%), 51.28% (95% CI; 34.78% to 67.58%) and 35.9% (95% CI; 21.2% to 52.82%), respectively. The frequency of G and C alleles was 30.8% and 69.2%, respectively. Among the non-diabetics, 26.3% of patients had one coronary stenosis, 31.6% and 41.2% of cases had two and three coronary stenosis, respectively. All non-diabetic patients with GG genotype (100%) had two or three coronary stenosis but 25% ones with CC genotype and 37.5% ones with CG genotype had one involved coronary artery. A significant difference was found in severity of coronary involvement among different genotypes in non–diabetic patients (p=0.04), but there was no significant association in diabetic patients. Our findings suggest that the role of resistin in atherogenesis is apart from insulin resistance and diabetogenesis. Its contribution to other factors involved in atherosclerosis should be investigated.

Keywords: atherogenesis, atherosclerosis, inflammation, insulin resistance, resistin

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P-10-1003-1

Study of induction of apoptosis in lung of SM induced-chronic bronchitis patients

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Sulfur mustard (SM) is a bifunctional alkylating agent that was used extensively against both Iranian military and civilian population by Iraqi forces in Iran-Iraq war. It causes acute and chronic effects on different organs following exposure. Late pulmonary complications of SM include airway hyperreactivity, chronic bronchitis and bronchiolitis obliterans. Apoptosis is a genetically regulated physiological process of cell suicide that is central to the development and homeostasis. The aim of the present work was to assess the cell death process induced in brochoalveolar lavage fluid (BAL) of SM induced-chronic bronchitis patients. For this propose, BAL samples were taken from 9 normal veterans as control group and 18 patients in the case group exposed to SM. Fas and Fas L levels and Caspase 3 activity were determined by spectrophotometric assays. The results show that Fas and Fas L levels were significantly increased in chemical casualties, comparing with the control. There was no significant change observed BAL Caspease 3 activity. These results provide possibilities of existence of caspase independent apoptotic pathway in lung of SM induced-chronic bronchitis patients. However, for further investigation this study should be repeated by other methods.

Keywords: sulfur mustard, apoptosis, chemical casualities

P-10-666-4

Analysis of vitamin D receptor gene polymorphisms in type 1 Diabetes Mellitus in two populations of Iran: Mashhad and East Azarbaijan

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The overall annual incidence of type 1 diabetes mellitus (T1DM) has risen from approximately 16 cases per 100,000 population in the 1990s to 24.3 per 100,000 population currently and is probably still increasing. Vitamin D is a known immune system modulator that acts via the vitamin D receptor (VDR). The VDR gene is a candidate gene for susceptibility to autoimmune disorders. T1DM is an autoimmune disease characterized by destruction of insulin-producing pancreatic B cells. VDR gene polymorphisms may be related to T1DM. Different results have been reported in different ethnic backgrounds. The aim of this study was to test for association between common VDR polymorphisms and the genetic susceptibility to T1DM in the Mashhad and East Azarbaijan. Sixty nine T1DM patients and forty five unrelated healthy subjects from Mashhad and forty nine T1DM patients and fifty controls from East Azarbaijan were included and interviewed to obtain the clinical data. The DNA extracted from peripheral blood and assayed for the Bsm I, Fok I, Apa I and Taq I polymorphisms of VDR by PCR and restriction fragment length polymorphism analysis. Aa, FF, Bb genotypes were significantly higher in patients group of Mashhad and AA genotype of Apa I was significantly higher in patients of East Azarbayejan compared with control groups (p=0.003, 0.008, 0.014, 0.013 respectively). T1DM age of onset was higher in FF genotype of Fok I polymorphism in patients of East Azarbaijan (p=0.044). Nephropathy was more frequently seen in BB genotype of Bsm I in patients of East Azarbaijan and ketoacidosis was more frequently seen in Ff genotype of Fok I in patients of Mashhad (p=0.030, 0.04 respectively). VDR polymorphisms are associated with susceptibility to

type I Diabetes, higher age of onset, nephropathy and ketoacidosis in East Azarbaijan and Mashhad of Iran.

Keywords: type 1 diabetes mellitus (T1DM), vitamin D, polymorphism

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P-10-274-1

Does the transcription factor 7 like-2 gene (TCF7L2) associate with type2 diabetes in Iranian population?

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One of the most challenging health problems of the 21 century is diabetes. Diabetes type2 (T2D) is a multi-factorial disease in which environmental triggers interact with genetic variants in predisposition to the disease. A variant of TCF7L2 gene (rs 7903146), which is a transcription factor implicated in blood glucose homeostasis, has been associated with T2D in most nations. However a comprehensive survey of this variant has not been done for an Iranian population, therefore a case–control study was performed to assess the relationship between this gene variant and T2D. Diabetic and non-diabetic populations were selected according to WHO guidelines. Total genomic DNA was extracted from blood samples and its integrity was checked by gel electrophoresis. A pair of primers was designed to amplify a fragment in which there is a specific restriction site for RSA I enzyme. Using different conditions, the amplification of this region was optimized. Screening of normal and diabetic samples using PCR-RFLP was investigated to determine the frequency of different alleles for finding any potential association between the alleles and T2D. Insight gained from the study of TCF7L2 in type 2 diabetes will probably lead to the better management and treatment of this complex disease. The use of this genetic marker, which is powerful in prediction of improving diabetes risk in Iranian population demands further clinical investigation.

Keywords: glucose homeostasis, TCF7L2, type2 diabetes

P-11-1048-1

Association of VDR gene polymorphism with insulin resistance in diabetic patients

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It has been reported that vitamin D deficiency may predispose to glucose intolerance and type 2 diabetes mellitus. Since FokI variant of Vitamin D Receptor (VDR) associates with alteration of receptor function which affects the bioactivity of vitamin D, we analyzed the association of FokI variant of VDR with various glucose metabolism indices among patients with diabetes mellitus type 2 (DMT2). In a case series study, we recruited patients with DMT2 according ADA criteria. Age of diagnosis, BMI, FBS, lipid profile, insulin, HbA1C were measured. HOMA was calculated as index of insulin resistance. VDR genotyping was performed using PCR-RFLP method. Of totally 105 diabetic patients (mean age 55.03±10.43 years), 79.4% were female. Frequency of FF, ff and Ff genotypes were 71.6, 5.3 and 23.2%, respectively. In patients with Ff and ff genotype, the age of onset was lower, BMI>27 and poor-controled condition were more frequent compared with FF genotype. In patients with BMI<27, age of onset was significantly lower (42.51±7.61 vs. 52.12±12.83 years, p=0.02), HbA1C was higher (6.25±2.85% vs. 4.91±3.16%, p=0.04) and poor-control condition was more prevalent (80% vs. 42.1%) in Ff and ff patients compared to others. Also in this group HOMA was higher. Our findings show that VDR polymorphism is associated with age of diagnosis and insulin resistance in patients with diabetes mellitus type 2.

Keywords: vitamin D receptor, diabetes mellitus type 2, polymorphism, insulin resistance

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P-10-169-1

Functional C3435T polymorphism of MDR1 gene: An impact on genetic susceptibility and clinical outcome of colorectal cancer

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The human multidrug resistance gene 1 (MDR1) encodes a plasma membrane P-glycoprotein (P-gp) that functions as the transmembrane efflux pump for various structurally unrelated anticancer agents and toxins. Polymorphisms in the MDR1 gene may have an impact on the expression and function of Pgp, thereby influencing the susceptibility to various diseases, including cancer. Recently, a silent C3435T polymorphism in exon 26 of MDR1 has been reported to be associated with decreased expression of Pgp in TT genotype carriers and thus it may alter the physiological protective role of Pgp and influence disease risk. In this case–control-designed study, 118 unrelated colorectal cancer patients and 127 sex-and–age-matched healthy controls were enrolled. They were visited at two centers during a one year period (2006-2007). DNA of the whole blood sample was extracted and the polymorphic fragment was amplified by polymerase chain reaction using specific primers. The C3435T polymorphism was detected by the restriction fragment length polymorphism method. Significantly increased frequencies of the 3435T allele and the 3435TT were observed in patients with colorectal cancer compared with controls (3435T: P=0.03; OR, 1.43; 95% CI, 1.02-2.8; 3435TT: P=0.003; OR, 2.2; 95% CI, 1.3-3.74). In contrast, frequency of genotype TT was significantly higher in controls compared to colorectal cancer (TC: P=0.006; OR, 0.49; 95% CI, 0.30-0.82). This study suggests that C3435T MDR1 polymophism has an association with colorectal cancer. The results support the notion that P-gp plays a major role in the defense against intestinal bacteria or toxin.

Keywords: C3435T, P-gp, colorectal cancer

P-10-1077-1

Endothelial nitric oxide synthase haplotypes and circulating nitric oxide levels significantly associate with risk of essential hypertension

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Nitric Oxide (NO) a potent vasodilator plays a pivotal role in blood pressure regulation. Endothelial NO synthase gene (NOS3) polymorphisms influence NO levels. Here, we investigated the role of

the ˜922A/G, ˜786T/C, 4b/4a and 894G/T polymorphisms of the NOS3 and NOx levels in 800 consecutive unrelated subjects comprising 455 patients of essential hypertension and 345 controls. The polymorphisms were investigated independently and as haplotypes. Plasma NOx levels (nitrate and nitrite) were estimated by Griess method. Genotype frequencies for the –786TC, 4b/4a, 894G/T polymorphisms differed significantly (P<0.001) between patients and controls and associated with an increased risk of hypertension (OR=2.0, OR=3.8, OR=1.6, respectively). The 4-locus haplotypes ATaG (H1), ATaT (H2), GCaG (H3) were significantly associated with essential hypertension and served as susceptible haplotypes (P<0.0001). On the other hand, haplotypes ATbG (H4) and GTbG (H5) were negatively associated with hypertension and served as protective haplotypes (P<0.0001). NOx levels were significantly lower in patients than controls (P<0.0001). The individual polymorphisms showed marginal association with NOx level, however, the susceptible haplotype H2 associated significantly with lower NOx levels in patients (P<0.001) and conversely the haplotype H4 with higher NOx levels in controls (P<0.001). In conclusion, the 4b/4a and likely -786T/C polymorphisms were identified as the determinants modifying the risk of hypertension. This study identifies the NOS3 variants and haplotypes as genetic risk factors and as useful markers of increased susceptibility to the risk of essential hypertension.

Keywords: hypertension, NOS3, polymorphisms, haplotypes, plasma NOx

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P-10-15-2

Alpha-Tocopherol and oxidative stress in coronary artery disease among Iranians population

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Oxidative modification of low density lipoprotein (LDL) is implicated as an important early event in atherosclerosis. Observational and experimental studies indicate that α-TOH inhibit atherogenesis through inhibition of oxidative modification of LDL. This study was designed to assess the degree of association between α-TOH and CAD in a sample of the Iranian population. The study was performed as a case-control study with 61 patients with angiographically proven CAD and 58 apparently age-and smoking status-matched controls. Individuals with CAD were divided into three groups: those with 70% stenosis in one vessel, two vessels, or three vessels. Plasma concentration of α-TOH, glutathione (GSH) and malondialdehyde (MDA) was measured by HPLC. Plasma α - tocopherol and glutathione were significantly decreased in CAD patients compared with controls (24.82µmol/L vs. 27.84µmol/L and 2.55µmol/L vs. 4.84µnmol/L, respectively). MDA concentration was significantly higher in patients (1.18nmol/ml) compared with controls (0.51nmol/ml). Significant correlation was identified between MDA levels and severity of CAD (P<0.001). An inverse correlation was found between α -TOH and MDA (r=-0.14) and GSH and MDA (r=-0.262). normolipidemic patients of CAD group had higher TC and MDA and lower vitamin E and GSH (all P< 0.05) Additionally, the ratio of LDL-C/HDL/C and TC/HDL were higher and that of vitamin E/TC and vitamin E/total lipids were lower in CAD patients group than in control subjects These data are compatible with the hypothesis that an increased intake of antioxidant, primarily as α - tocopherol, is associated with a reduced risk for coronary disease.

Keywords: oxidative stress, tocopherol, coronary artery disease

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O-10-484-1

Association between severity of angiographic coronary artery disease and paraoxanase-1 promoter gene polymorphism T(-107)C in Iranian population

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The oxidation of low-density lipoproteins and cell membrane lipids is believed to play an integral role in the development of fatty streak lesions, an initial step in coronary artery disease (CAD). Paraoxonase-l (PONl) is an enzyme associated with the high-density lipoprotein (HDL) particle. PONI protects LDL from oxidative modification by hydrolyzing lipid peroxides, suggestive of a role for PONI in the development of CAD. The present study tested the hypothesis that PONI promoter polymorphism T(-107)C could be a risk factor for severity of CAD in Iranian population. PONI promoter genotypes were determined in 300 consecutive subjects (>40 years old) who underwent coronary angiography (150 subjects with>50% stenosis served as cases [CADj+ and 150 subjects with <20% stenosis served as controls [CAD-). PONI promoter genotypes were determined by PCR and BSTUI restriction enzyme digestion. CAD+ subjects did not show any significant differences in the distribution of PONI promoter genotypes as compared to CAD- subjects (P=0.075). However the analysis of PONI promoter genotypes distribution showed a higher percentage of (-107) TT among CAD+ compared with CAD- (P=0.027). After controlling for other risk factors, the T(-107)C polymorphism had interaction with age (P=0.012), but did not show any interaction with other risk factors such as body mass index, gender, smoking, diabetes, level of HDL-C, LDL-C, triglyceride and total cholesterol. These data suggest that the TT genotype may represent a genetic risk factor for coronary artery disease in Iranian population.

Keywords: promoter gene polymorphism, paraoxonase-l, coronary artery disease

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P-10-484-4

Association of Cys 311 Ser polymorphism of paraoxonase-2 gene with the risk of coronary artery disease

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Recently another member of the paraoxonase gene family designated paraoxonase-2 has been identified. Paraoxonase-2 has antioxidant properties similar to paraoxonase-1 and paraoxonase-3. However, in contrast to paraoxonase-1 and paraoxonase-3, paraoxonase-2 is not associated with high-density lipoprotein and may only exert its antioxidant function at the cellular level. We assessed the frequency

and genotype distribution of cys 311 ser paraoxonase-2 polymorphism in 300 subjects (>40 years old) with angiographic documentation of coronary artery disease (150 patients with >50% stenosis served as cases and 150 individuals with <20% stenosis served as controls) to determine the possible association between this mutation and susceptibility for coronary artery disease. The paraoxonase-2 genotypes were determined by polymerase chain reaction and DdeI restriction enzyme digestion. The cases (coronary artery disease positive patients) showed significant differences in the distribution of cys 311 ser paraoxonase-2 genotypes as compared with the controls (coronary artery disease negative subjects, P=0.015). The analysis of paraoxonase-2 genotypes distribution showed higher percentage of CC genotype among coronary artery disease positive compared with coronary artery disease negative (P=0.008). After controlling for other risk factors, the cys 311 ser polymorphism had not correlation with age, body mass index, gender, smoking, and diabetes, level of high-density lipoprotein, low-density lipoprotein, triglyceride, and total cholesterol. Our data indicate a major effect of the paraoxonase-2 polymorphism on coronary artery disease risk in patients referred to Shariati Hospital in Tehran.

Keywords: coronary artery disease. Paraoxonase-2, polymorphism