

Clinical Biochemistry and Biochemical Markers of Disease

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Lipid metabolism in patients with nonalcoholic fatty liver disease

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Nonalcoholic Fatty Liver Disease is fatty inflammation of the liver. It's related to insulin resistance and metabolic syndrome. Nonalcoholic Steatohepatitis (NASH) is most extreme form of NAFLD which is regarded as cirrhosis of the liver. It has been suggested that the imbalance between products of lipids peroxidation and antioxidants cause dysregulation of normal metabolism and physiology. The aim of the present study was to examine the significant parameters of oxidative stress and antioxidants in patients with nonalcoholic fatty liver disease (NAFLD). Forty male patients with histologically proven NAFLD were included. All patients underwent a percutaneous liver biopsy under ultrasonic guidance. The liver specimens were embedded in paraffin and stained with hematoxylin and eosin, Masson-trichrome and reticulin silver stain. Diagnosis was based on histopathological results, steatosis, fibrosis and cirrhosis. Thirty body mass index (BMI) matched and age matched healthy male subjects were enrolled in this study. None of the healthy control had any known disease and none was taking any medications. Routine biochemical findings of the healthy control subjects were also within the normal range. Total plasma cholesterol, triglyceride (TG) high density lipoprotein (HDL) low density lipoprotein (LDL) was measured by enzymatic colorimetric method. Serum insulin, C-peptide was measured by ELISA methods. Plasma malonaldehyde (MDA) levels were measured by spectrophotometric method. Coenzyme Q10 levels were measured by high performance liquid chromatographic (HPLC) method. Copper-Zinc superoxidase Dismutase activity was measured by the SOD-525 method in spectrophotometer. Catalase activity was determined by measuring residual hydrogen peroxidase after incubation with the enzyme. The means levels of age, body fat, total cholesterol, LDL and glucose did not significantly differ among the patients and healthy groups. Patients with NAFLD had lower HDL level, coenzyme Q10, SOD and catalase activity than the healthy group. On the contrary, Patients with NAFLD showed higher TG, MDA, insulin, C-peptide than the normal group. These results may be concluded that disturbances in BMI, body fat and lipid metabolism contribute to altered oxidation status in NAFLD and insulin resistance may be related to decreased antioxidants in NAFLD as well as products of lipid peroxidation

Keywords: lipid metabolism, fatty liver, coenzyme Q10, SOD, malonaldehyde

P-10-110-1

Fatty acid composition of HDL and coronary artery disease

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The fatty acid content of high density lipoprotein (HDL) partially reflects that of the diet and has been reported to be associated with several important biological functions, which may serve as risk marker for coronary heart disease. The aim of this study was to investigate whether fatty acid composition of HDL phospholipids correlates with angiographically documented coronary artery disease (CAD). The population included 212 patients, undergoing clinically indicated coronary angiography. All patients underwent coronary angiography; normal arteries were defined as stenosis <40% and abnormal as stenosis >50% or occlusions. The severity of CAD was expressed by the number of affected vessels. The fatty acid composition of HDL phospholipids was determined by gas liquid chromatography (GLC). Multivariate analyses were used to test the independence of associations between the presence and severity of CAD as outcome variables and fatty acid composition of HDL phospholipids. Patients with CAD showed significantly lower levels of linoleic acid (P=0.041), eicosapentaenoic acid (EPA) (P=0.027) and docosahexaenoic acid (DHA) (P=0.026) than patients without CAD. In the multivariate analyses that included those variables found to be statistically significant in the univariate analyses, the association of linoleic acid (OR=0.89, P<0.05), EPA (OR=0.41, P<0.05) and DHA (OR=0.48, P<0.05) with the presence and of EPA ($\beta=0.23$, P<0.01) and DHA ($\beta=0.17$, P<0.05) with the severity of CAD remained inversely significant. This study shows that polyunsaturated fatty acids including EPA and DHA content of HDL particles are independently associated with the presence and severity of angiographically documented CAD.

Keywords: fatty acids, HDL particle, CAD

P-10-118-1

Study of antioxidant enzyme levels in mild and severe preclampsia and normal pregnancies

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Preeclampsia (PE) is a disease characterized by hypertension and proteinuria in the third trimester of pregnancy. Preeclampsia is a major

cause of maternal mortality, and fetal death, especially in developing countries, but its aetiology remains unclear. Oxidative stress has been increasingly postulated as a major contributor to endothelial dysfunction in preeclampsia. Thus, this study was performed to analyze the susceptibility of erythrocytes and to evaluate antioxidant enzymes activity such as glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) in blood samples of women with preeclampsia and normal pregnancy. In this cross-sectional-comparative study, circulating level of GPX, SOD, CAT and malondialdehyde (MDA) were analyzed in 30 healthy pregnant women, 25 women with mild PE and 26 with severe PE hospitalized in Rasoule Akram and Akbarabadi hospitals of Iran University of Medical Sciences. Our results showed that the level of MDA, GPX, SOD and CAT in severe and mild PE pregnant women were significantly higher ($p < 0.05$) than healthy pregnant women. Increased level of malondialdehyde and antioxidant enzymes activity in pregnant women with mild and severe PE probably shows that oxidative stress markers play a significant role in pathophysiology of PE. Therefore, determination of antioxidant enzyme activity in these patients and using supplemental dietary with antioxidants such as vitamins C and E, may play a role in prevention of pre-eclampsia in women at high-risk for this condition.

Keywords: preeclampsia, oxidative stress, catalase, glutathione peroxidase, superoxide dismutase

P-10-103-1

Relationship between serum hyaluronic acid level and the stages of liver fibrosis in patients with Chronic Hepatitis

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Liver biopsy is the gold standard for assessing liver disease stage but it is prone to some serious complications. Noninvasive methods have been proposed as surrogate markers for liver fibrosis. It was shown that serum hyaluronic acid (HA) level increases with the development for liver fibrosis. The aim of this work was to determine serum HA level cutoff point for predicting liver fibrosis and highlighting its diagnostic value for differentiation of various fibrosis stages and inflammation grades of liver disease in chronic hepatitis patients in an Iranian population. Serum HA levels in chronic hepatitis (hepatitis B, C and autoimmune hepatitis) patients ($n=62$) and controls ($n=20$) were compared by ELISA and stages of fibrosis were assessed according to the modified Knodell score system. Mean HA concentration in patients (113.4 ± 59.2 ng/ml) was greater than the control group (46 ± 10.5 ng/ml, p -value < 0.001). Differences among various stages of hepatic fibrosis (stage 1-6) and HA serum levels were statistically significant in almost all fibrosis stages as compared to healthy controls (p -value < 0.05). As degree of liver fibrosis stage and inflammation grade increased, there was a gradual rise in serum HA level. Cutoff point of serum HA concentration (59.5 ng/ml) was shown with a reasonably good sensitivity (82.3%) and specificity (85%). The findings suggest that serum HA level is a useful non-invasive marker of liver fibrosis with a

strong positive correlation between serum HA level and the degree of liver fibrosis stages and inflammation grades.

Keywords: chronic hepatitis, hepatic inflammation grade, hepatic fibrosis stage, hyaluronic acid

P-10-103-2

Attenuation of serum laminin level upon treatment of chronic hepatitis

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Increased levels of Laminin (LN) - one of the main glycoproteins of the basement membrane - were observed in the more advanced stages of fibrosis in patients with hepatic disease. The aim of this work was to determine serum LN level cutoff point for predicting liver fibrosis, highlighting its diagnostic value and determining the effect of treatment on its level. Serum LN levels in chronic hepatitis patients ($n=62$) and controls ($n=20$) were compared by ELISA and stages of fibrosis were assessed according to the modified Knodell score system. Mean serum LN concentration in patients (91.9 ± 20.9 ng/ml) was greater than the control (46.2 ± 10.2 ng/ml, p -value < 0.001). Serum levels of LN in all stages of hepatic fibrosis were significantly higher than those of the healthy controls (p -value < 0.05). A cutoff point of 52 ng LN/ml of serum was obtained for the discrimination of various stages of liver fibrosis showing a good sensitivity (96.8%) and specificity (80%). After 6 months of treatment, a gradual decrease in serum LN level was observed, however the level was still higher than that of the healthy group (p value < 0.05). The findings of this study suggest that serum LN level is a useful non-invasive marker of liver fibrosis. There was strong positive correlation between serum LN level and the degree of liver fibrosis and inflammation (stages and grades). Serum LN level may also be used for the follow-up of liver fibrosis in patients with chronic liver disease as well as for the assessment of liver fibrosis and monitor the recovery of disease during the treatment protocol, where liver biopsy is contraindicated.

Keywords: chronic hepatitis, hepatic fibrosis, laminin

P-10-110-2

Effects of changes in dietary fatty acid composition on HDL composition and serum lipid profile

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Apart from being a source of energy, dietary fatty acids are known to affect various aspects of lipoprotein metabolism, which makes the

evaluation of their metabolic effects even more important. The aim of this study was to investigate the metabolic response to dietary induced changes in fatty acid composition of HDL particles. The population included 85 healthy subjects assigned to two consecutive 28-d experimental period. All subjects consumed a high polyunsaturated to saturated fatty acids ratio (P: S) with P: S of 1.2 for the first period and a low P: S with a P: S of 0.3 for the next 28-d period. At the beginning and end of each dietary period, fatty acid composition of HDL particles and lipid profile were measured. There was enrichment in polyunsaturated fatty acids (PUFA) in the HDL phospholipids after the high P: S diet. Changes in diet were associated with significantly higher concentrations of triglyceride and significantly lower concentrations of HDL-C and apoA-I ($P < 0.01$) after the low P: S diet. In addition to changes in fatty acid composition of HDL particles, the diet enriched with PUFA also has beneficial effects on lipid and apolipoprotein profile.

Keywords: fatty acid, HDL, lipoprotein, diet

P-10-456-1

Interleukin-6 (IL-6) in end stage renal disease (ESRD)

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Interleukin-6 is a 22-27 kDa polypeptide secreted from activated monocytes, macrophages, fibroblasts, adipocytes and endothelial cells in response to various stimuli, such as TNF- α , IL-1 β , bacterial endotoxins, physical exercise and oxidative stress. The potential causes of elevated plasma IL-6 levels in end-stage renal disease (ESRD) patients may be related to the loss of kidney function, uremia and dialysis related factors. Even before the initiation of dialysis therapy, patients with decreased renal function already demonstrate signs of inflammation and the deterioration of renal function which has been associated with a significant increase in serum cytokine levels. The aim of this study was to determine the pre and post serum IL-6 levels in patients with ESRD (undergoing hemodialysis). The study population consisted of 78 patients (48 males, 30 female, ages 18-66) at hemodialysis unit, Tehran University Medical School. Aliquots were taken from pre-and postdialysis patients. Plasma samples of ESRD patients were stored at -70°C and later used for IL-6 measurements. Plasma IL-6 was measured by commercially available highly sensitive ELISA, and served as a marker of inflammation. Post dialysis IL-6 levels were significantly greater than predialysis levels (14.3 ± 4.2 vs 9 ± 3.5 pg/ml, respectively $P < 0.01$). IL-6, the major mediator of the acute-phase response, was elevated in the plasma of ESRD patients which is a strong predictor of outcome. A number of factors prevalent in patients with ESRD, such as hypertension, adiposity, insulin resistance, fluid overload and persistent infections, could all be associated with elevated IL-6 levels. In addition, reduced renal function, directly or indirectly, seems to be closely related to IL-6 elevation and genetic variations may be of importance. However, also factors associated with the dialysis procedure, such as bioincompatibility of dialyser membranes and dialysis solutions, may stimulate IL-6 production. The clinical consequences of elevated IL-6 levels and strategies to reduce IL-6 levels should be further evaluated to confirm the importance of this cytokine as a central regulator of the inflammatory response in ESRD.

Keywords: interleukin-6 (IL-6), end stage renal disease (ESRD)

P-10-281-1

The role of matrix-metalloproteinases in vascular remodeling and atherosclerosis

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Vascular remodeling, defined as lasting structural changes in the vessel wall in response to hemodynamic stimuli, plays a role in many (patho) physiological processes requiring cell migration and degradation of extracellular matrix (ECM) such as atherosclerosis. Since degradation of the extracellular matrix scaffold enables reshaping of tissue, participation of specialized enzymes called matrix metalloproteinases (MMPs) has become the object of intense recent interest in relation to physiological and pathological vascular remodeling. MMPs are a family of zinc-dependent endopeptidases comprised of more than 25 members divided into specific classes based on substrate specificity for various ECM components. They are secreted as zymogens and regulated at multiple levels. Experimental studies have revealed that some biological actions of MMPs aggravate a pathological condition, whereas others may be beneficial for the patient suffering from atherosclerotic disease. Infiltration of inflammatory cells, degradation of ECM of intima layer of artery, migration and proliferation of smooth muscle cells (SMC), and intraplaque angiogenesis that all occur in progressive processes of atherosclerosis, are facilitated by MMPs. Releasing of MMPs are induced by Lipid accumulation, formation of reactive oxygen species (ROS) and cytokines in activated-macrophages (Foam cells). Several MMP system components that are expressed in atherosclerotic tissue are MMP-1, -2, -3 and -9. The messenger molecules that mediate communication between the various cell types involved in vascular proteolysis in atherosclerosis are poorly understood. However, there has been considerable interest in the role of the CD40/CD40L system. Therefore, a better understanding of the biological consequence and regulation of MMP activity may represent a potential therapeutic target for treatment of atherosclerosis.

Keywords: atherosclerosis, vascular remodeling, extracellular matrix, matrix metalloproteinases, angiogenesis

P-10-107-1

The effect of feeding aerial part of *Silybum marianum* on blood glucose and lipids in diabetic rats

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Use of medicinal plants for attenuation of hyperglycemia and restoration of lipids to normal level is clinically important. In this respect, *Silybum marianum* (SM) is a plant that can lower lipid peroxidation and lipids in an experimental model of hyperlipidemia. Therefore, the effects of chronic oral administration of this plant on serum glucose, triglyceride, total cholesterol, and HDL- and LDL-cholesterol level of diabetic rats were investigated. For this purpose, female rats ($n=36$) were randomly divided into 4 groups; i.e., control,

SM-treated control, diabetic, and SM-treated diabetic groups. The treatment groups received oral administration of plant-mixed pelleted food (6.25%) for 4 weeks. Serum glucose, triglyceride, total cholesterol, LDL- and HDL- cholesterol levels were determined before the study, and at 2nd and 4th weeks after the study. Serum glucose level in diabetic group increased 2 and 4 weeks after the experiment as compared to data one week before the study ($p < 0.001$) and SM treatment of diabetic rats only had a mild non-significant effect. In addition, triglyceride level in diabetic group increased 4 weeks after the experiment in comparison with related data one week before the study ($P < 0.05$) and there was a significant lower level of triglyceride in SM-treated diabetic rats ($p < 0.05$). Furthermore, a similar significant reduction was obtained for treated-diabetic group as compared to diabetic group regarding serum cholesterol level ($p < 0.05$). On the other hand, HDL- and LDL- cholesterol levels were significantly higher ($p < 0.05$) and lower ($p < 0.05$) in SM-treated diabetic group as compared to untreated diabetic group respectively. Oral chronic administration of SM had no significant hypoglycemic effect and led to appropriate changes in blood lipid profile.

Keywords: Silybum marianum, glucose, lipid, diabetes mellitus, rat

P-10-196-1

Investigation of iodine carrier proteins in human

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According to many researches all the trace elements have at least one carrier protein, while for iodine it is only mentioned that excess is excreted in the urine. On the other hands, metabolism of all the cells is controlled by thyroid hormones and iodine is a necessary component of these hormones. The questions arises here that if this precious element has no carrier, why in East Asian countries, peoples who mostly consume sea foods rich in iodine suffer more from thyroid diseases and why excess of iodine leads to thyroid disease. To find out possible answers to these questions, this study was designed. Peripheral blood samples were collected from 14 patients suffering from thyroid cancer. To investigate the effect of residence time on binding proteins to iodine, patients were divided into two different groups who consumed radioactive iodine 131 at two time points (1 and 3 hours after oral consumption). Immediately after collection sera were prepared using standard procedure and the dose of radiation was determined in a part of serum. Other two equal portions were used by two different methods; protein precipitation by tri-chloro-acetic acid (TCA) and polyacrylamide gel electrophoresis to separate serum proteins. In first method serum was added to TCA 10% and was centrifuged. The sediment was washed twice with TCA and dose of radioisotope was determined in the final precipitation, using a gamma counter. In second method, proteins were separated using 7% gel and a voltage of 120 volts. Different bands were separated and the radioactivity in each band was determined. Evaluated results were expressed as mean \pm standard deviation. The mean of total iodine binding to serum proteins by TCA method in two different groups, were 4.13 ± 0.80 and 10.75 ± 2.21 , respectively ($P < 0.001$). Mean of total iodine binding to serum proteins evaluated by electrophoresis method in two mentioned groups, were 3.80 ± 0.71 and 9.65 ± 2.05 , respectively ($P < 0.001$). Iodine bound to albumin in two different groups was statistically significant ($P = 0.004$), but no significant difference was found for other bands. In conclusion, nearly 10% of daily consumed iodine binds to many proteins that transport it in blood stream. Iodine binding is affected by residence time of iodine in blood.

Iodine binds to serum albumin more than other serum proteins. Prealbumin, α globulins, apolipoproteins, β globulins and γ globulins are in subsequent orders, respectively.

Keywords: iodine, iodine carrier proteins, PAGE, protein electrophoresis

P-10-206-1

Urine iodine determination using microwave digestion method

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More than one billion people live in iodine deficient areas so Iodine deficiency disorders (IDD) are common. Urine iodine level is a good index for IDD diagnosis and monitoring. For urine interferences, its digestion is an obligatory step in urine iodine determination methods. High temperature and long time heating are considered as weaknesses of the present methods. Using microwave for digestion of urine and decomposing of interfering substances is the aim of this study, for shortening of digestion time and increasing safety. One hundred random urine samples were processed by conventional acid digestion and also new microwave optimized digestion methods. After digestion iodine levels of the samples which were digested by two mentioned methods, were determined using chemical colorimetric kinetic reaction according to the famous Sandell Kolthoff reaction. Only 10 min was adequate for completely digestion of urine samples. Precision of new digestion step was 7.8% and 8.9% for intra and inter assay coefficient of variation. The final results of iodine content of samples digested by the two methods had acceptable correlation ($r^2 = 0.902$). The obtained results of this study showed that microwave procedure for urine digestion is acceptable and is comparable and can replace the conventional procedure.

Keywords: microwave, digestion, urine, iodine

O-10-278-1

Long fasting dependent changes in the serum levels of cholesterol, triglyceride, HDL-c, LDL-c, estrogen and progesterone in young women during Ramadan

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Long starvation during hallowed Ramadan month affects the health and psychological and social well-being of growing individuals. However, there are many hypotheses about hallowed Ramadan month starvation and its effect on the human serum lipid and lipoprotein concentration. In addition, the direct effect of serum lipid changes on the atherosclerosis has been established. But there are few studies about effects of female hormonal changes the lipid profile. In this study, we investigated the relation between Islamic starvation with changes in women serum lipid and lipoprotein concentrations and women sex hormones changes in the healthy people. Concentrations of cholesterol, triglyceride, HDL-c, LDL-c, estrogen and progesterone

before and after Islamic starvation were determined by of chemical and enzymatic methods. Results have showed that during Islamic starvation cholesterol concentration increased and triglyceride concentration decreased but none of these changes were statistically significant. HDL-c concentration after starvation increased significantly. During starvation, the decrease in estrogen concentration and the increase in progesterone concentration were significant. Our results prove the positive effect of starvation on the HDL-c concentration and its effect in decreasing the risk of atherosclerosis and related diseases.

Keywords: long fasting, cholesterol, triglyceride, HDL-c, LDL-c, estrogen, progesterone, Ramadan

P-10-232-2

Structural heterogeneity of phosphoglycan from Iranian Leishmania major

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Lipophosphoglycans (LPG) are the major cell surface antigen of *Leishmania major* that has unique structural features. In addition *L. major* secretes soluble lipophosphoglycan (sLPG) in the culture medium that is similar to membrane lipophosphoglycan (mLPG). Structural and compositional analysis indicates that sLPG has molecular weight higher than mLPG and this increase is due to glycan composition. Compositional analysis indicates that LPG contains more than 80 % glycan and 20% lipid and phosphate. Protozoan parasites of the genus *Leishmania* secrete a range of proteophosphoglycans (PPG) known to be important for successful colonization of *Leishmania* in the sandfly and for virulence in the mammalian host. PPGs are a large family of extensively glycosylated proteins with some unusual and unique features. In discontinuous SDS-PAGE, PPG could not enter the resolving gel but after mild acid hydrolysis several bands resolved. Agarose gel electrophoresis and immunoblot analysis using monoclonal antibody (WIC 79.3) indicated that the PPG preparation consisted of heterogeneous molecules. Compositional analysis showed that the PPG preparation contained 67% glycan, 28% protein and 5% phosphate. Thus in *Leishmania* phosphoglycans are very heterogeneous from molecular weight 10 to 1000 KDa.

Keywords: *Leishmania major*, lipophosphoglycan, structural heterogeneity, proteophosphoglycan

P-10-132-1

Serum alkaline phosphatases and dietary zinc in a group of healthy reproductive age women

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Serum alkaline phosphatase is a member of a family of zinc metalloprotein enzymes that function to split off a terminal phosphate group from an organic phosphate ester. This enzyme requires zinc for its proper action. The aim of our cross-sectional study was to evaluate

serum alkaline phosphatase and dietary zinc relationship in a group of reproductive age women. Eighty reproductive age (17-50 years) women were participated in this study. They were separated into two groups: Obese group with stage 1 and 2 obesity (BMI: 30-34.9 and 35-39.9 kg/m² respectively) and non-obese group with normal weight (BMI: 18.5-24.9 kg/m²). Serum alkaline phosphatase was measured by enzymatic method. Dietary zinc intake was assessed by 3 days dietary foodrecord and was analyzed by NUTRITIONIST III Software. Pearson correlation coefficient was used to investigate association between variables. In our study, there was no significant difference between serum alkaline phosphatase in obese and non-obese individuals. Mean dietary zinc in obese women (6.39±5.3 mg/dl) was positively correlated with serum alkaline phosphatase activity (79.7±34.5 IU/l); however this correlation was not confirmed for non-obese women. There is a significant positive relationship between dietary zinc intake and serum alkaline phosphatase activity in obese individuals. Therefore, adequacy of zinc intake may be necessary for the proper action of this enzyme.

Keywords: alkaline phosphatase, dietary zinc intake, obesity

P-10-172-1

Study of glutathione-S-transferase enzyme activity in rodent and human malaria (*Plasmodium berghei* & *Plasmodium falciparum*)

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Glutathione-S-transferase (GST, EC 2.5.1.18) is a family of enzymes that utilize glutathione in reactions contributing to the transformation of a wide range of compounds, including carcinogens, therapeutic drugs, and products of oxidative stress. *Plasmodium berghei* Glutathione-S-transferase (PbGST) is an enzyme which is involved in drug resistance in the malarial parasite. The activity of this enzyme was estimated during treatment with Eosin B, the new antimalarial drug. The study was performed in vivo in mice infected with *Plasmodium berghei* and in vitro in human RBCs with *Plasmodium falciparum*. Toxicity test, rate of effect of drug in vivo and variation of PbGST function were carried out. Initial studies with *P. berghei* and *P. falciparum* gave showed 47% inhibitory effect of Eosin B as compared with 41% in arteether (artemisinin derivative) on in vivo testing of rodent malaria. In vitro testing for GST activity with the two drugs showed significant difference in PbGST and PFGST. Further research is required to observe the effect of eosin B as an antimalarial drug in human *Plasmodium* and determine its drug target so as to replace chloroquine in resistant strains.

Keywords: malaria, glutathione-S-transferase, eosin B, *Plasmodium falciparum*, *Plasmodium berghei*

P-10-227-1

Lipoprotein a and other serum lipids and lipid lipoproteins in patients with beta-thalassemia major

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Beta-thalassaemia is an inherited blood disorder with need of repeated blood transfusions. Serum lipid abnormalities have been repeatedly reported in these patients. The aim of this study was to evaluate plasma lipids and Lipoprotein (a) [Lp(a)] in thalassaemic patients who referred to Yazd blood transfusion center. In this cross sectional case control study, fasting serum lipids, lipoproteins and Lp(a) from 58 patients (32 females, 26 males) with beta-thalassaemia major, were compared to 68 (40 females, 28 males) age matched healthy control. Beta-thalassaemia patients had significantly lower serum levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) compared with control (113.8±17.7, 32.3± 8, 58.2±14.7 vs. 158.3±30.6, 43.8±9.2, 96.2±26.4, p<0.001), while serum triglycerides (TG) and Lp(a) levels did not show significant differences between two groups. Nineteen percents of patients and 27% of controls had serum Lp(a) levels equal to or higher than 30 mg/dl. There were no significant correlations between lipids and lipoproteins with age in the two groups, but TG and TC correlated significantly (r=0.5, p<0.001) only in control group. Our results indicated that in patients with beta-thalassaemia major cholesterol and its carrier lipoproteins are lower than healthy controls, but triglycerides and Lp(a) does not show significant differences.

Keywords: beta-thalassaemia, lipids, lipoproteins, lipoprotein (a)

P-10-303-1

Expression and purification of antigen 85 complex (A, B, C) and MPB-64 from Mycobacterium Bovis AN5

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Bovine Tuberculosis, a disease caused by Mycobacterium Bovis (M. Bovis), is closely related to human Tuberculosis (TB). Both diseases are the main causative agents in animals and human death. Antigen recognition at various stages of tuberculosis infection has been focused in a number of studies. Various protective antigens by M. Bovis have been identified which include antigen-specific T-cell mediated immunity. Among those antigens, MPB-64 and 85 complexes (A, B, C), the secretory proteins, produce the strong antigen-specific responses in TB infected animals. In this study we managed to produce recombinant secretory antigens 85 complex (A,B,C) and MPB-64 to determine which one of these proteins or the combination could be considered as major target recognized by T-cell involved in protective immunity. Throughout this study Genomic DNA from M. Bovis standard strain AN5 was prepared by Karaj Razi Vaccine & Serum Research Institute. Desired genes were amplified by Polymerase Chain Reactions (PCR) using designed primers. Following purification, the PCR products were digested with suitable restriction endonucleases, ligated into PGEX4T1 plasmid and transformed into Escherichia Coli strain DH5

a. Recombinant plasmids were sequenced to confirm the insertion. Recombinant clones were over expressed in E. coli strain BL-21. Purification of the proteins were done by affinity chromatography on glutathione agarose in order to make GST-tag proteins that could be used as diagnostic tools in clinical setting.

Keywords: cloning, purification, antigen 85 complex (A, B, C), MPB-64

P-10-291-1

Relationships between free leptin and insulin resistance in women with polycystic ovary syndrome

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Patients with polycystic ovarian syndrome have increased insulin resistance and high incidence of obesity. The obese gene product, leptin play a central role in food intake and obesity and circulate in both free and bound forms. The free form is the biologically active leptin. We assessed the correlation of metabolic parameters with free and bound form levels in 27 polycystic ovarian syndrome (PCOS) women (aged 26±5.6 years) and 27 healthy controls (aged 25±4 years). Total leptin and insulin levels were measured using ELISA-kits. Free leptin form was purified by gel filtration chromatography and their collected fractions were measured by ELISA-kit. Insulin resistance was calculated by homeostasis model assessment (HOMA). In PCOS patients and control groups a correlation was found between leptin and body mass index (BMI). A significant difference was found between leptin and free leptin in PCOS subject and control (P=0.05). Significant correlations were found between free leptin and leptin with insulin resistance in PCOS subject (r=0.53 p=0.004, r=0.69 p=0.00) and control groups respectively (r=0.57, P=0.003, r=0.71, p=0.002). The biologically active free form of leptin is responsible for insulin resistance and leptin action in the body.

Keywords: polycystic ovarian syndrome, insulin resistance, obesity, leptin

P-10-320-1

Elevated circulating inflammatory markers in patients with cardiac syndrome X

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Cardiac syndrome X (CSX) is defined as a typical angina pectoris, positive treadmill exercise test and normal coronary angiogram. Despite the extensive studies, the pathophysiological mechanisms in CSX, however, remain unclear. More recently, the data has been suggested that chronic inflammation has been associated with CSX. The evidence for this hypothesis included that inflammatory marker are increased, and associated with the disease activity in patients with CSX. The present study was, therefore, to investigate whether inflammatory markers such as high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) might be involved in the pathogenesis of CSX. 60 patients with CSX

and 60 age and gender matched normal controls were prospectively enrolled in this study. Plasma levels of hs-CRP, IL-6 and TNF- α were measured by enzyme linked immunosorbent assay (ELISA) method. Data were compared between patients with CSX and normal controls. Patients with CSX were detected to have significantly higher plasma hs-CRP, IL-6 and TNF- α levels in comparison with normal controls (hs-CRP: 4.8 ± 1.03 vs. 0.84 ± 0.1 ($\mu\text{g/dl}$); IL-6: 33.64 ± 2.7 vs. 8.9 ± 1.5 (pg/ml); TNF- α : 24.17 ± 1.75 vs. 4.44 ± 1.04 (pg/ml); $p < 0.01$, respectively). Our data suggested that low-grade, chronic inflammation might contribute to the development of CSX manifested by increased plasma levels of inflammatory markers.

Keywords: cardiac syndrome X, chronic inflammation, high sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha

P-10-328-1

Study of calcium level, prothrombin time and activated partial thromboplastin time in patients with Hodgkin's disease at the Children's medical Center of Tehran

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Hodgkin's disease is a neoplastic disorder originating in lymphoid tissue, usually occurring in all age groups, and initially localized, but subsequently spreads to contiguous lymphoid structures and ultimately disseminates to nonlymphoid tissues. The aim of this study were to evaluate calcium level, prothrombin time and activated partial thromboplastin time in patients with Hodgkin's disease at the Children's medical Center of Tehran. In the present study, the sera and plasma of all patients with diagnosed Hodgkin's disease who referred to Children's medical Center of Tehran were collected and tested for detection of Ca level, P.T and A.P.T.T. The statistical tests were used to analyze the results. The age range of patients was 2-13 years, with a median of 7.6 years, mean 7.5 years. There were 51 male (73%) and 19 female (27%). Stages III, IV disease (advanced-stages) were occurred in 61.4% of the patients. P.T ($P < 0.01$) was more than normal range, increase of A.P.T.T (14%) and Ca level were seen in patients. The results of present study showed increase of P.T, A.P.T.T and Ca level in advanced stages.

Keywords: Ca, P.T, A.P.T.T, Hodgkin's disease

P-10-329-1

Analysis of p53 mutations in human colorectal cancer in Kerman province

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p53 is an important tumor suppressor gene that is mutated in more than half of all human cancers. p53 is often mutated in final stages of tumor when it can not induce apoptosis in cancer cells thus tumor spread to other tissues. Mutations in p53 also lead to chemotherapy resistance in cancer and so they are valuable marker for diagnosis and effective therapy. In this study 52 samples of colorectal carcinoma tissues in stage IIIA were obtained from pathology archive during 1995-2008 as paraffin embedded block. 6 samples of fresh tumor also were obtained from patient undergoing surgery due to aggressive

colorectal carcinoma. Normal tissue from both archive and fresh samples were also collected for comparison. The DNA was extracted by DNeasy kit (Qiagen). Three exons 5, 7 and 8 of the p53 gene were amplified by a mixture of Taq polymerase and pwo polymerase (Roche). The PCR products were observed on agarose gel electrophoresis and were sequenced in MWG OPERON Company. The results showed 18% of patient had mutation in exon 5, 30% in exon 7 and 18% in exon 8. A new ACC to AAC mutation in codon 140 of exon 5 was found in a tumor sample that was not reported in all tumor samples and cancer cell line previously. Also a deletion in codon 282 exon 8 was reported for the first time in colorectal tumor samples.

Keywords: colorectal cancer, mutation, paraffin embedded tissue, p53

P-10-155-1

Comparison the level of serum adenosine deaminase activity in normal pregnancy and gestational diabetes mellitus

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To our knowledge there is not any report regarding adenosine deaminase activity (ADA) in gestational diabetes mellitus. The aim of the present study was to investigate the level of serum ADA activity in normal pregnancy, gestational diabetes mellitus and normal non-pregnant women. Twenty patients with gestational diabetes mellitus, 40 normal pregnant and 20 normal nonpregnant women were enrolled the study. The catalytic concentrations of ADA were measured in all women by enzymatic assay using commercial available kit. The results showed that ADA activity was significantly higher in GDM (24.30 ± 8.04 IU/L) and pregnant women (23.88 ± 8.66 IU/L) than those of normal non-pregnant women (11.85 ± 3.23 IU/L) ($p < 0.001$), but the level of ADA was not significantly different between GDM and normal pregnant women ($P = 0.85$). The clinical significance of an increase in plasma adenosine deaminase activity in normal pregnancy and gestational diabetes mellitus remains to be cleared.

Keywords: adenosine deaminase, gestational diabetes mellitus, pregnancy

P-10-337-1

The effect of mitoxantrone on histone proteins

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Mitoxantrone is a clinically useful antitumor antibiotic for treatment of leukemia and breast cancer. Binding of mitoxantrone to cellular DNA has been studied in detail. In the cell nucleus, DNA is associated with histone proteins making chromatin structural units, the nucleosomes. In the present study, we have investigated the effect of mitoxantrone on histone proteins in solution, using thermal denaturation, circular dichroism and fluorescence spectroscopy techniques. The results show that, upon addition of various concentrations of mitoxantrone, the ellipticity at 206-208 and 220-222 nm is increased but at higher concentrations of drug the ellipticity is decreased. This alteration in ellipticity is accompanied by the variation in the alpha-helical structure of the protein. Fluorescence emission intensity of histones is decreased

upon mitoxantrone binding because of quenching of drug with the tyrosine residues of the protein and this process is concentration dependent. Thermal denaturation of histones in the absence and presence of mitoxantrone shows that in the presence of the drug T_m is increased to higher degrees. From the results it is suggested that mitoxantrone binds to histone proteins and stabilizes their structure. Therefore, histone proteins may play an important role in the mitoxantrone-chromatin interaction in the cell

Keywords: fluorescence spectroscopy, histone proteins, mitoxantrone

P-10-304-3

In vivo study the effect of oxamate on spermatogenesis and LDH-C4 activity in mouse

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LDH-C4 is a specific isoenzyme, distinct from other LDH isoenzymes with regard to its locality and kinetic properties. Because about 80% of LDH in spermatozoa contains LDH-C4, it must be strongly related to very specific metabolic processes connected with sperm. The aim of present study was to evaluate in vivo effect of oxamate as contraceptive on the spermatogenesis and lactate dehydrogenase-C4 (LDH-C4) activity in mouse sperm. In this study 20 adult Rat were divided in to 4 cages, one cage as a control group and 3 others as experimental groups. Experimental groups received intraperitoneal injection of different concentration of Oxamate (150, 300, 600 mg/kg/day) for 45 days. Sperms were collected from cauda epididymides, counted and LDH-C4 was purified by ammonium sulfate precipitation, DEAE-Sephadex A-50 anion exchange chromatography. Results showed that oxamate at the dose level employed had a significant reducing effect on sperm production and LDH-C4 activity, at the concentration of 600 mg/kg had 63% reduction effect on LDH-C4 activity. We conclude that oxamate in vivo can reduce LDH-C4 activity and spermatogenesis and this reduction is concentration dependent.

Keywords: oxamate, LDH-C4 activity, spermatogenesis

P-10-332-1

Adenosine deaminase in patients with primary immunodeficiency syndromes: The analysis of serum ADA1 and ADA2 isoenzymes activities

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Adenosine deaminase (ADA) is involved in purine metabolism and plays a significant role in the mechanisms of the immune system. We aimed to investigate the activity of ADA and its isoenzymes in patients with

various primary immunodeficiency (PID) syndromes. The serum of 76 children with various PID syndromes and 30 healthy control subjects were examined. Total ADA (tADA) and its isoenzymes activities were determined using the kinetic method described by Eliss. ADA2 activity was measured in the presence of a specific ADA1 inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA). Our results indicated that tADA and ADA2 levels were higher in patients with Chronic Granulomatous Disease (CGD), Leukocyte Adhesion Deficiency (LAD), hyper IgM (HIM) and Wiskott-Aldrich Syndrome (WAS) than those of corresponding controls ($P < 0.01$). There was a significant elevation of tADA and ADA1 activities in IgA deficient patients as compared to healthy individuals ($P < 0.01$). In comparison to healthy control group, the ADA activity did not differ significantly in patients with common variable immunodeficiency (CVID), IgG deficiency and hyper IgE. Our results hypothesized that altered ADA activity may be associated with altered immunity. Therefore, serum ADA level could be used as an indicator along with other parameters in diagnosis and follow up of the patients with CGD, LAD, IgA deficiency, HIM and WAS.

Keywords: adenosine deaminase, ADA isoenzyme, primary immunodeficiency syndromes

P-10-89-1

Potential misleading results from thyroid immunoassays: A case series and literature review

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In our country, the laboratory diagnosis and monitoring of thyroid disease are based on the thyroid panel including thyrotropin (TSH), thyroxine (T4), and triiodotyronine (T3) (both total and free) and the most commonly used assays are radioimmunoassay (RIA), enzyme immunoassay (ELISA) and chemiluminescence (CLIA). However, some serum samples will demonstrate a nonspecific binding with assay reagents that can interfere with the measurement of these hormones. Here we explain two cases with false positive results and review the nature of these disturbances; their occurrence, prevalence, and detection. This report describes a false elevation of TSH in the plasma of two patients due to the presence of antibody interference. In both cases who were clinically euthyroid with normal other thyroid function tests, repeated analysis of the samples in question obtained similar TSH values. There was no evidence of analytical assay problems, as assessed by an examination of recent quality-control test results, proficiency test results, and calibration curves. Immunoassay antibody interferences are unpredictable and can result in either falsely increased or falsely decreased test results, depending on the nature of the interfering antibody and the assay format. Since the interference is unique to a particular patient sample, the problem is not easily identified by routine quality assurance checks. The three major possible sources of antibody interference in thyroid hormone immunoassays are autoantibodies, heterophile antibodies, and rheumatoid factors (RF). Interferences in immunoassay laboratory tests continue to be a significant challenge between clinicians and clinical laboratories. Also a raised awareness to this type of analytical problem is important if laboratory operators are to avoid changing the correct test results.

Keywords: thyroid panel immunoassays, false positive, antibody interference

P-10-16-1

Determination of thymidine phosphorylase activity in leukocytes of Iranian MNGIE patients and their plasma thymidine by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

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Thymidine Phosphorylase (TP) catalyzes the conversion of thymidine into thymine. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disease which is caused by mutations in the nuclear gene encoding TP; severe impairment of TP enzyme activity and accumulation of thymidine in plasma. The clinical manifestations of MNGIE are recognizable and homogenous, but not in the early stages. In patients who are suspected of having MNGIE, determination of TP activity in buffycoat and thymidine levels in plasma is diagnostic. The methods that usually used for measurement of activity of TP and plasma thymidine haven't enough velocity, accuracy and sensitivity. The activity of TP was measured in buffycoat of patients with clinical features suggestive of MNGIE by RP-HPLC. Also plasma thymidine of patients was assessed by the same method. Our patients had detectable plasma thymidine (>10 μ mol/Lit) and it was undetectable in healthy controls. TP activity in buffycoat of patients decreased to less than 10% relative to controls. These results propose a diagnostic algorithm for definitive diagnosis of MNGIE based on the measurement of plasma thymidine, TP activity in buffycoat, or both. In this study, we have set up a sensitive and fast assay of the TP activity by using RP-HPLC in Iran. In addition, we have established references values for the TP activity and plasma thymidine in the Iranian patients.

Keywords: thymidine phosphorylase, thymidine, MNGIE, HPLC, buffycoat

P-10-177-1

Immunobiochemistry of nitric oxide, microelements and liver enzymes and inhibitory evaluation of visceralization in Leishmania major infected Balb/c mice under treatment with Paromomycin

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This study has been carried out to compare the pathophysiological signs, production of nitric oxide, serum concentration of microelements and liver enzymes including aspartate transaminase, alanine transaminase and alkaline phosphatase in BALB/c mice infected with Leishmania major of cutaneous leishmaniasis. Experimental Leishmaniasis was initiated by subcutaneous injection of promastigotes into the basal tail of test groups. The development of lesions was determined weekly by a digital caliper. After 10 weeks, all mice were killed and target tissues including spleen and liver from each mouse were removed, weighed. Griess microassay method was applied for measurement of NO concentration in plasma, liver and spleen suspensions. Serum microelements including Zn and Cu were determined by the Atomic Absorption Spectrophotometer. Serum

AST, ALT and ALP were determined by Auto Analyzer Technical RA1000. After 3 weeks of 0.4 and 0.6 mg Paromomycin injection, the following results have been achieved: Although, lesion size in control group have had an increase trend, it has reduced in test groups (0.4, 0.6 mg), particularly 0.6 mg dosage showed an anti-leishmanial activity with a significant reduction of lesion size with (P<0.001). Both hepato/splenomegaly have decreased in test group with (P<0.05). NO in Liver and Spleen increased with (P<0.01) and NO in serum increased with (P<0.001). The laboratory evaluation of anti-leishmanial effects of Paromomomycin in L. major infected Balb/c mice. Therefore, in addition to current therapies against CL, injectable Paromomomycin is introduced as a relevant and suitable drug for the treatment of CL in rodent model of leishmaniasis.

Keywords: alanin transaminase, alkaline phosphatase, aspartate transaminase, Cu, Zn

P-10-388-1

The effect of anticancer drug, daunomycin, on chromatin proteins in alveolar macrophages

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Daunomycin is a clinically useful anthracycline antibiotic that its antitumor activity has been attributed to the interaction of the planar aromatic ring between adjacent base pairs of the DNA double helix, and the resulting inhibition of the synthesis of DNA and RNA. In the present study we attempted to investigate the effect of daunomycin on histone and non-histone proteins of alveolar macrophages using SDS-polyacrylamide gel electrophoresis and western blot techniques. Alveolar macrophages were prepared by lavage from rat lung and then treated with various concentration of daunomycin for desired periods of time. The proteins were then extracted from drug treated and the controls and analyzed. The results showed that low concentration daunomycin had no considerable effect on the chromatin's protein content of the cells but at higher concentrations the amount of extractable proteins were decreased. Between them, apart from core histones, histone H1 and its sub fraction, H10 and HMG proteins decreased as drug concentration was increased. Western blots also confirmed the results. We concluded that daunomycin exerts its action via chromatin proteins and compaction of chromatin probably is a result of cross linking between protein-proteins or protein-DNA at the chromatin level.

Keywords: macrophage, daunomycin, core histones, histone H1, HMG proteins

P-10-201-1

Anti-leishmanial effects of L-arginine as nitric oxide inducer and Indomethacin as prostaglandin inhibitor in Balb/c mice infected with Leishmania major MRHO/IR/75/ER

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Cutaneous leishmaniasis is still one of the health problems. Nitric oxide has a key mechanism in the elimination of parasite from the body by its anti-leishmanial activity prostaglandin is a critical inhibitory factor of

infected MQ to decrease their anti-leishmanial activity. This study is designed to induce NO by L-arginine precursor and inhibit PG production by anti-inflammatory Indomethacin in L. major infected Balb/c, in order to evaluate the effects of NO and PG on, size of lesion and proliferation of amastigotes inside macrophages. Liver, spleen and lymph nodes were also studied as target organs to detect amastigotes. In addition to serum, liver and spleen suspensions were investigated for NO induction by using Griess microassay and serum PG was determined by ELISA. The results indicated that NO production was inhibited by Leishmania in infected Balb/c mice as compared with naive animals. Serum NO was inhibited by a combination therapy of L-arginine and Indomethacin ($P < 0.05$). Although NO was decreased ($P < 0.05$) in liver by Indomethacin, however it increased in spleen after L-arginine and Indomethacin application ($P < 0.05$). Both L-arginine and Indomethacin had significant inhibitory effects on visceralisation of leishmania in target organs. Only L-arginine decreased proliferation of promastigotes in macrophages ($P < 0.05$). Pathophysiological signs including hepatomegaly, splenomegaly, survival rate and body weight all were affected in this experiment. Statistical analysis of data revealed an association between NO induction and PG inhibition in leishmaniasis. These data may indicate a possible candidate for L-arginine and Indomethacin as novel drugs for the treatment of leishmaniasis in mouse model.

Keywords: Balb/c, indomethacin, L-arginine, nitric oxide, prostaglandin

P-10-417-1

The effect of Diazinon on some liver enzymes of rats

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Diazinon (DZN), an organophosphate pesticide has been used in agriculture and domestic for several years. Although it is well known that primary target of DZN is acetylcholine esterase (ChE), but it has several toxicological effects on other enzymes. We examined, the in vivo effect of DZN on the serum activities of ChE and enzymes concerning liver damage. For this propose, Wistar rats (200-250 g) were randomly divided into five groups as followed: control and sham (corn oil), and three groups) of diazinon receiving different dose (30, 50, 100 mg/kg) by intraperitoneal injection. 24 hours after injection, serum samples were taken from 7 rats in each group. Then serum enzymes activities including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and gamma-glutamyltransferase (GGT) and ChE were determined by biochemical methods. The results show that at concentration higher than 30mg/kg, the enhanced activity of ALT, AST, LDH, and GGT were observed, but ChE activity was decreased as compared to the control group. From these results, it can be concluded that DZN causes liver damage due to the enhancement of hepatic enzymes activities.

Keywords: Serum, Rat, Dizinon, liver enzymes

P-10-185-1

Study of Realationship between Thyrotropin and Leptin Levels in Major Thalassemic Patients

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Thyrotropin hormone (TSH) has been recently suggested to induce interleukin-6 secretion by adipocytes, thus leptin secretion from adipocytes may be affected by TSH. Because of leptin effect on growth and reproduction, major thalassemic patients' short stature can be a side effect of leptin deficiency due to changes in TSH concentration. We evaluated the relationship between TSH and leptin levels and the changes in body mass index (BMI) in major thalassemic patients. Blood samples were collected from 30 major thalassemic patients and 30 normal subjects (range: 10-20 y). BMI was calculated by dividing body weight (Kg) by square height (m). Both leptin and TSH were measured by Enzyme Linked Immunosorbent Assay (ELISA) method. Mean BMI and mean concentration of leptin in thalassemic patients were significantly lower than normal subjects (p value=0.049, 0.005, respectively). No significant difference was noticed between TSH concentrations in thalassemic patients and normal group (p value=0.147). The positive relationship between BMI and leptin concentration in normal subjects (p value<0.01) was disappeared in thalassemic patients (p value=0.35). There was not marked relation between TSH and leptin concentrations both in thalassemic patients and normal subjects (p value= 0.909, 0.214, respectively). But TSH concentrations in thalassemic patients had significant positive correlation with BMI. The results obtained in this study indicate that in thalassemic patients, growth can be influenced by TSH and leptin concentrations but leptin secretion is not affected by TSH.

Keywords: BMI, major thalassemia, leptin, TSH

P-10-417-2

The effect of diazinon on biochemical indices in serum of rats

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Diazinon (DZN) is one of the most widely used organophosphate insecticides in agriculture and public health programs. It becomes biotransformed to more potent oxon metabolite that inhibits acetylcholinesterase. The aim of this study was to investigate the effects of DZN on some biochemical indices in serum. For this propose, Wistar rats (200-250 g) were randomly divided into five groups as followed: control and sham (corn oil), and three groups) of diazinon receiving different dose (30, 50, 100 mg/kg by intraperitoneal injection. 24 hours after injection, serum samples were taken from 7 rats in each group. Then serum biochemical parameters including glucose, urea, uric acid, triglyceride and cholesterol were measured by biochemical methods. The results show that DZN increased serum

urea, triglyceride, and uric acid levels. The results suggest that the effect of DZN is dose dependent. DZN probably causes kidney damage and affects blood lipids levels.

Keywords: Serum, rat, dizinon, biochemical indices

P-10-364-1

Comparative study of the Gln/Arg192 polymorphism frequency of paraoxonase I in patients with coronary artery stenosis and control subjects

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Serum paraoxonase (PON1) is an HDL (High density lipoprotein) associated esterase that prevents the oxidation of LDL (low density lipoprotein). A common polymorphism in coding region of the paraoxonase gene involving a Gln (Q) to Arg (R) interchange at position 192 has been demonstrated to affect PON activity. It has been recently shown that R alloenzyme is less efficient at preventing LDL from oxidation and this finding may explain why in some studies the paraoxonase RR genotype has been found at an increased frequency in coronary artery disease (CAD); therefore, to investigate the significance of this polymorphism in pathogenesis of CAD we performed comparative study of this polymorphism in patients and control subjects. PON1 genotypes were determined in 174 subjects who underwent coronary angiography. CAD (>50% stenosis) was detected in 99 subjects (patients) and 75 subjects with <10% stenosis served as controls. PON1 genotypes were determined by PCR and AlwI restriction enzyme digestion. The frequencies of the QQ, QR and RR genotypes were found as 28.3%, 50.5% and 21.2% in patient group respectively and 45.3%, 42, 7% and 12% in control subjects respectively ($\chi^2=6.12$; $p=0.046$). The association of this polymorphism with the severity of stenosis was also evaluated and as results showed that the distribution of PON1 genotypes was not significantly different compared with the severity of stenosis ($\chi^2=2.67$; $p=0.27$). These results suggest that Gln/Arg 192 genotype is a risk factor for stenosis but does not have any effect on the severity of this disease.

Keywords: CAD, PON1, polymorphism

P-10-315-2

Evaluation of urinary tract infection in jaundice neonates

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Neonatal jaundice (hyperbilirubinaemia) is a frequent problem in neonatology as it affects 60% of full-term infants and 80% of preterm infants in the first three days of life. There are various causes of hyperbilirubinaemia in neonates and treatment may be varying in them because of this. Urinary tract infection (UTI) is a common clinical problem in febrile neonates younger than 8 weeks old. Some studies have noted that hyperbilirubinaemia may be one of the first signs of a bacterial infection in neonates. Jaundice may be the first sign of a UTI in asymptomatic infants before other signs and symptoms become evident. In the present study we evaluated the incidence of UTI in newborns with asymptomatic, hyperbilirubinemia in the first two weeks

of life. Of the 59 icteric neonates, 4 (6.7%) were diagnosed to have UTI. Escherichia coli was the prominent pathogen isolated. UTI can be present in newborns with asymptomatic, unexplained, hyperbilirubinemia. Although U/A is not a sensitive test for UTI, it is suggested that performing U/A and U/C as screen tests in icteric infants is important.

Keywords: hyperbilirubinemia, UTI, U/A, U/C, neonates

O-10-313-1

Characterization of purified recombinant ESAT6, CFP10 and ESAT6/CFP10 fusion protein of Mycobacterium Tuberculosis C

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Mycobacterium tuberculosis (M.tb) is major cause of human tuberculosis (TB) which kills millions of people annually. The two secretory proteins of M.tb recently focused on, are Early Secretory Antigenic Target 6 (ESAT6) and Culture Filtrate Protein 10 (CFP10). These proteins are potent T-cell antigens recognized in over 70% of tuberculosis patients. In addition, the two proteins are dominant gamma interferon (IFN- γ)-induced antigens, which has led to their proposed usage as diagnostic reagents for human tuberculosis via ELISPOT (Enzyme Linked Immuno Spot) assay. To see whether each one of these proteins or the combination can detect M.tb from nontuberculosis mycobacteria with high specificity in biological fluids, we produced the proteins from M.tb C strain that was provided by Razi Institute. esat6, cfp10 and esat6/cfp10 fusion gene were amplified by Polymerase Chain Reaction (PCR). Cloning of the genes into pGEX4T1 plasmid was performed. Recombinant plasmids were sequenced to confirm the insertion. E.coli strain BL21 was transformed with the recombinant plasmid. The expressed proteins were purified by affinity chromatography with glutathione agarose beads in order to produce GST-tagged proteins, and then analyzed by SDS-PAGE and western blotting. Purity and antigenicity of the proteins were confirmed by western blotting. In this study ESAT6, CFP10 and ESAT6/CFP10 fusion protein obtained successfully. In order to compare the proteins alone or their combination in detection of TB, we can use the proteins ESAT6, CFP10 and ESAT6/CFP10 in ELISPOT assay.

Keywords: ESAT6, fusion protein, tuberculosis, IFN- γ , ELISPOT

P-10-476-4

Prevalence of iron deficiency anemia among adolescent schoolgirls from Kermanshah, Western Iran

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Iron deficiency anemia is a major health problem in developing countries. Anemia reduces physical work capacity and cognitive

function and adversely affects learning and scholastic performance in schoolgirls entering adolescence. A cross-sectional study was conducted to determine the prevalence of iron deficiency, iron deficiency anemia and anemia among adolescent school girls aged 14–20 years from 20 different high schools located in three educational areas of Kermanshah, the capital of Kermanshah province in Western Iran. The prevalence of anemia (Hb, 12 mg/dl) among adolescent school girls was 21.4%. Iron deficiency using a ferritin level, 12µg/l was found in 23.7% of studied girls. There were 47 girls (12.2%) with iron deficiency anemia (Hb, 12 g/dl and ferritin, 20µg /l). Around 57.3% of anemic girls were iron deficient. There were no significant differences between the presence of anemia and the level of education of parents. The mean levels of hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) in studied adolescent girls from Western Iran were found to be lower than the standard for females aged 12–18 years. In conclusion, regarding the detrimental long-term effects and the high prevalence of iron deficiency, iron deficiency anemia as well as anemia in Kermanshah, Western Iran and this prevention, in particular, could be a high priority in the programs of health system of the country and a supplementation of weekly iron dose is recommended.

Keywords: iron deficiency, anemia, adolescent girls, Western Iran

P-10-486-1

Comparison of severity of DNA-damage in gastric tissue, levels of nitric oxide and oxidative stress in gastric juice of smoker and non-smoker patients with dyspepsia

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Cigarette smoking is associated with an increased risk of peptic ulcer and gastro-intestinal cancer. Oxidative and nitrosative stress accompanied by toxic materials in smoke and tar has a significant role in production of carcinogenic complexes and injury to DNA and cellular proliferation in gastric cancer. The study was designed to compare the rate of injury to DNA in gastric tissue, the levels of nitric oxide (NO) and severity of oxidative stress in gastric juice of smoker and non-smoker patients with active peptic ulcer. In this study 43 smoker patients with active peptic ulcer (14 female & 29 male) referring to gastroenterology clinic with mean age of 45.30±13.16 as case group, 43 non-smokers without peptic ulcer (13 female & 30 male) with mean age of 42.67±16.04, 43 smoker without peptic ulcer (16 female & 27 male) with mean age of 44.58±12.07 and 43 non-smoker patients with active peptic ulcer (20 female & 23 male) with mean age of 45.37±13.39 were selected as control groups of 1, 2 and 3, respectively. Rate of gastric mucosa DNA damage in the four groups were measured colorimetrically, the levels of nitric oxide in gastric juice using Griess colorimetric method, the activities of superoxide dismutase and glutathione peroxidase and level of total antioxidant capacity in gastric juice were determined by colorimetric method. The DNA damage in gastric mucosa of smoker patients with active peptic ulcer was higher than those of the three control groups (p<0.0001 in all case). Comparing with the control groups 1 and 2, significant elevation in the mean level of nitric oxide in the case group was noticed (p<0.0001 in both cases). In the gastric juice of the case

group the activities of superoxide dismutase and glutathione peroxidase were higher than those of the control groups (p<0.0001 in all cases), while the levels of total antioxidant capacity in the case group were lower than those of the three control groups (p=0.0001, p=0.0001 and p=0.049 respectively). Results of this study approve the direct relation between increase in DNA damage and toxic complexes existing in smoke and tar of cigarette, especially NO radicals. It seems that reactive species of oxygen and nitrogen such as peroxide, peroxy nitrite and new unknown complexes that form mediate the reaction between tar and nitric oxide and play an important role in DNA damage and diseases related to tobacco products such as gastric cancer.

Keywords: cigarette Smoking, DNA damage, nitric oxide, oxidative and nitrosative stress, active peptic ulcer

O-10-487-1

Adiponectin, triglyceride and cholesterol in acute myocardial infarction

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Adiponectin which is secreted by adipose tissues has been reported to have direct antiatherosclerotic effects also plasma adiponectin levels have been shown to be decreased in patients with cardiovascular diseases and hypertension. To determine the relationship between plasma adiponectin levels and serum triglyceride and cholesterol concentrations in patients with acute myocardial infarction and in healthy control group, this case-control study was carried out on 43 patients with acute myocardial infarction (AMI) and 43 age, sex, and other classic cardiovascular risk factors matched healthy persons with normal coronary artery angiography as control group in Jahrom University of Medical Sciences- IRAN, 2006-2007. Blood samples were taken from the patients at first 24h of admission. Plasma adiponectin levels were measured by ELISA method in the patient and the control groups. Plasma adiponectin levels in the patients and the control group were 3.328±1.716 µg/ml and 5.014±1.456 µg/ml, respectively (P<0.001). Serum triglyceride concentrations in the patients and the control group were 146.05±99.595 and 144.23±99.824, respectively (P<0.933). Serum cholesterol concentrations in the patients and the control group were 146.30±45.209 and 171.84±33.765 respectively (P<0.046). There was a significant reverse relationship between plasma adiponectin levels and serum triglyceride (r=-0.056, P=0.002) and cholesterol (r=-0.323, P=0.035) concentrations in the patients, but this relationship was not found in the control group. The results suggest that low plasma adiponectin level may be an acute myocardial infarction risk factor. Further comprehensive studies are needed to explore effect of serum triglyceride and cholesterol concentrations on plasma adiponectin level.

Keywords: adiponectin, acute myocardial infarction, serum triglyceride and cholesterol concentrations

O-10-492-1

In-ovo injection technology: Effects of different Aromatase inhibitors on sex differentiation

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The influence of in-ovo administration of aromatase inhibitors on sex differentiation was investigated. Five hundred fertilized breeder eggs of Ross strain were used as animal model in this experiment. Eggs were divided into five groups which were treated with four different anti-aromatase inhibitors, namely, tomoxifen (TMX), garlic extract (GAR79), tomato extract (TOM86) and clomiphene citrate (CLS). Moreover, water-injection was considered as control (DW). Eggs were sanitized and prepared for incubation in a regular automatic hatchery. At day 5 of incubation period, eggs injection site were cleaned with 70% ethylic alcohol, bored by a needle and anti-aromatases were injected using insulin syringe through thin end of the eggs and then sealed by melted paraffin. Hatched day old chickens were feather sexed to determine Male/Female ratio. Further study was performed to control 42 day old Chicken's gonads alteration when treated with different anti-aromatases. Results showed that GAR79 with highest significant ($p < 0.05$) anti-aromatase activity affects male production (80.43%), followed by TOM86, TMX and DW respectively. It is concluded that GAR79 and TOM86 are of importance with high potential anti-aromatase effects on sex differentiation for different purposes

Keywords: in-ovo-injection, aromatase inhibitors, sex differentiation

P-10-237-1

Role of acylated ghrelin and leptin in puberty and reproductive system of homozygote beta-thalassemic patients

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Recently published animal studies consider leptin and ghrelin as hormones that play a role in puberty and fertility. To determine the function of these hormones in the maturity and fertility of thalassemic patients who are known cases of delay in puberty and fertility, circulating levels of leptin, ghrelin and some reproductive hormones were determined for 98 (59 male and 38 female) beta-thalassemic patients (18-23 years old) and the results were compared with corresponding values obtained for healthy matched control ($n=50$, 27 male and 23 female). Beside lower BMI, all the hormones evaluated for the patients were significantly lower than control groups ($P < 0.001$). Furthermore, leptin/ghrelin ratio of female patients was less than the corresponding values for the control group ($P < 0.0001$). Finally, significant negative correlations ($p < 0.05$) were detected between circulating levels of acylated-ghrelin and LH, FSH, estradiol, or testosterone of male patients. In conclusion, inadequate production of leptin and ghrelin could change the harmony of reproductive hormones in beta-thalassemic patients affecting menstruation cycle of female and pubertal timing of male patients.

Keywords: acylated-ghrelin, leptin, puberty, reproductive hormones, and beta-thalassemia

O-10-237-2

Excess iodine and autoimmune thyroid diseases

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The mechanisms by which excessive iodine is related to the development of thyroid autoimmunity are still unknown although several hypotheses have been put forward; therefore, to determine possible mechanism by which iodine could develop thyroid autoimmune disease, a series of in-vivo, ex-vivo and in-vitro experiments were carried out. Consumption of excess iodine by rats, for 45 days did not affect circulating levels of total and free T4 or T3, but caused pathological changes of thyroid gland resembling alterations usually observed in autoimmune thyroid diseases. Our in-vitro experiments showed albumin has an affinity to bind iodine instantaneously, at 37°C with a saturable mechanism and results of cell culture experiments showed a significant ($P < 0.001$) decrease in the Km of iodine uptake and increase ($P < 0.001$) in the amount of iodine uptake of FRTL-5 cells, when albumin was used as a carrier system for the iodine. Finally, addition of radioactive iodine to the sera of healthy human showed that 10.42±2.76% of total I125 binds to different proteins, while consumption of I131 by thyroid patients showed that 18.88±9.19% binds to proteins with different pattern from the one detected for the healthy subjects. In conclusion the present results may be the first report that introduces carrier systems for the iodine (pre-albumin, albumin, globulin, and lipoproteins) and over iodination of these proteins or changes of their concentration may activate circulating immune cells to attack thyroid gland as a largest iodine store to initiate autoimmune thyroid diseases.

Keywords: excess iodine, autoimmune thyroid diseases, FRTL-5, iodine uptake and iodine carrier proteins

O-10-497-1

A comparative study on the effect of mitomycin C on, chromatin and histone proteins

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Mitomycin C is a potent anticarcinogenic antibiotic which exhibit a broad spectrum of activities against solid tumors. In the cell, DNA has been considered as the main target of mitomycin C which intercalates into DNA double helix and cross links DNA strand. In the cell nucleus, DNA is complexed with histones and makes a defined structure called nucleosomes. Therefore, in fact, chromatin but not DNA is the main target for anticancer drugs. In the present study, we attempted to investigate the effects of anticancer drug, mitomycin C on chromatin and histone protein in solution employing UV/Vis and fluorescence spectroscopy and thermal denaturation techniques. The results showed that in the presence of mitomycin C, the absorbance of chromatin at 210 nm and 260 nm was decreased. Fluorescence emission spectra represented quenching of DNA chromospheres with drug and decreased fluorescence intensity in a dose-dependent manner. Binding of mitomycin C to chromatin shifted DNA thermal denaturation to higher temperature. Also, mitomycin C shows very low affinity to histone proteins. From the results presented above it seems that although histone proteins show binding sites for this drug, the

interaction of histone proteins with the DNA diminishes the binding affinity of mitomycin to chromatin.

Keywords: mitomycin C, chromatin, DNA, histone proteins

P-10-379-1

Barley oxalate oxidase: Purification and study of some properties

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Oxalate oxidase (EC 1.2.3.4) catalyses the conversion of oxalate and dioxygen to CO₂ and H₂O₂. It is used for clinical detection of oxalate in urine and plasma. The enzyme activity has been detected in several species, with the barley enzyme being the best characterized. The barley enzyme has been shown to be 96% identical with a wheat (*Triticum*) protein called germin. Oxalate oxidase activity increases in extracts of barley (*Hordeum vulgare*) leaves in response to the powdery mildew fungus and this is proposed as a source of H₂O₂ during plant pathogen interactions. The germin-like oxalate oxidase are homo-oligomeric, water-soluble, heat-stable, protease-resistant, SDS-tolerant glycoproteins originally known to be expressed in cell walls of cereal embryos at the onset of germination. Oxalate oxidase was purified to homogeneity in several steps from roots of barley seedlings. The purification method comprised (i) ammonium sulphate fractionation. (ii) Thermal treatment and (iii) ion exchange chromatography. For enzyme assay the formation of H₂O₂ by oxalate oxidase was coupled to the horseradish peroxidase-mediated oxidation of DMA (N,N dimethylaniline) and MBTH (3-methyl-2-benzothiazolinone hydrazone). The assays were monitored by an increase in absorbance at 590nm at 37 C. The purified enzyme appeared as a single band with a molecular mass of 26000 Da on coomassie-stained SDS-page. According to this study we purified oxalate oxidase enzyme with over than 620-fold and 40% recovery.

Keywords: oxalate oxidase, barley, purification, germin

P-10-379-2

Conjugation of oxalate oxidase and horseradish peroxidase

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Measurement of oxalate in blood is required for the diagnosis and medical management of primary and secondary hyperoxaluria, chronic renal failure and calcium oxalate nephrolithiasis. Various methods are available for the measurement of oxalate in serum/plasma such as colorimetric method, gas chromatography, isotope dilution and mass spectrometry, high performance liquid chromatography (HPLC) and enzymic colorimetry. The enzymic colorimetric method is simple, sensitive and specific. This method employing oxalate oxidase is based on the following reaction. (COOH)₂ + 2O₂ + 2CO₂ + H₂O₂ MBTH + H₂O₂ + DMA Indamines dye (purple color) + 2H₂O (MBTH=3-Methyl-2-Benzothiazolinone Hydrazone; HRP= Horse Radish Peroxidase; OXO=Oxalate oxidase; DMA= N, N-Di Methyl Aniline). Since oxalate measurement needs two enzymes (oxalate oxidase and peroxidase), and at present these enzymes are used separately, we decided to prepare two enzymes conjugated for different purposes to be used as a complex. This work has more effect on reducing experiment errors and increase speed and accuracy. Oxalate oxidase from barley roots were extracted and purified. Then it was used for conjugation with horseradish

peroxidase. The crude enzyme was purified by ammonium sulphate precipitation, thermal treatment and ion exchange chromatography on DEAE-Sepharose column. In the second part, oxalate oxidase was conjugation to horse radish peroxidase by periodate procedure. The yield of conjugation was analyzed by SDS-PAGE. This conjugate appeared as a single band with molecular mass of 175-185 kDa in non-reducing SDS-PAGE with coomassie-stain. Conjugate produced with periodate retained about 60% of enzymes activity. According to data from this study, the purified oxalate oxidase and conjugate OXO-HRP can be used for production of kits to measure oxalate in biological fluids.

Keywords: oxalate oxidase, horse radish peroxidase, conjugation

P-10-237-3

Circulating acylated-ghrelin and resistin of normotensive and none obese diabetic patients (type I and II)

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Resistin and ghrelin are hormones that have roles in the weight regulation, as well as glucose and lipid homeostasis. Data from human studies regarding resistin and ghrelin in diabetic patients are scarce especially in the cases of non-obese patients. To illuminate some of the missing points, eighty diabetic patients and the same number of matched healthy individuals participated in this study according to the inclusion criteria [age, gender, body mass index (BMI), blood pressure (BP) and diabetes type]. Fasting and postprandial glucose, hemoglobin A1c (HbA1c), insulin acylated-ghrelin, and resistin were evaluated for all the participants while HOMA and QUICKI were calculated. Beside increases in fasting and postprandial glucose, HbA1c and insulin, calculated HOMA increased while QUICKI decreased, among diabetic patients (P<0.001). Patients also had reduced acylated-ghrelin that was more predominate among type I cases (P<0.001), while resistin was significantly reduced among the female patients (P<0.001). Finally, there was a significant negative correlation between insulin and circulating resistin of older healthy subjects [female (R=-0.72, P<0.001) and male (R=-0.59, P<0.01)], which was absent for the patients. In conclusion none of diabetes indicators correlated with the reduction of ghrelin and resistin that may indicate these reductions are the results of protective phenomena due to excess glucose, increase insulin, or high circulating lipids usually observed among diabetic patients. This hypothesis could be confirmed by the strange negative correlation between insulin and resistin among the older (38-55 years) healthy individuals.

Keywords: diabetes (type I and II), acylated-ghrelin, resistin, insulin, HOMA, QUICKI, HbA1c

P-10-294-1

Correlation between fasting ghrelin hormone and body mass index (BMI), age in adult women

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Ghrelin is a 28 amino acid hormone that is secreted by gastrointestinal duct especially of gastric fundus mucosa. It is implicated in energy homeostasis and increase in food intake and reduction of fat utilization. Studies showed that level of ghrelin is related to body mass index (BMI). However, increase of age can affect secretion of ghrelin

because gastrointestinal disability occurs in older age. Thus we determined the relation of ghrelin with body mass index and age in adult women. In this study 33 women were selected with randomized sampling and data including weight and height collected with questionnaire. Then blood samples were collected in tubes containing an antiprotease. Level of ghrelin was determined with ELISA kit. Then data were analyzed with SPSS software. Mean of age of women was 34±6.9 (20-61 old years) and BMI 27.0±3.6 (kg/m²). There was negative relation between ghrelin and age but it was not significant ($r=0.06$, $p=0.72$). BMI had direct relation with ghrelin but was not significant ($r=0.24$, $p=0.17$). Ghrelin had a significant increase between two group of BMI (46.1±24.2 for BMI≤25 vs.117.8±143.8 for BMI>25; $p=0.02$). These data provide evidence that ghrelin can decrease in the elderly that can be a result of decrease of appetite. However ghrelin is important in control of obesity and decrease of ghrelin leads to low food intake. To better understand these effects more study is recommended.

Keywords: age, body mass index, ghrelin

P-10-517-1

The effect of curcumin on insulin amyloid formation

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Amyloid fibrils are highly ordered aggregated states of peptides and proteins and appear in a variety of diseases. Insulin is very prone to form amyloid fibrils under slightly destabilizing conditions such as acidic pH and elevated temperatures. Insulin fibrils have been found at the site of injection of a diabetic patient and in insulin infusion pumps. Compounds that prevent protein aggregation could be of use in these cases. In the present study, the effect of curcumin was investigated on insulin under conditions that promote the conversion of the soluble protein into amyloid fibrils. Curcumin is the active ingredient of turmeric, and was added to the medium containing insulin. Insulin amyloid fibrils were analyzed by transmission electron microscopy, Congo Red binding, Thioflavine-T fluorescence and circular dichroism in the presence and absence of curcumin. The results showed that under in vitro amyloidogenic conditions curcumin decreases fibril formation when present at low concentrations, in micromolar ranges. Curcumin has a symmetrical structure resembling Congo Red but with two aromatic moieties replaced by polar groups, and an overall smaller structure. It is suggested that, like Congo Red, curcumin binds to beta sheets and blocks their self assembly. The effects of curcumin thus may not depend on insulin sequence but on its fibril-related conformation. In summary, the phenolic yellow pigment curcumin was found to be able to suppress amyloid accumulation in insulin. Since there are other reports indicating its effect on abeta fibrillation, the present study is suggestive of a general effect for this compound.

Keywords: curcumin, insulin, amyloid

O-10-285-1

Pre and post kidney transplantation assessment of serum gamma glutamyl transferase and glutathione reductase

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Increased oxidative stress may contribute to the development of accelerated coronary vascular disease in kidney transplant patients. Recently serum gamma glutamyl transferase (GGT) has been proposed as a new sensitive and reliable marker of oxidative stress. Our goal was to evaluate pre and post kidney transplantation changes in the activities of serum GGT and glutathione reductase (GR). Recipients of first kidney (Men, age range: 30-60 years, n=22) were enrolled. Fasting blood samples were obtained on day-1 (pre-transplantation) and then every day from day 1 to 14 post- operationally and there after weekly on days 21, 28, 35 and 42, respectively. GGT and GR activities were measured spectrophotometrically. A group of healthy individuals were included as a control. GGT activity showed an increasing pattern during the study. The activity was 9.67±4.3 U/L on day-1 and 20±10.2 U/L on day 42 (51.5% increase). Besides, GGT activities of patient group were significantly higher most of the time except on day 1, 2 and 3 in comparison to those of the control subjects. Monitoring of GR activity during pre and post operation revealed the same pattern as GGT. When compared to the control group, GR activity was markedly higher in patient ($p<0.000$) during the whole period. In current study an increasing pattern of GGT and GR activities in serum were observed after kidney transplantation. This result confirms the presence of high oxidative stress after this operation which may have a role in tissue injury and dysfunction. Measurement of serum GGT as a marker of oxidative stress is reliable, simple and inexpensive.

Keywords: gamma glutamyl transferase, glutathione reductase, kidney transplantation, oxidative stress

O-10-533-1

A new approach for rapid isolation and purification of alkyl hydroperoxide reductase (AhpC) from Helicobacter pylori

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Alkyl hydroperoxide reductase (AhpC) of *Helicobacter pylori* is known as the virulence factor because of its activity in *H. pylori* survival against oxidative environment of gastric mucosal layer. This conserved antigen has been described as a specific and unique enzyme for *H. pylori* and therefore, both *H. pylori* AhpC and Anti-AhpC could be useful in the development of serologic and stool antigen tests, to detect and monitor *H. pylori* infection. In this study, a new convenient approach has been used to purify it. The isolation and purification of AhpC from *H. pylori* were attempted by various techniques including ammonium sulfate precipitation, dialysis, preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electroelution and in any step, enzymatic activity of AhpC was

determined. AhpC was purified 100-fold with an overall recovery of 60% from clinical isolates of *H. pylori*. This approach is simple, time and cost-saving for purification of AhpC enzyme from *Helicobacter pylori*.

Keywords: alkyl hydroperoxide reductase, AhpC, *Helicobacter pylori*, enzyme purification

P-10-541-1

Effects of conjugated linoleic acid in association with vitamin E on serum Apo B and MDA in type 2 diabetic patients

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It has been reported that Conjugated Linoleic Acids (CLAs) have several health related benefits. CLAs have been shown to have antiadipogenic, antiatherogenic, anti diabetogenic and anti-inflammatory properties. However, results from human studies on risk markers of diabetes are ambiguous. The objective of the present study was to determine the effect of CLAs supplementation (providing equal proportions of c9, t11 and t10, c12 CLA) alone and in association with vitamin E on serum lipid profile, MDA, apo-B100, systolic and diastolic blood pressures in patients with type 2 diabetes mellitus. The study was performed as an 8-weeks randomized double-blind, placebo-controlled parallel intervention. Participants were 41 (19 men and 22 women) type 2 diabetic persons (35 to 50 Y, 30>BMI>25). They were stratified according to sex, age and BMI into three groups. One group was given 3.0 g CLA/d (3×1g capsules; a 50:50 isomer blend of c9, t11 and t10, c12 CLA) with 100 IU/d vitamin E, and the second group, 3.0 g CLA/d with vitamin E placebo. Third group was given CLA placebo (soy bean oil) and vitamin E placebo. Blood pressure and serum lipid profile, MDA, and Apo B were measured before and after the intervention. Obtained data showed no significant differences in serum triacylglycerol, total cholesterol, LDL-C, HDL-C, apo-B100 and MDA on the effect of the intervention. Also, no differences were seen in systolic and diastolic blood pressure before and after the intervention between groups. CLA supplement alone and in association with vitamin E is not acceptable recommendation for correction serum lipid profile, apo-B100 and MDA in type 2 diabetic patients.

Keywords: conjugated linoleic acid, vitamin E, type 2 diabetes mellitus, MDA, apo-B100

P-10-260-1

Determination of salivary amylase activity before and after exam in medical science students

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Salivary amylase activity has been measured during physical stress and exercise by investigators and its changes have been reported. The aim of present study is to measure salivary amylase activity before and

after basic sciences and pre internship exams in medical students in order to introduce the proper index for stress. This study was performed experimentally. Saliva samples as much as 0.2-0.5 ml were taken from medical students before and after basic sciences and pre internship exams (these exams were held in 1385-1386). Salivary amylase activity was measured using spectrophotometry. Salivary amylase activity, before and after the exams, were 36.24±26.99 Iu/l and 20.81±17.09 Iu/L, respectively (p<0.05). Findings of the present study indicate that salivary amylase activity increases in pre-exam stressful period compared with post-exam period. There is a statistically significant difference between the two groups (p<0.05). Therefore, salivary amylase activity measurement is a proper indicator for evaluation of stress.

Keywords: stress, α-amylase, saliva

P-10-547-1

Use of glucose isomerase for isomerization and production of fructose syrup was performed by selected industrial strain of streptomyces olivochromogenes ptcc1456

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Growth of microorganism and production of enzyme in different culture media was studied and effect of different parameters such as phosphate and aeration was evaluated. The growth of microorganism at 30°C caused a production of high amount of enzyme. The production of enzyme was considered in three culture media (A, B, C). Medium (A) was selected for the higher production amount of enzyme. The higher amount of enzyme production was seen in medium A which was 34.9% GIU/ml after 76 hours. The use of baffles in culture flasks increased the amount of enzyme production, five times more. The production of enzyme increased, 1.13 times more, in phosphate deficient medium (p>0.05). The cells containing enzyme (intra cellular glucose isomerase) were separated by centrifuge, 3000/min, and extraction and release of enzyme was performed by ultra sonication, a physical-mechanical method. This method released about 91.9% of total intracellular enzyme. The best length of time for sonication was found to be 5 minutes. Experiments showed that optimum pH and temperature of the enzyme were 7.3-7.8 and 76°C, respectively. The highest activity of the enzyme was observed at pH 5.6 for up 130 min. At the time of isomerization reaction the existence of magnesium ions was necessary and its omission caused a decrease of enzyme activity in isomerization process, but this effect was not necessary for the enzyme activity. Result showed that treatment of glucose syrup at temperatures of 40.9 and 76°C, by the enzyme, caused 46%, 48% and 51% isomerization, respectively.

Keywords: glucose isomerase, fructose syrup, sonication

P-10-583-1

Study of Paraoxonase1 gene polymorphism in a healthy population of Khoramabad

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Human serum paraoxonase (HuPON1: EC 3.1.8.1), a calcium-dependent esterase, is synthesized in the liver and widely distributed in tissues including liver, kidney, intestine, and serum, where it is associated exclusively with high-density lipoprotein (HDL). PON1 belongs to the family of serum paraoxonases consisting of PON1, PON2, and PON3. The 3 human PON genes are located adjacent to each other at bands q21-q22 on chromosome 7. PON1 plays an important role in prevention of atherosclerosis and also protection against organophosphate-induced neurotoxicity. PON1 shows 2 common polymorphisms: Q/R at position 192 and M/L at position 55. In this study, PON1-192 and 55 polymorphisms were investigated in 64 healthy Iranian individuals. Genomic DNA was isolated from whole blood by the Bartlett and White method, and PON1 genotypes were determined by polymerase chain reaction (PCR) amplification followed by restriction isotyping and gel electrophoresis. The chi-square test was used to evaluate the Hardy-Weinberg equilibrium. The genotype frequencies for PON1-Q192R were approximately 47% (QQ), 41% (QR) and 12% (RR) and for PON1 M- 55L, 44% (LL), 44% (ML) and 12% (MM). Thus, the frequency of alleles R, L, Q, and M were 0.33, 0.66, 0.67, and 0.34, respectively. The QQ/ML haplotype was the most frequently seen in Iranians and the RR/MM and QR/MM haplotypes were absent in this population. In conclusion, the frequencies of PON1-192 and 55 polymorphisms in this Iranian population were different from those seen in other Asian populations from Japan and China but similar to European (Caucasians).

Keywords: atherosclerosis, haplotype, paraoxonase, polymorphism, polymerase chain reaction

P-11-38-1

Serum lipid profile alterations in acute leukemia before and after chemotherapy

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The relationship between serum lipid levels and cardiovascular diseases has been shown in many studies, but there has been far less investigation into their relationship with non-cardiovascular diseases. Abnormal serum lipids profiles have been reported in human malignancies. Attempts have been made to correlate serum lipids in leukemia patients with the disease activity and response to chemotherapy. The aim of this study was to evaluate a possible prognostic significance of serum levels of total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), and triglyceride (TG) in patients with acute leukemia before and after chemotherapy. Fasting blood samples were obtained in 39 previously untreated patients at the time of diagnosis and after chemotherapy with complete remission. Venous blood samples were taken and serum was analyzed for lipid profile. Total cholesterol, serum triglyceride, HDL-C and LDL-C were estimated by enzymatic kits. Thirty nine proved cases of ALL and AML with mean age of 24.87 years (9-52 years) were studied out of which 53.8% (21) were males and 46.2% (18) were females. An altered serum lipid profile was observed at the time of

diagnosis of patients. Statistically significant values included elevated TG (194.41±114.16 mg%), reduced TC (128.97± 53.30 mg %), reduced LDLC (82.84±42.22) and reduced HDLC (25.30± 7.66 mg %). After complete remission the lipid profile significantly was different, a reduction in TG (127.84±53.69) and elevated TC (168.01±72.82), LDLC (105.10±40.03), and HDLC (37.38±11.16) were observed. These results support the idea that cholesterol and its fractions (LDLC, HDLC), and TG determination might be considered as useful biochemical and prognostic markers in hematologic neoplasms.

Keywords: acute Leukemia, total cholesterol, serum triglyceride, HDL-C, LDL-C

P-10-540-2

Comparison of adenosine deaminase activity between COPD patients and healthy subjects

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COPD is a subset of obstructive lung diseases that also includes cystic fibrosis, bronchiectasis and asthma. Altered serum adenosine deaminase (ADA) levels have been recorded in various diseases. There are two isoenzymes of ADA in serum; ADA1 and ADA2. The study was planned to evaluate alterations in serum ADA and its isoenzymes levels, if any, in COPD patients and healthy subjects. In this research ADA activity was determined in serum of 90 persons (30 COPD, 30 nonsmoker control and 30 smoker controls). We used colorimetric method for measuring of ADA activity. ADA activity in the COPD and smoker control groups was significantly lower than in non smoker group (18.99±7, 19±9.1 and 22.95±6.7 U/L, respectively). The mean differences between the patient group (COPD) with non smokers control group are statistically significant for ADA2 (p<0.05), whereas there weren't any mean differences between three group for ADA1. In summary, ADA activity decreases in COPD patients. It can be concluded that decrease in enzyme activity with increasing of adenosine may play an important role in the formation of pulmonary injury in COPD patients and their remedy procedure.

Keywords: Chronic Obstructive Pulmonary Disease (COPD), adenosine deaminase (ADA), adenosine, colorimetric method

P-10-596-1

Effect of Gabapentin on liver function tests and some biochemical factors

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Gabapentin is a new anti-epileptic drug. In vitro electrophysiology studies indicate that gabapentin produces blocking of voltage-sensitive sodium channels, resulting in stabilization of hyperexcited neural membranes and inhibition of propagation of synaptic impulses. In this research the effect of gabapentin has been studied on the liver tissue and the activity of liver enzymes (AST, ALT and ALP) and some blood biochemical factor including protein, albumin and BUN have been

measured. In this research 50 adult male rats wistar strain have been used in five groups of ten. Respectively, three experimental groups received 50, 100, 150 mg/kg of gabapentin via intraperitoneal injection for 28 days. Only distilled water as a solvent was injected to the sham group and the control group didn't received any matter. The blood samples were prepared for evaluating the above mentioned factors in serum and the results were analyzed by RA 1000 instrument and among experiments were evaluated by using ANOVA, Duncan, Tukey and t-Test. According to the results, the activity of ALT, AST and protein increased. Albumin and BUN decreased significantly ($P < 0.05$), whereas in plasma the concentration of creatinine, bilirubin and ALP did not show any significant change. We suggest that gabapentin in short-term increases the activity of liver enzyme (ALT, AST) and serum concentration of protein and decreases BUN and albumin concentrations. Gabapentin doesn't affect ALP.

Keywords: gabapentin, liver, rat

O-10-597-1

Purification of three major forms of β -hCG from urine and production of polyclonal antibodies against them

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Urine of pregnant women contains multiple hCG-related molecules. Some partially degraded forms of hCG and its subunits have become of potential clinical importance and nowadays measuring of hCG and its variants are widely used for various purposes. However many efforts have been made for purification of hCG and some of its free subunits and fragments but still it seems that preparing preparations of hCG and hCG β from urine, free of disturbing contaminants to use as clinical procedures and also production of antibodies against the pure forms needs to be explored. In this work an optimized method for purification of major β -hCG forms from pregnant women urine based on ultrafiltration method follows by three successive steps: 1. affinity chromatography on a Concanavalin A-Sepharose column, 2. ion exchange chromatography on a DEAE-Sephacel column and 3. Preparative gel-electrophoresis was proposed and specific polyclonal antibodies against the purified forms were produced. We also propose for the first time a method based on affinity chromatography using trypsin ligand for extraction of β -hCG from urine. Three β -hCG forms with the MW of 28, 32 and 35 kDa were purified and used for production of polyclonal antibodies. Also we could extract a 37 kDa form of β -hCG that probably acts as a trypsin inhibitor.

Keywords: purification, β -hCG, DEAE-Sephacel, preparative gel-electrophoresis, polyclonal antibody, trypsin inhibitor

P-10-600-1

Association of glutathion S-transferase and chromosomal aberrations as a means to determine occupational exposure

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The recognition and therapeutic uses of rays in medicine has drawn attention to its biological effects and dangers for people exposed to it. Research has shown that cell nucleus and chromosomes are the main target of damage due to x-ray. This damage causes chromosome instability and other damages like ring decentric and acentric. Chromosome damager is important because they are related to many diseases including malignancies. On the other hand, x-ray may cause changes in the activities of enzymes involved in the protection of cell for detoxicating. On of these important enzymes is GST. In this study the relationship between GST and chromosome disorder s in environmental lymphocyte in radiotherapists in governmental hospitals of Tehran are studied in comparison with control group This study aims at determining the relationship between GST enzyme activities and the frequency of chromosome aberrations s in environmental lymphocyte in radiotherapists in governmental medical centers with a length of service of more than 5 years. 33 radiotherapist including 19 female with mean age of 31.5 ± 15 and males with mean age of 37.6 ± 14 , having over 5 years of service were the member of the experimental group. 37 of the personnel of the same hospitals including 22 females with an age range of $36/8 \pm 14$ acted as the control group. The conditions for entering the study were length of service over than 5 years, not using drugs, not using acetaminophen, not using antibiotics for one month before sampling, not having a blood related disease and not having a background of X-raying. The same conditions applied to control group except that they didn't work in radiotherapy centers since the sample was small, all the conditions were checked. 5ml blood heparinized was taken from both groups to investigate enzyme activities and chromosome aberrations s. the GST enzyme activities and chromosome aberrations s were investigated with Habig and banding-trypsin G methods, respectively. The study showed that Ring, decentric and acentric chromosome aberrations and GST enzyme activities are significantly more in experimental group than in control group ($P = 0.04$). Results show an increase in the above-mentioned factors in radiotherapists.

Keywords: chromosome aberrations, glutathione s- transferase, radiotherapy

P-11-148-3

Relationship between serum lipid levels and lipid oxidation parameters in type-2 diabetic patients

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Oxidative stress and lipid oxidation participate in many pathological process including diabetic chronic complications. However incidence of oxidative stress and Hyperlipidemia are common in diabetic patients, there are little available data about the relation of plasma lipid levels and serum lipid oxidizability in these patients. The aim was to evaluate the relation of serum lipid levels and lipid oxidation parameters of whole serum in a group of diabetic patients. In this descriptive study

fasting serum lipids and lipoproteins from 60 type-2 diabetic patients were determined and in-vitro copper induced whole serum lipid oxidation was evaluated. Serum lipids were determined by routine lab methods and serum lipid oxidation monitored by the change of conjugated dienes in diluted serum after addition of Cu²⁺. 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. SPSS software and Pearson correlation test were used for data analysis. From lipid oxidation parameters only OD-max showed significant positive correlation with total cholesterol ($r=0.54$, $P=0.01$) and triglyceride ($r=0.4$, $P=0.05$) levels. The results indicate that in diabetic patients, serum levels of lipids, only affects the final extent of in-vitro lipid oxidation and it has little effect on the resistance to and rate of this process.

Keywords: diabetes, oxidation parameters, serum lipid levels

P-10-600-4

Association of glutathion S-transferase and occupational exposure X-ray

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The recognition and therapeutic uses of rays in medicine has drawn attention to its biological effects and dangers for people exposed to it. X ray may cause changes in the activities of enzymes involved in the protection of cell for detoxification. One of these important enzymes is GST. In this study the relationship between GST and X-ray in radiotherapists in governmental hospitals of Tehran is studied and compared to a control group. It aims at determining the relationship between GST enzyme activities and the X-ray in radiotherapists in governmental medical centers with a length of service of more than 5 years. 33 radiotherapist including 19 females with an age mean of 31.5 ± 15 and 14 males with an age mean of 37.6 ± 14 , having over 5 years of service were the members of the experimental group. 37 of the personnel of the same hospitals including 22 females with an age range of 36.8 ± 14 acted as the control group. The conditions for entering the study were length of service over than 5 years, not using drugs, not using acetaminophen, not using antibiotics for one month before sampling, not having a blood related disease and not having a background of X-raying. The same condition is applied to control group except that they didn't work in radiotherapy centers since the sample was small, all the conditions were checked. 3ml blood heparinized was taken from both groups to investigate enzyme activities. The GST enzyme activities were investigated with Habig methods. Results of the study showed that GST enzyme activities and exposure X-ray are significantly more in experimental group than in control group ($P=0.01$). The results show the increase in the above-mentioned factors in radiotherapists.

Keywords: glutathione s- transferase, radiotherapy

P-11-611-2

Low power laser irradiation on the rate of wound healing in diabetic rats. Does it effect?

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A number of published studies demonstrate the beneficial effects of LLLT on the diabetic wounds. We studied the effects of a combination 670 nm and 810 nm diode lasers on the diabetic wound healing parameters in the rats. Of the nineteen rats, 10 underwent the induction of diabetes. Five rats in each diabetic and non-diabetic side underwent low power laser therapy using 670nm diode laser (500mW, 10J, 48s) to the wound context; and 810nm (250mW, 12J, 50s) to the wound margins. Wound area measurement promoted using computer software after digital microscope photography through the days 0, 3, 6, 9, 12, 15, 20 and 24. Comparing two non-diabetic groups, they are not significantly different in their successive measured wound areas, percent open wound area, and wound healing rate over the study period ($P=0.302$, $P=0.604$, and $P=0.364$, accordingly). After 7 days of low level laser therapy in the non-diabetic group, the urine excretion raised evidently in comparison to the control group. Overall, our study showed no better results of measured wound healing parameters in the low level laser therapy group compared to the control ones. Through the measurement of daily urine volume, as an incidental observation, we saw a significant increase in the urine volume of the laser non-diabetic group in comparison to the other groups.

Keywords: diabetic wound, low level laser therapy, wound area, urine volume

P-10-63-1

Inhibition of angiogenesis in diabetes by amygdalin

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Amygdalin or vitamin B17 is a cyanogenic glycoside compound that inhibits cellular oxidizing enzymes and is useful for treatment of cancer or diabetes. Suppression of angiogenesis during diabetes is a recognized phenomenon that is used within the context of diabetic retinopathy and hasn't diminished vision as a complication of treatment. DMEM and Hams F12 cultures were combined together and then 20% fetal calf serum containing vascular endothelial growth factor (VEGF) was added to them. Amygdalin was injected to male streptozotocin-induced diabetic rats. Aortic artery was excised as 2 mm rings from rats and embedded in fibrin gel and incubated at 37°C for 7-14 days. The angiogenesis expression in aortic rings was compared between amygdalin-given and non-given groups of diabetic rats by optic microscope. The endothelial cells culture analysis of aortic rings in diabetic rats revealed the progression of new endothelial cells in non-given group and not progression in another group. The present in vitro assay showed the inhibition of angiogenesis in aortic rings of diabetic rats by amygdalin. This study can be used in application of amygdalin for pharmaceutical purposes and clinical treatment of

diabetic retinopathy. Therefore, the claim that amygdalin has beneficial effects for diabetic patients is supported by this study.

Keywords: amygdalin, angiogenesis, diabetes, retinopathy, rat aortic rings

O-10-584-1

Production and characterization of antimelatonin monoclonal antibodies

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Anti-melatonin monoclonal antibodies (MAbs) were prepared following coupling melatonin to bovine serum albumin (BSA) by Mannich reaction. Balb/c mice were immunized via injection of the melatonin-BSA intraperitoneally. The spleen cells producing high titer of antibody were fused with myeloma cells of SP2/ origin. After two limiting dilutions, two stable clones (AS-H10 and AS-D26) exhibiting best properties were selected for further studies. The class and subclass of two MAbs were found to be IgG(1) and IgG(2a) with lambda and kappa light chains, respectively. Antibodies secreted by these two clones showed high affinity of about 10(9)M. Study of the specificity criteria showed that these clones had no cross reactivity with indolic, aromatic, and imidazole ring-containing compounds, and had high specificity towards melatonin. The calibration curve was constructed with a sensitivity range of 10 ng/mL to 10 mug/mL. In conclusion, these MAbs may be useful for immunoassay of melatonin.

Keywords: melatonin, monoclonal antibodies

P-10-9-1

Assessment of the levels of breast milk biochemical compositions and its influencing factors in mothers referring to Tehran northern and southern health care centers

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All mothers referred to the selected clinics in Tehran northern and southern health care centers entered the study. The levels of breast milk biochemical compositions and the influencing factors between the two centers showed a significant difference between the centers with respect to breast milk Mg levels (P=0.5). The mothers occupation in the northern and southern groups showed that most of them in northern (%96.6) and southern (%94.1) groups, were housewives; and there was not a significant difference between the two groups with respect to occupation (P=0.17). The relation between the biochemical

compositions of breast milk and the mothers occupation in the two groups was not significantly different, statistically (P>0.05). The levels of breast milk triglyceride were associated with dietary carbohydrate in southern group, significantly (P=0.43). The levels of breast milk and dietary Ca, Mg, Cu and Zn were not significant (P>0.05).

Keywords: breast milk, macronutrient, micronutrient

P-10-524-1

Characteristics of type1 diabetic patients with a long duration of diabetes

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Duration of diabetes is an important risk factor for the development of both microvascular and macrovascular complications. However, most clinic populations will have a small cohort of patients with a long duration of diabetes. The aim of the present retrospective study was to assess the characteristics of such a cohort. Demographic and biochemical data and the prevalence of microvascular complications (retinopathy, neuropathy, and nephropathy) were established in 60 Type 1 diabetic patients with a long duration of diabetes. 31 male and 29 female patients aged 65±11.5 years with duration of diabetes 50.7±5.2 were studied. HbA1c was 8.5±1.2, with BMI 26.7±4.5 kg/m², serum, creatinine 120±70 mmol/l, HDL 1.7±0.6, triglycerides 1.3±1.1 mmol/l. Hypertension was diagnosed in 78.3% of patients. 21% of patients without neuropathy had no hypertension. The percentage of diabetic patients with retinopathy was 83.3%, neuropathy 61.7% and nephropathy 31.7% with only 4 patients (6.6%) with no microvascular complications. While the majority of patients (60%) were on ASA and Statin, just 33% were only on ASA and 20% only on Statin. Type 1 diabetic patients with a long duration of diabetes (~50 years) appear to have relatively poor glycaemic control, treated for hypertension and hyperlipidaemia. Further studies are needed to define the characteristics of those who do not develop complications despite long duration of diabetes.

Keywords: diabetes, complications, risk factors

P-10-626-1

Evaluation of the effects of fasting on the oxidative system and susceptibility of erythrocytes membrane to oxidant during Ramadan time

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Fasting in the holy month of Ramadan is one of the religious duties in Islam and most people fulfill this religious duty in our country, Iran. It seems that the level of some blood components antioxidant defense and lipid peroxidation, change during this month. In this study lipid peroxidation was compared before and during the month of fasting by determining malondialdehyde (MDA) of erythrocyte and antioxidant defense including reduced glutathione (GSH), glutathione peroxidase activity (GPX) of erythrocyte, and total antioxidant of plasma. The relationship between fasting and change in erythrocyte MDA and antioxidant defense was investigated. Thirty healthy 35±15 year old

men intending to fast during the holy month of Ramadan were included in the present study. The erythrocyte level of MDA, GPX, GSH and antioxidant of plasma were determined 4 days before the fasting month and also three more times - 9th, 19th, and 28th day of Ramadan. Erythrocytes MDA, as well as susceptibility of erythrocyte lipids to oxidation were determined by Thiobarbituric method, reduced glutathione of erythrocyte by colorimetry method, glutathione peroxidase activity of erythrocyte by NADPH oxidation assay method and total antioxidants of plasma were determined by FRAP assay. The results of biological parameters were calculated by mean of statistical software program (SPSS) and Paired T-test and Friedman test. The amount of oxidative index, showed that the erythrocyte MDA level and susceptibility of erythrocyte lipids to oxidant were decreased significantly during Ramadan ($P < 0.0001$). The GSH and GPX level increased significantly during Ramadan ($P < 0.05$). Total antioxidant levels of plasma increased significantly during Ramadan ($P < 0.0001$). Since MDA and susceptibility of erythrocyte lipids to oxidant decreased and defense antioxidant increased, during Ramadan, it seems that fasting a month may have preventive effects on oxidative stress and degenerative disorders.

Keywords: Ramadan fasting, GPX, GSH, MDA, total antioxidant of plasma

P-10-263-1

Lipid and protein peroxidation in patients with beta-thalassemia major and intermedia

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Iron overload and autoxidation of globin chains are suggested mechanisms for oxidative stress in beta-thalassemia patients. The aim of this study was to evaluate the extent of lipid and protein peroxidation in patients with beta-thalassemia intermedia (TI) and compare the results with sex and age matched beta-thalassemia major patients (TM). The levels of serum thiobarbituric acid-reactive substances (TBARS), protein carbonyls, and ferritin were measured in 32 untransfused TI patients and 31 regularly transfused TM patients. Blood from TM patients was taken just before transfusion. Serum ferritin was measured by ELISA and levels of TBARS and protein carbonyls were measured by spectrophotometric methods. Serum ferritin and TBARS concentrations were significantly higher in the TM patients than in the TI patients ($P < 0.001$). There was no significant difference in levels of protein carbonyls. Our results suggest that the measurement of peroxidation products may be a simple measure of iron toxicity, in addition to the conventional indices of iron status in thalassemia patients. Also, we confirm that TM patients suffer from oxidative stress more than TI patients despite the chelation therapy.

Keywords: beta-thalassemia major, beta-thalassemia intermedia, oxidative stress, protein carbonyls, TBARS

P-10-12-2

Effects of vitamin E on in vitro oxidation and glycation of low density lipoprotein

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The modification of the lipid and apoprotein components of low density lipoprotein (LDL) occurs in the arterial walls. The objective of this study was to investigate the effects of aspirin on LDL oxidation and glycation in vitro. First, LDL was isolated from plasma by ultracentrifugation. Then, each one of different concentration of aspirin were added to LDL fraction and incubated for 3 hr at 37°C and pH=7. The LDL oxidation levels were estimated by measuring conjugated diene (CD) and thiobarbituric acid reactive substances (TBARS) after Cu+2 ions were added. Also, the LDL glycation levels were determined by calorimetric assay after glucose was added. Results showed that all different concentration of aspirin decreased LDL oxidation and glycation reactions, significantly. Their inhibitory effects were proportional to the doses. The results of this investigation demonstrated that aspirin probably inhibited LDL oxidation and glycation, and thus has a role in ameliorating atherosclerotic and diabetic risks of patients.

Keywords: low density lipoprotein, modification, vitamin E

P-10-511-1

Effects of homocysteine on biomarkers of oxidative stress in fructose-fed rats

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Large doses of fructose for a long time induce oxidative stress in humans and laboratory animals. Also it has been suggested that Homocysteine enhances oxidative stress. This study was done to investigate co effects of dietary Homocysteine thiolacton hydrochloride (50mg/kg.w/day) on plasma and tissues biomarkers of oxidative stress in fructose- fed rats (60%). Thirty two male adult Wistar rats of body weight 212±12 g were divided into 4 groups; i.e., Control, Control+Homocysteine, Fructose, Fructose+Homocysteine, for a 6 weeks period. At the end of the experimental period, heparinated plasma was used for measurement of total antioxidant capacity by appropriate kits. The heart and kidney were removed immediately and homogenized in phosphate-buffered saline (pH7.4). Glutathione peroxidase (GPX), superoxide dismutase (SOD) and malondialdehyde (MDA) were measured in tissues homogenate supernatants by appropriate kits. MANOVA, T2 Hotteling test, were used for statistical analysis in SPSS. The Result indicated that fructose, significantly, increased MDA in heart ($p=0.000$) and kidney ($P=0.000$), reduced activity of GPX ($P=0.000$ and $P=0.001$), SOD ($P=0.000$ and $P=0.000$) in heart and kidney, respectively, and total antioxidant capacity in plasma ($P=0.000$). Homocysteine significantly increased MDA in heart

($P=0.070$), decreased SOD in heart ($P=0.011$) and kidney ($P=0.002$), GPX in kidney ($P=0.016$) and non-significantly total antioxidant in plasma ($P=0.063$). In conclusion, although, each of fructose and homocysteine alone increased MDA, reduced activity of GPX and SOD in heart and kidney and total antioxidant capacity in plasma but additive effects of homocysteine with fructose were not significant and homocysteine didn't enhance the effects of fructose.

Keywords: homocystein thiolacton hydrochloride, oxidative stress, total antioxidant capacity

P-10-12-3

Comparison of plasma nitric oxide levels in radiologists and laboratorists

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The various nitric oxide (NO) levels play the key role in atherogenesis, carcinogenesis and inflammatory diseases progression. In this study, the plasma NO levels were measured in radiologists and laboratorists. The present study was undertaken on 50 radiologists and 50 laboratorists and their age and sex matched healthy volunteers were chosen from those working in Hamedan hospitals. Their venous blood samples were collected and the plasma fractions were separated. Then, the plasma NO levels were measured by Griess method and the data analyzed by SPSS program. Results showed that the plasma NO levels in radiologists ($21.32 \pm 0.96 \mu\text{mol/L}$) increased compared to laboratorists subjects ($16.20 \pm 0.73 \mu\text{mol/L}$) with $P < 0.05$ significance. In this study it was demonstrated that the plasma NO levels in radiologists were higher than laboratorists. Therefore, radiologists are more susceptible to the risk from various plasma NO levels.

Keywords: laboratorist, nitric oxide, radiologist

P-10-12-4

Determination of plasma malondialdehyde levels as lipid peroxidation laboratory factor in gestational diabetic patients and comparison with non-diabetic subjects

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The lipid peroxidation reaction displays a key role in atherogenesis, carcinogenesis and infectious diseases. In this study, the plasma malondialdehyde levels were measured in gestational diabetic patients and non-diabetic subjects. The present study was undertaken in 50 gestational diabetic patients and 50 non-diabetic subjects. Their age and sex matched healthy volunteers were chosen from consultants of Hamedan Fatemyeh hospital. Their venous blood samples were collected and plasma fractions separated. Then, the plasma malondialdehyde levels were measured by calorimetric method and the data analyzed by SPSS program. Results showed that the plasma malondialdehyde levels in gestational diabetic patients ($1.49 \pm 0.46 \mu\text{mol/L}$) increased compared to non-diabetic subjects ($0.58 \pm 0.15 \mu\text{mol/L}$) with $P < 0.05$ significance. In this study it was demonstrated that the plasma malondialdehyde levels in gestational diabetic patients were higher than non-diabetic subjects. Therefore, gestational diabetic patients have more sensitivity to the risk from lipid peroxidation.

Keywords: gestational diabetes, oxidative stress, reactive oxygen species

O-10-614-1

Synthesis and evaluation of superoxide dismutase activity of 2-hydroxyacetophenone semicarbazones and thiosemicarbazones complexes

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Free radicals represent one of the main causes of cellular damage and numerous degenerative diseases. Superoxide dismutase (SOD) is an antioxidant enzyme known to protect cells from the toxic effects of superoxide ion by its dismutation into dioxygen and hydrogen peroxide in biological systems. In order to develop new superoxide dismutase mimics, we have synthesized and evaluated the 2-hydroxyacetophenone semicarbazones and thiosemicarbazones complexes for their SOD activity. The novel analogues of 2-hydroxyacetophenone semicarbazones and thiosemicarbazones were synthesized and their copper and zinc complex were obtained by means of coordination with cupric chloride and zinc chloride, respectively. All these compounds have been characterized by IR while ligands have also been characterized by ¹H NMR spectral studies. All test compounds were determined for superoxide dismutase activity by indirect assay using nitroblue tetrazolium (NBT) reduction method. Six copper complexes showed potent SOD activity in vitro. The copper complexes 2-hydroxy-4-methoxyacetophenone semicarbazones and 2-hydroxy-4-methoxyacetophenone thiosemicarbazones were the most active compounds with $IC_{50} = 0.2 \pm 0.13$ and $IC_{50} = 0.27 \pm 0.06$, respectively. These results indicate that the coppered 2-hydroxyacetophenone semicarbazones and thiosemicarbazones may be lead SOD mimics.

Keywords: free radical, semicarbazones complexes, superoxide dismutase, thiosemicarbazones complexes

P-10-383-1

The analysis of serum adenosine deaminase and its isoenzymes activities in patients with acute lymphoblastic leukemia

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Adenosine Deaminase (ADA; EC 3.5.4.4) is an amino hydrolase enzyme. The highest ADA activity was found in lymphocytes. In serum, ADA is known to be divided into two isoenzymes, ADA-1 and ADA-2. In this study, the total ADA and its isoenzymes activities were assayed using (EHNA) that inhibits ADA-1. The evaluation was carried out using

automatic analyzer (RA-1000). 54 patients with ALL included 18 new cases, 22 relapses and 14 remission subjects (1-15 years old). 30 healthy control subjects were also evaluated in this study. The mean±SD values of total ADA in new cases, relapses, remissions and control subjects were found to be 32.68±11.02 IU/L, 34.50±12.71 IU/L, 14.50±1.82 IU/L, 15.40±2.74 IU/L, respectively. The mean±SD ADA-1 activities were measured as, 21.95±9.31 IU/L, 23.16±8.94 IU/L, 5.07±1.20 IU/L, 5.83±1.78 IU/L, respectively. The mean±SD values of ADA2 were 11.45±4.23 IU/L, 11.33±6.85 IU/L, 9.71±2.12 IU/L, 9.56±1.94 IU/L, respectively. Serum total ADA, ADA-1 activities in the ALL patients with new cases and relapses were significantly higher than of healthy control individuals ($p < 0.001$). The values decreased to subnormal levels, during remission phase. b. Sera of the ALL patients showed that the elevated amount of serum ADA was due to increased level of ADA1 due to lymphocytes. The finding shows the correlation of values with immune system. We concluded that serum total ADA and ADA-1 activity might serve as a useful indicator for evaluating the disease activity in patients with ALL.

Keywords: adenosine deaminase, acute lymphoma Leukemia, isoenzymes

P-10-649-1

Sex differences in adenosine deaminase activity of stroke patients

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Adenosine deaminase (ADA, EC 3.5.4.4) catalyzes the irreversible hydrolytic deamination of adenosine to inosine. The purpose of this study was to determine the plasma activities of total adenosine deaminase (ADAT), and its isoenzymes, ADA1 and ADA2, and ADA1/ADA2 ratio of male and female ischemic stroke patients. We determined activities of plasma ADAT, ADA1, ADA2 and ADA1/ADA2 ratio in 30 patients (15 men and 15 women) with acute ischemic stroke within 12 h of the onset of the attack, as well as in 30 control subjects (15 men and 15 women) of comparable age. There were significant differences between the ADA1 activity and ADA1/ADA2 ratio in male and female stroke patients ($p < 0.05$). Compared with male stroke subjects, females had higher ADA1 activity and ADA1/ADA2 ratios. There were no significant difference between activities of ADAT and ADA2 in men and women of the stroke and control groups. In addition, the Canadian Neurological Scale in men was significantly higher than that of women in the stroke group ($p < 0.05$). Our results suggest that the primary mechanism in men with ischemic stroke might involve the reduction of ADA1 activity. The reduction is probably an adaptation mechanism for induced increase in adenosine availability and protection of brain to ischemic injury.

Keywords: adenosine, adenosine deaminase, stroke

P-10-672-1

The association between DNA methyltransferase 3b C-149T genotypes and promoter methylation of P14, E-cadherin and APC2 tumor suppressor genes among Iranian colorectal cancer patients

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Colorectal cancer (CRC) is the third most common cause of cancer death worldwide. Gene silencing due to DNA hypermethylation is a major mechanism for loss of tumor suppressor genes function. Overexpression of DNMT1 and DNMT3B is a common finding in human tumors. The C-149T transition in the promoter of the DNMT3b gene, which significantly increases its transcriptional activity, leads to aberrant de novo DNA methylation status. However, its role in the epigenetic silencing of tumor suppressor genes in CRC is still not well characterized. We investigated the association between DNMT3b genotypes and genes promoter methylation of p14, E-cadherin and APC2 tumor suppressor genes. We genotyped DNA obtained from 110 sporadic CRC patients by PCR-RFLP. Methylation status of p14 and APC2 promoters in tumors was determined by methylation specific PCR. The frequency of DNMT3b genotypes in patients were 23.1% CC, 49.4% CT and 27.5% TT. Hypermethylation was observed more frequently in APC2 promoter than E-cadherin and p14 gene (92.6%, 40.4% and 16.7%, respectively). The DNMT3B CT genotype showed significant association with APC2 gene promoter methylation ($p = 0.023$). We found no significant association between sex, age, smoking status, and site of tumor with hypermethylation of any of the above-mentioned gene in the study group.

Keywords: CRC, methylation, DNMT3b, APC2, P14, E-cadherin

P-10-157-1

Evaluation of renal function in hyperthyroidism patients and normal individuals who referred to Bou Ali Lab of Zanjan city

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Hyperthyroidism is one of the important and common diseases of endocrine glands, such that nowadays many peoples in the world suffer from that and it imposes economic load upon societies. The major purpose of this study is that hyperthyroidism has significant effects on the metabolism of various organs especially renal function, based on existing controls and papers. Renal function analysis has an important role in filtering metabolic wastes and can be clinically useful. In this case-control study, BUN & creatinine indices in two groups of hyperthyroidism persons and control persons are evaluated and compared by calorimetric method. Also, the complete urinalysis was performed in both groups. Hyperthyroidism patients (mostly females and aged 26-66) which were confirmed by endocrinologist were compared to equal number of control group (most female and aged 12-75). Statistical analysis indicated that BUN concentration of serum in Normal persons (14.32 3.48) and that of hyperthyroidism patients (14.89 2.49) had no significant difference, while creatinine concentration of serum in hyperthyroidism patients compared to normal persons had significant difference in all sex and age group. According to above results this study indicates that because of heart

output and therefore GFR increase, and also the presence of muscular atrophy in hyperthyroidism persons, the creatinine concentration of serum has decreased significantly.

Keywords: BUN, creatinine, GFR, hyperthyroidism

P-10-408-1

Antibody titres to heat shock protein 27 are elevated in patients with acute coronary syndrome

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IgG antibody titres to heat shock protein 27 (anti-Hsp27) were measured to determine whether these titres were affected in patients admitted with acute coronary syndrome. Blood samples were taken from 94 patients admitted with acute coronary syndrome. Anti-Hsp27 IgG titres were determined using an in-house enzyme-linked immunosorbent assay (ELISA) in the first and second 12 h after the onset of symptoms and compared with values for 81 age- and sex-matched control subjects. Median antibody titres to Hsp27 in the first sample from patients whose diagnosis was a myocardial infarction (n=42) was 0.41 absorbance units (range 0.28–0.57) and for those with unstable angina (n=52) was 0.31 (range 0.20–0.42), both being significantly higher than for controls (n=81), which was 0.08 (range 0.05–0.15) (P<0.05). However, titres fell in the second samples collected in the coronary syndrome patients and were then no longer significantly different from controls (P>0.05). Myocardial infarction patients also had significantly higher anti-Hsp27 titres in the first 12 h than patients with unstable angina (P<0.05), but again the difference in the second sample did not reach statistical significance (P>0.05). Serum antibody titres to Hsp27 rise and fall rapidly after the onset of acute coronary syndrome, and may be an early marker of myocardial ischaemia as patients with myocardial infarction or unstable angina both had high titres.

Keywords: acute coronary syndrome, antibody titre, ELISA, heat shock protein 27, myocardial infarction

P-10-697-1

Effect of garlic/ezetimibe combination on lipid profile and atherosclerosis risk factors in hypercholesterolemic mice

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Using herbs for lowering blood glucose and lipids to normal levels is clinically important. In this respect, garlic is one of the medicinal plants, which has shown hypocholesterolemic effects. Ezetimibe is also a novel and effective lipid lowering medicine that is well tolerated by

the patients and has a safety profile similar to that of placebo. This study was aimed to evaluate the combination effect of aquatic extract of garlic and ezetimibe on lipid profile and glucose in hypercholesterolemic mice. A total of forty N-mary male mice were randomly divided into five groups. Group 1 received: chow+2% cholesterol+0.5% cholic acid, group 2: chow+4% garlic extract+2% cholesterol+0.5% cholic acid, group 3: chow+0.005% Ezetimibe+2% cholesterol+0.5% cholic acid, group 4: chow+4%garlic+0.005% Ezetimibe+2% cholesterol+0.5% cholic acid, and group 5: chow only. After four weeks mice were sacrificed, blood was collected, liver weight was measured and lipid profile and glucose levels were determined enzymatically. Compared with hypercholesterolemic mice, ezetimibe plus garlic significantly decreased cholesterol level (P<0.000), low-density lipoprotein cholesterol levels (P<0.000), liver weight (P<0.001), %liver/body weight (P<0.02) and atherogenic index (P<0.005). The findings showed that the combination of garlic and ezetimibe was more effective than garlic and ezetimibe alone in improving the lipid profile.

Keywords: garlic, Ezetimibe, hypercholesterolemic mice

P-10-704-1

Synthesis, characterization of 1,10-phenanthrolinedithiocarbamate Pd(II) nitrate complexes and their interaction with calf thymus DNA

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Two new palladium (II) dithiocarbamate complexes of general formula [Pd(Phen)(R-dtc)]NO₃, where R is piperidine- or nonyl-dithiocarbamate and phen is 1,10'-phenanthroline have been synthesised. These complexes were characterized by FT-IR, UV-Vis and 1H NMR spectroscopy as well as conductivity measurement and elemental analysis. 1H NMR and particularly infrared spectral studies of these palladium complexes suggested that the dithiocarbamate ligands coordinate as symmetrically bidentate and the C–N bond of dithiocarbamate bounded to palladium have more double bond character as compared to that of dithiocarbamate sodium salt. The molar conductance values of these palladium complexes in water suggest them to be 1:1 electrolyte. The above two complexes have partially been interacted with calf thymus DNA. Both the complexes interact with DNA and their binding with DNA is sufficiently strong. Moreover, both of these complexes can denature the DNA and their [L]_{1/2} (the concentration of the complexes in the midpoint of transition) are 0.48 and 0.46 for piperidine- and nonyl-dithiocarbamate complexes respectively.

Keywords: dithiocarbamate, palladium (II) complex, DNA binding

P-10-428-1

Biochemical markers troponin I, leptin and CK-MB in diagnosis of coronary artery disease

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Coronary artery disease (CAD) has a high prevalence and percentage worldwide. Establishment of new methods in prognosis of the diseases is important for the scientists. The aim of this study was to assess the role of these biochemical markers in diagnosis of CAD patients. This has been a case series study on 100 patients suffering for CAD. In their serum, troponin I, leptin and CK-MB was measured. Troponin I levels were determined by two-site immunoluminometric assay. Leptin and CK-MB levels were determined by ELISA. These results show that measurement of troponin I and CK-MB as a gold standard is a suitable diagnostic method for MI development. Also two biomarkers; i.e., leptin and troponin I are suitable markers in diagnosis of CAD patients. Troponin I is not found in other tissues throughout the body other than cardiac tissue, therefore, troponin I is proposed as an ideal marker for infinitive diagnosis of AMI.

Keywords: cardiac troponin I (cTnI), leptin, myocardial infarction (MI), CK-MB, coronary artery disease (CAD)

P-10-147-1

Small dense low density lipoprotein, lipids, and lipoproteins levels in patients with coronary artery stenosis and healthy individuals

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Low density lipoprotein (LDL) particles are heterogeneous with respect to their size, density and lipid composition. Among LDL particles, the smaller and denser LDLs (Small dense (sd) LDL) are believed to be atherogenic, since these particles are taken up more easily by arterial wall. They are readily oxidized and have reduced affinity for LDL receptor and increased affinity for arterial proteoglycans. Therefore, they are strongly associated with development of coronary artery disease (CAD). The aim of this study was to compare sdLDL levels in CAD patients and healthy individuals. In this comparative cross-sectional and case-control study the sdLDL levels were determined in 86 patients with coronary stenosis, 35 patients without coronary stenosis identified by angiography and 30 healthy individuals. SdLDL was measured by a direct homogenous LDL-C assay in the supernatant of sera that remained after heparin-magnesium precipitation. The results of ANOVA test showed that the sdLDL levels were higher in patients with coronary stenosis than in patients without coronary stenosis and healthy individuals (respectively, 21.54 ± 7.1 , 16.88 ± 4.4 and $15.45 \pm 5 \text{ mg/dl}$, $p=0.001$). In addition, Linear regression analysis and Pearson correlation coefficient revealed that sdLDL levels were positively correlated with serum triglyceride ($r=0.494$), total cholesterol ($r=0.354$) and LDL-C ($r=0.749$) and inversely correlated with HDL-C ($r=-0.586$) ($p<0.01$). The results suggest that patients with increased levels of sdLDL are at high risk for coronary artery stenosis.

Keywords: small dense LDL, coronary artery stenosis, lipoprotein

P-10-420-1

Evaluation of effects of Clofibrate, Nicotinic acid and their combination on the concentration of lipoproteins, triacylglycerol and total cholesterol in patients with cardiovascular diseases

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Some studies have shown that the use of antihyperlipidemic drugs such as Nicotinic acid, Clofibrate, and their combination reduce the risk of cardiovascular diseases. These drugs also reduce atherogenic lipids and lipoproteins and increase HDL-Cholesterol. 92 patients suffering from cardiovascular diseases were investigated: 78 patients received Clofibrate (1gr twice daily), 7 patients received nicotinic acid (increasing doses of nicotinic acid up to 2-21 gr daily) and 7 patients received combination of Clofibrate and nicotinic acid (Clofibrate 1 gr twice daily and plus increasing doses of nicotinic acid up to 2-2.1 gr daily). The serum concentration of total cholesterol, triglyceride, LDL-C and HDL-C were measured before treatment, six and fifteen weeks after treatment, respectively. Treatment with Clofibrate, after six weeks, significantly ($p<0.001$) reduced the total cholesterol (13%), triglyceride (20%), LDL-C (16%). This drug also significantly increased the HDL-C (23%). After 15 weeks treatment with Clofibrate a further decrease in total cholesterol (25%), triglyceride (55%), LDL-C (27%) and further increase in HDL-C (32%) were also shown. Treatment with Nicotinic acid after 6 weeks significantly ($p<0.001$) reduced the total cholesterol (6%), triglyceride (11.5), LDL-C (6%). Nicotinic acid also significantly increased the HDL-C (14%). After 15 weeks treatment with the combination therapy a further decrease in total Cholesterol (30%), triglyceride (54%), LDL-C (29%) and further increase in HDL-C (28%) were also observed. Combination therapy (Clofibrate plus Nicotinic acid) for the patients suffering from cardiovascular diseases is more effective than either of the drugs alone in reducing levels of the atherogenic factors, total Cholesterol, LDL-C.

Keywords: HDL-C, LDL-C, triglyceride, nicotinic acid, clofibrate

P-10-502-1

Relationship between seminal antioxidant enzymes and the phospholipid and fatty acid composition of spermatozoa

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Sperm cell membranes are susceptible to peroxidative damage through an excess of reactive oxygen species. The objective of this study was to determine seminal plasma glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity and relate these to phospholipid profiles and phospholipid-esterified fatty acid composition of spermatozoa. Seminal plasma GPX and SOD activities, phospholipid, phospholipid-esterified fatty acid composition and malondialdehyde (MDA) of spermatozoa were assayed in 10 normozoospermic and 25 asthenozoospermic subjects. Mean seminal GPX and SOD activity in normozoospermic men were not significantly different from asthenozoospermic men. A significant positive correlation was

observed between seminal plasma GPX activity and phosphatidylcholine content ($r=+0.77$, $P=0.037$) and there was a significant negative correlation with lysophosphatidylcholine content ($r=-0.89$, $P=0.02$) in normozoospermic sperm samples. Positive correlations were found between SOD activity and polyunsaturated fatty acid composition of spermatozoa. MDA content in the spermatozoa of asthenozoospermic subjects was significantly higher than in normozoospermic males ($P<0.05$). Negative correlations were found between MDA content and seminal SOD activity and arachidonic acid content of spermatozoa from normozoospermic samples ($r=-0.5$; $P=0.046$, $r=-0.9$; $P=0.001$, respectively). Seminal plasma GPX and SOD provide protection against lipid peroxidation of phospholipid and phospholipid-bound fatty acids in normozoospermic samples.

Keywords: glutathione peroxidase, phospholipid, polyunsaturated fatty acid, semen, superoxide dismutase

P-10-731-1

Urinary albumin excretion and hs-CRP levels as risk factors of coronary disease in premenopausal women

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Urinary albumin excretion is independently associated with C-reactive protein levels in over weight and obese nondiabetic premenopausal women. C-reactive protein (CRP) and microalbuminuria are considered markers of chronic inflammation of the arterial wall and of endothelial dysfunction, respectively. Increases of CRP levels and urinary albumin excretion (UAE) rate have both been reported to be independently associated with a higher risk of cardiovascular morbidity and mortality in the general population. The aim of this study was to evaluate the possible correlation between UAE and CRP concentrations in over weight and obese premenopausal women. A cross-sectional study was done in 100 overweight and obese premenopausal women, aged 18-45 years. Other measurements included, blood pressure, fasting plasma levels of glucose and lipids. Urinary albumin excretion was positively correlated with body mass index (BMI) ($P<0.01$), diastolic blood pressure ($P<0.05$), triglycerides ($P<0.01$) and CRP levels ($P<0.05$), and negatively with HDL cholesterol ($P<0.001$). After multivariate analysis, diastolic blood pressure, HDL cholesterol, and CRP levels maintained their significant correlation with UAE. The study shows a strong relationship between UAE and CRP concentrations, irrespective of age and other variables. On this basis, it can be argued that inflammation of the arterial wall, as indicated by higher CRP plasma levels, and endothelial dysfunction, as shown by higher UAE rate, might represent simultaneous phenomena in the development of atherosclerosis in overweight and obese premenopausal women.

Keywords: urinary albumin, excretion, C-reactive protein, atherosclerosis

O-10-740-1

Seminal protein profiles of the varicocele and healthy men by SDS-PAGE

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Varicocele recognized is a major cause in the etiology of male infertility, but its exact mechanism of action on sperm function and infertilization is still controversial, it is also clear that a significant proportion of men with a varicocele are fertile. Twenty normozoospermic semen samples with age range 24-35 years old, including 10 healthy and 10 varicocele men were collected into sterile containers after a period for 2-3 days of abstinence (attending the infertility clinic). Sperm parameters analyzed according to World Health Organization guidelines reported in 1999. The seminal plasma protein content of each sample was determined by the Bradford method. SDS-PAGE on 10% polyacrylamide resolving gel was performed with a same amount of protein concentration (5.7 mg/ml). Bradford protein assay revealed no significant difference (p value=0.832) between varicocele (8.26 ± 3.34) and healthy (8.56 ± 1.35) men. Protein bands were detected with molecular weights in the range of 14.4-94.95 kDa in both groups. Pattern of seminal plasma proteins by SDS-PAGE showed various expressions of bands in patients between 18.4-38.5 kDa as compared to healthy men. Although the etiology of varicocele is multifactorial, but low and high individual gene expression could be one of the effective factors in varicocele patients and protein profile may be useful for identification of seminal secretory markers which merits further investigation.

Keywords: healthy men, normozoospermic, protein profile, SDS-PAGE, varicocele

O-10-495-1

Ischemia modified albumin in acute coronary syndrome

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Ischemia Modified Albumin (IMA) is a sensitive and early biochemical marker of ischemia. The aim of this case-controlled study was to evaluate the sensitivity and specificity of IMA in the diagnosis of acute coronary syndrome. Serum IMA levels measured in 70 patients presenting to the emergency department within three hours of acute chest pain and 90 age and sex matched healthy controls. The levels of IMA were determined by addition of known amount of Cobalt (II) to a serum specimen and measurement of the unbound cobalt (II) by colorimetric assay using dithiothreitol (DTT). Cardiac troponin I (cTnI) levels were assayed and electrocardiogram (ECG) recorded in patients with acute chest pain. Results of IMA, ECG, and cTnI, alone and in combination, were correlated with final diagnoses of unstable angina and myocardial infarction. Mean serum IMA levels were significantly higher in acute coronary syndrome patients (0.685 ± 0.16 absorbance units (ABSU)) compared to control group (0.243 ± 0.06 ABSU). However, there was no statistically significant difference between the mean IMA levels in unstable angina patients (0.678 ± 0.32) and MI patients (0.685 ± 0.32 ABSU). The optimal diagnostic cutoff point for IMA in this study population was found to be 0.49 ABSU by ROC analysis. IMA was 86% sensitive and 88% specific in the diagnosis of

acute coronary syndrome. Findings indicate that IMA may be used as an alternative biochemical parameter for clinical diagnosis of acute coronary syndrome. However, IMA levels can not discriminate unstable angina patients from MI patients.

Keywords: acute coronary syndrome, ischemia modified albumin

P-10-500-1

C-reactive protein associated with coronary artery disease in Iranian patients with angiographically defined coronary artery disease

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Several cross-sectional and cohort studies have reported an association between serum markers of inflammation such as C-reactive protein, and coronary heart disease in Caucasian populations. We aimed to investigate the relationship between levels of serum C-reactive protein (hs-CRP) and the presence of coronary artery disease (CAD) in patients undergoing coronary angiography. Serum hs-CRP, fasting lipid profile and blood glucose levels were measured in 462 patients (262 males and 200 females) undergoing routine coronary angiogram. Anthropometric features including blood pressure were determined using standard procedures. Demographic characteristics, including smoking habit were assessed by questionnaire. Of the 462 subjects undergoing angiography, 335 (72.5%) had CAD and 110 (23.8%) had a normal angiogram. Mean age ($p < 0.000$), waist circumference ($p < 0.042$) and median values of hs-CRP ($p < 0.000$) and triglycerides ($p < 0.014$) and height ($p < 0.035$) and Weight ($p < 0.031$) and FBS ($p < 0.041$) were higher in the patients with CAD than in the subjects with a normal angiogram. Serum hs-CRP concentrations were significantly higher in low density lipoprotein (LDL) ($r = 0.148$, $p < 0.008$). Total cholesterol ($r = 0.127$, $p < 0.021$), and serum HDL-c ($r = 0.207$, $p < 0.000$), systolic blood pressure ($r = -0.115$, $p < 0.041$) correlated with serum hs-CRP. Serum hs-CRP is an independent predictor of angiographically defined CAD in an Iranian population. Measurement of the serum hs-CRP level may improve risk stratification among patients suspected of having CAD. The strong correlations between serum hs-CRP with LDL and smoking may be due to the putative pro-inflammatory effects of these two parameters. The association with serum triglycerides may be indirect and related to insulin resistance and adiposity

Keywords: angiography, coronary artery disease, C-reactive protein

P-10-188-1

Phenotyping human serum paraoxonase (PON1): The ratio of arylesterase to paraoxonase activity

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Insecticides such as organophosphate (OP) and carbamate compounds are used in pest control for agriculture and household purposes and intoxication with these compounds has become a serious global health problem. Human paraoxonases-1 which is attached to high density lipoproteins (HDL) seems to be one of the most important detoxifying enzymes. Patient samples were obtained from angiography department of Baqiyatallah hospital and apparently healthy control samples were also selected for phenotyping. Using paraoxon as the substrate, 3 peaks of paraoxonase activity were found: RR phenotype had an activity more than 190 U/L, QR phenotype activity was 95-189 U/L and QQ phenotype had an activity less than 95 U/L. Thus, Phenotyping this enzyme might help to differentiate the susceptible people, working in toxic environments. The current study was to evaluate PON1 phenotype and the frequencies of polymorphisms at PON1 192 in a Persian population. The studied population consisted of individuals from distinct families ($n = 63$) of either gender, 59.24 ± 9.1 years old. Serum PON1 activity was assayed using phenylacetate and paraoxon as substrates.

Keywords: insecticides, arylesterase to paraoxonase ratio, phenotype

P-10-623-2

The cytotoxicity and genotoxicity effect of cadmium on mice liver

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Cadmium is one of the most toxic environmental pollutants affecting cytogenetically the various mammalian organs. The toxic effects of heavy metals have been studied over many years; inconsistent results have been obtained about their mutagenic and carcinogenic properties. In this study 15 mice (25-30gr) were divided in to 3 groups (1 control and 2 treated groups). The test groups were exposed to different concentrations of cadmium chloride solution (5ml/kg of 300 μ M) injected intraperitoneally every 24 hours for five times. 24h after last injection, the mice were killed and their livers were obtained. The cytotoxicity & genotoxicity damage in mice liver exposed to cadmium solution were evaluated using cammet assay for genotoxicity, and measurement of malondialdehyde (MDA) and glutathione (GSH) concentrations as well as alanine transaminase (Alt) and aspartate transaminase (Ast) activities, for cytotoxicity & oxidative stress. Our results showed significant increase in malondialdehyde concentration and lipid peroxidation, whereas liver glutathione concentration was decreased than the normal values, the liver enzyme activities and genotoxicity was increased appropriately with lipid peroxidation and toxicity with cadmium in liver cells. The results clearly showed that cadmium induced cytotoxicity and genotoxicity and damaged the mice livers. We suggest that the cadmium effect probably is through the oxidative damage and therefore using antioxidant could reduce the liver cadmium toxicities.

Keywords: cadmium, cytotoxicity, genotoxicity, commet assay, malondialdehyde

P-11-763-1

Determining the risk of coronary heart diseases (CHD) using 1H NMR spectra of plasma lipoprotein with the help of pattern recognition techniques

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1H NMR spectroscopy of human plasma in parallel with pattern recognition techniques could provide specific information on relation between different classes of lipoproteins and that of classification of healthy volunteers and those exposed to the risk of coronary heart diseases (CHD) due to variation in metabolites fingerprint content of blood plasma. Principal analysis (PCA) as a dimension reduction tools was applied on normalized spectral 1H NMR data. Abstract factors from PCA were used to build a classification model using the linear discriminant analysis (LDA) as supervised classification model. Result confirmed the ability of 1HNMR spectroscopy and multivariate pattern recognition techniques to build a model to properly classify patient with risk of CHD up to 97% for test sets. We conclude that 1H NMR spectroscopy with the help of LDA modeling could be useful techniques in classifying the CHD with that of normal in relation with metabolite fingerprint.

Keywords: coronary heart disease (CHD), 1H NMR, lipoprotein, pattern recognition

O-11-763-2

Diagnosis of Thalassemia using 1H NMR spectrum of human blood serum and linear vector quantization

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High resolution 1H NMR spectroscopy of biofluids is good representative for metabolic pattern and offers a high potential noninvasive technique for pathological diagnosis. Diagnosis of thalassemia as an inherited autosomal recessive blood disease can be performed by using 1H NMR spectra of human blood serum in parallel with pattern recognition techniques which gives valuable information and leads to proper diagnosis of thalassemia. 1H NMR spectra from twenty eight samples were collected from 15 adult male and female thalassemia patients as experimental set and 13 healthy volunteers as control set. Principal component analysis (PCA) as a dimension reduction tool was used for transforming spectra to abstract factors. The abstract factors introduced to Linear Vector Quantization (LVQ), which is an artificial neural network (ANN) technique for classification purposes, in order to establish adequate model for discrimination between healthy and sick samples. Assessing the quality and robustness of the built model was performed by using test set (left out samples in training algorithm). Therefore, three different arrangement of test set with 7 members were used. Number of abstract factors from PCA and number of supposed clusters (neurons) in LVQ algorithm are important parameters and optimized to minimize the number of misclassifications in test set. Built model using 6 abstract factors as

input for LVQ which trained with 7 clusters successfully classified all the members of the test sets except just one member of third test set. We conclude that 1H NMR spectroscopy and LVQ class modeling assisted by PCA could be useful techniques to effectively classify thalassemia and normal individuals.

Keywords: NMR, Linear Vector Quantization, thalassemia, principal component analysis (PCA)

P-10-707-1

A study on biological significance of activin A and follicle stimulating hormone (FSH) on the growth and maturation of mouse preantral follicles and enclosed oocytes in vitro cultures

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In vitro maturation (IVM) of oocytes is a promising technique to reduce the costs and avert the side-effects of gonadotropin stimulation for in vitro fertilization (IVF). We investigated the effects of FSH and activin A on the in vitro maturation of mouse follicles and enclosed oocytes. Intact preantral follicles were isolated from the ovaries of 6 week-old female mice and cultured in TCM-199 medium. Special quantities of FSH and activin A were added to the culture medium (containing 25-30 follicles) during separate experiments: 5, 20, 40, 60, 100, 140, 180 and 220M IU/l of FSH and 20-180ng/ml of activin A. Follicles were cultured, for 6 days, in an incubator at 37°C, 92% humidity and 5% CO₂ in air. FSH concentration of 100 IU/l showed significantly increased follicle diameter, survival, germinal vesicle breakdown (GVBD) and oocyte maturation rates. Activin A concentration of 100ng/ml resulted in a significant increase in follicle diameter (170 µm) with the survival rate of 73% as compared to the control (100 µm and 25%, p<0.05). The number of oocytes matured and the percentage of germinal vesicle breakdown (GVBD) were 61 and 70%, respectively, as compared to the control (20 and 29%, p<0.05). Follicle diameter (190 µm) and survival rate (85%) increased significantly in the presence of 100mIU/ml of FSH as compared to the control (p<0.05). But the administration of activin A+FSH increased the effect of both factors on follicular diameter (205 µm as compared to 100 µm in control, p<0.01), Follicle survival, oocyte maturation and GVBD rates were 91, 81 and 89%, respectively (p< 0.01). These results have suggested that exposure to FSH and activin A before the formation of antral cavity had positive effect on follicle survival and oocyte robustness.

Keywords: follicle stimulating hormone, activin A, preantral follicles

O-10-707-2

An in vitro investigation of the effects of chorionic gonadotropin and FSH on the maturation of mice follicles

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Progress in the treatment of human and animal infertility is directed towards the development of effective culture conditions for in vitro maturation of oocytes. In recent study, the effects of FSH and

chorionic gonadotropin on the in vitro maturation of mouse follicles and enclosed oocytes were investigated. For experiment, intact preantral follicles were isolated from the ovaries of 6 week-old female mice and cultured in TCM-199 medium. Special quantities of FSH and chorionic gonadotropin were added to the culture medium (containing 25-30 follicles) during separate experiments: 5, 20, 40, 60, 100, 140, 180 and 220 IU/ml of FSH and 1.4 IU/ml hCG. Follicles were cultured, for 6 days, in an incubator at 37 °C, 92 % humidity and 5% CO₂ in air. FSH concentration of 100 IU/ml showed significant increased follicle diameter, survival, germinal vesicle breakdown (GVBD) and oocyte maturation rates. In the medium containing FSH and 1.4 IU hCG, the ovulation percentage reached a maximum of 97% as compared to that seen for FSH-containing medium only (81%) or in control experiment (10%). So, the effect of FSH + hCG was highly significant over the control medium ($p < 0.0001$). Recombinant hCG and FSH are effective in promoting oocyte maturation in a clinical IVM program when administered in combination. In the medium containing 100 IU/ml FSH, 1.4 IU hCG and other growth parameters, the ovulation percentage reached a maximum of 97% as compared to that seen for FSH-containing medium only (81%) or in control experiment (10%). So, the effect of FSH + hCG was highly significant over the control medium ($P < 0.0001$). It is concluded from the experiment that FSH and hCG increase the ovulation percentage of follicles and enclosed oocytes but if they are supplied in a combination, this growth rate can increase significantly.

Keywords: follicle stimulating hormone, ascorbic acid, preantral follicles

P-10-322-1

Gamma glutamyltransferase (GGT) is a novel risk factor for CAD

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Coronary artery disease (CAD) is a leading cause of mortality worldwide. In addition to traditional risk factors for CAD development recent epidemiological studies have revealed that GGT is a marker of oxidative stress and is associated with several CAD risk factors. The aim of this study was comparison of traditional CAD risk factors in tertiles of GGT. 149 patients with angiographically documented CAD were selected. Serum GGT activity was determined spectrophotometrically. Lipids, lipoproteins and apolipoproteins were analyzed by enzymatic or immunochemical techniques. Based on serum GGT activity, patients were divided in to 3 equal groups: Tertile 1: n=50, GGT activity < 8.2 U/L; tertile 2: n=50, GGT activity = 8.2-13.14 U/L and tertile 3: n=49, GGT activity > 13.14 U/L. Traditional CAD risk factors were compared in tertiles of GGT. Compared with tertile 1, patients in tertile 3, exhibited higher ratio of LDL/HDL, total cholesterol (TC)/HDL and TG/HDL (2.3±0.5 vs. 2.8±0.9, $p=0.006$; 3.8±0.6 vs. 4.6±1.0, $p=0.002$; 3.5±1.7 vs. 5.7±3.1, $p=0.000$, respectively). Also higher ratio of TC/HDL and TG/HDL in tertile 2 were noted in comparison with tertile 1 (4.5±1.3 vs. 3.8±0.6, $p=0.004$ & 5.3±3.2 vs. 3.5±1.7, $p=0.004$, respectively). Significantly higher level of HDL in tertile 1, compared with tertile 2 and tertile 3 was observed (45.6±7.1 vs. 41.4±7.1, $p=0.011$ & 45.6±7.1 vs. 39.8±7.3, $p=0.000$, respectively). Correlations were obtained between GGT and TG, HDL, LDL/HDL & apoB100/apoA1 ratio. Serum GGT activity is a low-cost assay. This assay may prove useful tool in clinical trial aimed at identifying individuals with risks for CAD.

Keywords: coronary artery disease (CAD), gamma glutamyltransferase (GGT), oxidative stress, traditional CAD risk factors

P-10-322-2

Inflammatory and oxidative stress markers in hypertensive patients

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Hypertension is an established major risk factor for coronary artery disease (CAD). The objective of current study was to compare the inflammatory and oxidative stress markers in CAD patients with and without the history of previous hypertension. 114 male patients with angiographically documented coronary artery disease (CAD) were recruited and divided in to two groups: 1) with the history of hypertension (n=37, mean age: 54.7±10.6) and 2) without the history of hypertension (n=77, mean age: 56.7±9.8). Oxidative stress markers i.e. serum gamma glutamyltransferase (GGT) and glutathione reductase (GR) activity and ferric reducing antioxidant power (FRAP) were determined spectrophotometrically. Inflammatory markers i.e. high-sensitivity C-reactive protein (hs-CRP) and soluble intracellular adhesion molecule-1 (sICAM-1) were determined by ELISA whilst fibrinogen levels were analyzed by the Clauss methods. Increased hsCRP and fibrinogen levels as well as GGT and GR activities were seen in group 1 compared with group 2 (1.39±0.22 mg/L vs. 1.21±0.19 mg/L, $p=0.021$; 453.9±129.5 mg/dl vs. 406.5±103.9 mg/dl, $p=0.041$; 15.7±9.0 U/L vs. 12.2±6.4 U/L, $p=0.022$ and 37.8±5.8 U/L vs. 34.8±6.9 U/L, $p=0.025$; respectively). Correlations were obtained between GGT & GR ($r=0.290$, $p=0.002$); GGT & hsCRP ($r=0.209$, $p=0.033$); GR & hsCRP ($r=0.379$, $p=0.000$); fibrinogen & GR ($r=0.303$, $p=0.001$) and fibrinogen & hsCRP ($r=0.391$, $p=0.000$). This study has shown that CAD patients with hypertension are exposed to increased inflammation and higher oxidative stress than control group. These findings indicate that simultaneous assessment of inflammatory and oxidative stress may prove useful in clinical trial aimed at treatment or prevention of CAD.

Keywords: coronary artery disease (CAD), hypertension, inflammation, oxidative stress

P-11-804-1

Malnutrition a risk factor for myocardial infarction in patients with type-2 diabetes

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In patients with type-2 diabetes mellitus, the association between malnutrition and cardiovascular disease may be stronger than that in non-diabetic individual; no studies have been done to determine the relationship between malnutrition and myocardial infarction in patients with type 2 diabetes. So, the objective of present study is to evaluate the relationship between malnutrition and M I in patients with type-2 diabetes. In this cross sectional comparative study, we assessed the nutritional status of MI patients with type 2 diabetes mellitus. Patients were malnourished (low body weight) if their body mass index was less

the 20 kg m⁻². Plasma concentrations of Total Protein, Albumin, Globulin, and A/G ratio were used as a measure of visceral protein stores. Fat stores were assessed from body fat. Selected area of this study was Southern Sindh, Pakistan. The blood samples were collected from the MI patients with type-2 diabetes. Number of patients includes 70, who are 45 to 60 years of age; out of them 48 were male and 22 female. 30 samples of age and sex matched controls (Non-diabetic MI patients) used in this study. Body mass index in controls was significantly high (22.9±4.1 SD) as compared to patients (18.2±3.7 SD). Protein levels also reveal significantly high values in controls than patients. There seems to be a clear relationship between malnutrition and an increased risk of M I in patients with type 2 diabetes.

Keywords: malnutrition, myocardial infarction, Diabetes Mellitus

P-10-830-1

Biochemical tests in hypothyroidism and hyperthyroidism: Identification, comparison and their relation

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Thyroid hormones are the most effective hormones in the general metabolism of body and catabolic as well as anabolic of biochemical reactions. The purpose of present study was to identify and compare difference in lipids and other serum biochemical parameter, among the patients with untreated hypo or hyperthyroidism. Therefore, 209 clients have been studied by random and they stratified in three groups of hypothyroidism, hyperthyroidism and normal healthy volunteer as control group. Blood samples were examined by laboratory testing and thyroid function tests were carried out for all cases. Total cholesterol, triglycerides, FBS (Fasting Blood Sugar), urea and uric acid were analyzed using routine chemical protocols. Total cholesterol was significantly lower among hyperthyroidism patients (p=0.02) while triglycerides showed no significant difference in corresponding values of control among the hypothyroidism patients (p>0.05). TG in 38.7% of hyperthyroid patients was higher than 200Mg. Levels of serum uric acid and urea were significantly higher in hyperthyroidism patients than other (p=0.00). There were no significant difference in FBS of proteins among the patients (hypo or hyper) compared to control (p>0.05). However; 17.6% of hypothyroid patients were diabetics. In conclusions, this analysis focused on finding hypo and hyperthyroidism for the purpose of treating hyperlipidemia and preventing the increase of urea, uric acid and glucose.

Keywords: hypothyroidism, hyperthyroidism, TFT (Thyroid Function Test), biochemical test

P-10-527-1

Effect of catechin on non-enzymatic glycation of human serum albumin

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Catechin is the main phenolic antioxidant in tea. Catechin has beneficial effects such as reduction of cholesterol and protection against cardiovascular disease that can occur in diabetes mellitus. As diabetes leads to glycation of various proteins and this in turn has some effects on the structure of proteins and their biochemical activity, the inhibition of this process seems very vital. For several years researchers in this field have done their best to recognize the antidiabetic compounds. The aim of this study is to determine the effects of catechin on albumin glycation in vitro. In the presence of various concentrations of catechin, human serum albumin was glycated and evaluated by Thio-barbituric acid method. The results showed that catechin has a statistically significant (P<0.05) effect on inhibiting or decreasing the reaction of albumin glycation. The findings of this research show that catechin can inhibit the reaction of glycation and, therefore, may decrease complications occurring in diabetes.

Keywords: albumin glycation, catechin, in vitro, thio-barbituric acid

O-10-807-1

The influence of the adiponectin gene on adiponectin concentration, metabolic factors and risk of type 2 diabetes in obese subjects

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Adiponectin is an adipose tissue secreted hormone with important metabolic effects. Information about influence of several single nucleotide polymorphisms (SNPs) of adiponectin gene on the serum adiponectin, and risk of type 2 diabetes (T2DM) are insufficient and mostly inconsistency. The aim of this study was to investigate any association between two SNPs (+45 T/G and +276 G/T) of the adiponectin gene with serum adiponectin levels, metabolic factors and risk of T2DM in the obese individuals. We have performed genotyping for two common SNPs of adiponectin gene in fifty unrelated obese patients with T2DM and fifty two obese nondiabetic control subjects by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Lipid profile was measured by enzymatic methods. Serum adiponectin, insulin, and glucose levels were measured by immunoassay, and glucose oxidase methods, respectively. The G allele and TG+GG genotype of SNP 45 were significantly more frequent than the T allele and TT genotype in T2DM patients compare to the nondiabetic group (P<0.05). Subjects with the G/G +TG genotype of SNP 45 were at increased risk for T2DM [Odds Ratio (OR) 2.574; 95% Confidence Interval (CI) 1.051-6.302; P=0.036] compared with those T/T genotype. There was no statistically significant difference in allele and genotype frequencies of SNP 276 comparing control group with T2DM group. We found that,

adiponectin SNP 45T/G, rather than SNP 276G/T, is more associated with risk of T2DM in obese individuals.

Keywords: adiponectin, SNP, PCR-RFLP, T2DM, obesity

P-10-807-2

Relationship of adiponectin to anthropometric indices and metabolic factors in obese subjects with type 2 diabetes

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Adiponectin is an adipocyte secreted protein with important biological functions. Hypoadiponectinemia is associated with obesity, insulin resistance, and type 2 diabetes. The aim of this study was to compare the serum adiponectin level in obese non-diabetic and diabetic subjects and its correlation with metabolic factors. This cross-sectional study was performed on 40 obese subjects with type 2 diabetes and 40 obese non-diabetics. Fasting lipid profile was measured by enzymatic methods. The NycoCard HbA1c protocol was used to measure HbA1c. The Serum adiponectin, insulin and glucose levels were measured by immunoassay, and glucose oxidase methods, respectively. Serum adiponectin level in non-diabetic ($6.44 \pm 2.47 \mu\text{g/ml}$) was significantly higher than diabetic ($4.6 \pm 1.84 \mu\text{g/ml}$). Furthermore, serum adiponectin concentration in females was significantly higher than males in non-diabetic (7.18 ± 2.68 vs. 5.61 ± 0.57) and diabetic groups (5.24 ± 1.83 vs. 4.07 ± 1.70). There was a negative and significant correlation between serum adiponectin level with triglyceride ($r = -0.341$, $p = 0.031$), waist to hip ratio ($r = -0.479$, $p = 0.002$) and body mass index ($r = -0.345$, $p = 0.029$) and positive correlation with HDL-C ($r = 0.337$, $p = 0.034$) in non-diabetic group. In diabetic group we found only a positive correlation between adiponectin and LDL-C ($r = 0.339$, $p = 0.032$). Obesity and type 2 diabetes are associated with low serum adiponectin concentration. Adiponectin is likely to be involved in the pathophysiology of the link between obesity and type II diabetes.

Keywords: adiponectin, type 2 diabetes, obesity, BMI

P-10-819-1

Proton-dependant efflux of Tetracycline in clinical isolates of *Helicobacter pylori*

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The function of 16S rRNA mutations in resistance to Tetracycline (Tet) has been described in *Helicobacter pylori*; however, the role of energy-dependant efflux in this resistance is not well understood. Tet-resistant (Tetr) strains were obtained by screening of 112 *H. pylori* isolates obtained during 1997-2008 from 250 children. MIC for Tet was determined by agar-dilution and cross-resistance to amoxicillin, metronidazole, and clarithromycin was determined by disk diffusion-method. Accumulation-assay was conducted by an addition of $20 \mu\text{g/ml}$ Tet to a bacterial-suspension in presence or absence of $200 \mu\text{M}$ carbonyl-cyanide *m*-chlorophenylhydrazone (CCCP). Fluorescence of accumulated-Tet was measured with a spectrofluorometer. Twenty Tetr isolates with MICs: 4-8 mg L-1 (35%), 16 mg L-1 (45%) and 32-64 mg L-1 (20%) were obtained, 13 of which showed cross-resistance

to Mtz. With CCCP, an inhibitor of Proton Motive Force (PMF), 2-16 folds increase in accumulated Tet was observed for 17 Tetr-isolates suggesting that efflux pumps energized by PMF was functional in them. Serial-passages of 5 isolates on increasing-concentrations of Tet resulted in the mutants with increased MICs by 2-4 folds of which 4 showed 2-4 folds increase in PMF-dependant Tet-efflux. This indicates that during passages, pressure effect of antibiotic have selected the strains with more active efflux pumps. These results indicate that Proton-dependant efflux mechanism plays a role in resistance of clinical-isolates of *H. pylori* to Tetracycline.

Keywords: *Helicobacter pylori*, children, Tetracycline-resistance, efflux, spectrofluorometry

P-10-807-3

Relative hypoleptinemia in obese subjects with type 2 diabetes mellitus

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Leptin, protein product of the *ob* gene, could have a significant role in the pathogenesis of obesity and type 2 diabetes mellitus. The aim of this study was to compare of serum leptin levels in obese non-diabetic and type 2 diabetic patients. This cross-sectional study was performed on 40 obese subjects with type 2 diabetes and 40 obese non-diabetics. Fasting lipid profile was measured by the enzymatic methods. The NycoCard HbA1c protocol was used to measure HbA1c. The Serum leptin, insulin and glucose levels were measured by enzyme immunoassay, and glucose oxidase methods, respectively. The insulin resistance index was calculated by homeostasis model assessment (HOMA-IR). The serum leptin level in non-diabetic group (31.42 ± 14.10) was significantly higher than in diabetic group (20.75 ± 16.03). Moreover, serum leptin level in women was significantly higher than men in diabetic (30.29 ± 17.70 vs. 12.94 ± 8.88) and non-diabetic (36.42 ± 10.65 vs. 25.89 ± 15.71) groups. There was a positive and significant correlation between serum leptin level with hip circumference in diabetic ($r = 0.417$, $p = 0.007$) and non-diabetic ($r = 0.514$, $p = 0.001$), and leptin with BMI in diabetic ($r = 0.644$, $p = 0.001$) and non-diabetic ($r = 0.447$, $p = 0.004$) groups. Type 2 diabetes was associated with marked reduction in serum leptin levels; in both men and women. Indicating leptin is likely to be a major link between obesity and type 2 diabetes in human beings.

Keywords: type 2 diabetes, obesity, leptin, BMI

O-10-795-1

Investigation the methylation status of JMJD1A and HUMARA genes in azoospermic patients

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CpG islands in or near promoter region of many genes are subject to methylation which can have impact on gene expression. Thus,

hypermethylation of promoter regions are known to be a cause of gene expression silencing and pathogenesis in many genetic disorders. JMJD1A is a crucial gene for the final step of spermatogenesis. On the other hand, during male meiosis both sex chromosomes are transcriptionally silenced and the period of silencing appears to be limited to the pachytene stage. After this stage, they reactivate in early spermatides. It has been shown that the locus of Androgen Receptor (HUMARA) is methylated at the pachytene stage. In this study we prepared testicular biopsy from Iranian azoospermic infertile men for investigation of methylation status of JMJD1A and HUMARA genes. Tissue sample of 5 obstructive azoospermic patients were used as normal controls. MS-PCR (Methylation Specific PCR) was set up to study the methylation status. Patients with non-obstructive azoospermia and infertility showed different pattern of methylation compared to normal controls. In brief, 100% of controls showed only unmethylated allele. In HUMARA patient group, 2 patients (11.8%) showed methylated allele, the rest (88.2%) showed unmethylated allele. In JMJD1A, patient group, 5 patients (16.6%) showed only methylated allele. 2 patients (7%) showed both methylated and unmethylated alleles and the other showed only unmethylated allele. This is the first evidence of involvement of epigenetic changes in JMJD1A promoter region in male infertility.

Keywords: male infertility, methylation, azoospermia, JMJD1A, HUMARA

P-10-824-1

Extraction of sesame cholesterol to survey the possibility of replacing it for horse serum (HS) in Mycoplasma cultures

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The possibility of replacing various sources of cholesterol instead of HS in Mycoplasma cultures (to produce Mycoplasma vaccines) because of limitation of horse keeping centers in Iran and lack of quick access to HS is of great importance. In this survey the amount of total cholesterol in sesame was measured according to the methods of Liebermann-Burchard method and Alcyon 300i (abbott) apparatus. Sesame's cholesterol was extracted and by Chloroform-Methanol-Acetone, Chloroform-Methanol and Acetone and measured by the two methods mentioned above. We used Thin Layer Chromatography (TLC) to observe cholesterol in the extract. Then we prepared separated suspensions of extracted cholesterol and sesame and used in culture media for mycoplasmas. Results of this study showed that the rate of extracted cholesterol in Chloroform-Methanol-Acetone method was more than the other methods. Mycoplasmas had the most proliferation in media with HS and media with sesame and extracted cholesterol had caused less proliferation. But the diameters of colonies in this media were bigger than HS. Finally, a better growth of microorganism can be achieved by creating a balance of the rate of supplement and extracted cholesterol from different sources for Mycoplasma cultures.

Keywords: cholesterol, sesame, mycoplasma

P-10-824-2

Estimation and extraction of cholesterol from different sources to be used in Mycoplasma cultures instead of horse serum (HS)

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The possibility of replacing various sources of cholesterol instead of HS in Mycoplasma cultures (to produce Mycoplasma vaccines) because of limitation of horse keeping centers in Iran and lack of quick access to HS is of great importance. In this survey the amount of total cholesterol in egg yolk (EY), dried milk and almond was measured according to the methods of Liebermann-Burchard method and Alcyon 300i (abbott) apparatus. Egg yolk cholesterol was extracted by the methods of Chloroform-Methanol-Acetone, Chloroform-Methanol and Acetone and measured by the above mentioned methods. We used Thin Layer Chromatography (TLC) to check the existence of cholesterol in the extracts. Separated suspensions of extracted cholesterol, egg yolk, dried milk, and almond were prepared and used in culture media for mycoplasmas. Results of this study showed that the amount of extracted cholesterol in Chloroform-Methanol-Acetone method was more than other methods. Mycoplasmas had the greatest proliferation in media with egg yolk and media with dried milk, almond and extracted cholesterol had less proliferation. But the diameter of colonies in this media was bigger than HS. Therefore, egg yolk is the best alternative for horse serum in mycoplasma cultures. Finally, a better growth of microorganism can be achieved by creating a balance of the rate of supplement and extracted cholesterol from different sources for mycoplasma cultures.

Keywords: cholesterol, egg yolk, dried milk, almond, Mycoplasma

O-10-283-1

Comparison of seminal folate and vitamin B12 between normozoospermic and azoospermic men

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Folate and vitamin B12 are important for cell proliferation during spermatogenesis and development of spermatozoa. Folate deficiency causes distribution of DNA synthesis. Cobalamin is an important factor in folate metabolism. It is known that vitamin B12 and folate deficiency may impair fertility. The aim of this study was to determine the vitamin B12 and folic acid levels in semen of normozoospermic and azoospermic men. Semen samples were collected from 74 azoospermic and 79 normozoospermic control subjects referred to the Avicenna Infertility Clinic (AIC) for infertility treatment. The concentration of vitamin B12 and folic acid in semen was analyzed, using radioimmunoassay (RIA). Finally, the results of two groups were compared using t-test. The results showed that the concentration of vitamin B12 was significantly higher in normozoospermic men compare to azoospermic men (517.2±386.3 vs. 250.2±204.6 pg/ml). The mean value of folate (ng/ml) was 18.6±16.9 in normozoospermic group and 14.9±12.7 in azoospermic group. However there was no significant difference in semen folic acid content between the two groups. Also, there was a significant correlation between folate and B12 (R=0.25). Azoospermia in infertile men have different etiology; the current results

show that vitamin B12 and folate deficiency are not the cause of azoospermia. Higher levels of vitamin B12 in normozoospermic may be the result of an increased demand for active spermatogenesis.

Keywords: folate, vitamin B12, normozoospermia, azoospermia, semen, male infertility

P-10-282-1

The role of estrogen in pathogenesis of migraine

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Migraine is a common episodic headache disorder that affects 12% of the general population. It is characterized by unilateral throbbing headache lasting from 4h to 3 years. It is divided into aura and without aura subtypes. Associated symptoms include nausea, vomiting and sensitivity to light, sound and head movements. 18% of the women and 6% of men suffer from migraine. Migraine can be considered a peculiar response of the central nervous system to a variety of stimuli. Migraineurs have a low threshold from migraine attacks, and this low threshold may be genetically determined. Migraine is understood as a neurovascular disorder. The headache is triggered by temporal, cortical or brainstem dysfunction. The neuronal disturbances lead to vasodilation and release of proinflammatory substances within the dura mater which in turn sensitize peripheral and central neurons within the trigeminal system. The influence of estrogen on migraine is evident by three fold greater prevalence among women than men, and by significant changes in migraine incidence with changes in female reproductive status. The role of estrogen in the pathogenesis of migraine is substantiated by studies of abrupt estrogen fluctuations. Estrogens enhance neuronal excitability and vasodilation, and both effects lead to an increased propensity for migraine. Estrogen affects vasculature through stimulation of nitric oxide release, especially during the luteal phase. Levels of neuropeptide Y, galanin and CGRP are modulated in concert with estrogen level fluctuations. Additional serotonin synthesis and degradation and neuronal firing appear to be influenced by estrogen. Epidemiological, pathophysiological, and clinical evidence link estrogen to migraine headaches.

Keywords: migraine, estrogen, vasodilation, trigeminal system, aura, CGRP

P-10-146-1

Association between paraoxonase-1 activity and the extent of coronary stenosis

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Paraoxonase (PON1) can prevent oxidized low-density lipoprotein formation and development of atherosclerotic lesions. However, studies on the association between PON1 activity and the extent of

coronary stenosis and underlying mechanism(s) are limited. In this study, the relationship between paraoxonase and arylesterase activities of PON1 and the severity of coronary stenosis together with determination of PON1 phenotypes in the studied patients have been investigated. Paraoxonase and arylesterase activities were measured in 61 patients with coronary stenosis of <50% and 63 patients with coronary stenosis of >70%. Individual human serum phenotyping for the PON1 Q192R polymorphism was achieved through dividing the paraoxonase activity in the presence of 1M NaCl by arylesterase activity. Patients with stenosis of <50% had significantly higher PON1 activity ($p<0.05$) and HDL-Cholesterol ($p<0.03$) compared to those with stenosis of >70%. No significant difference ($p>0.05$) was observed in the phenotype distribution of males and females. According to the current study, there are significant differences in paraoxonase and arylesterase activities and also HDL-C levels between patients with coronary stenosis of <50% and those with coronary stenosis of >70%. Therefore, this study provides further support for the important role of paraoxonase activity in coronary atherosclerosis.

Keywords: paraoxonase-1, coronary stenosis, high density lipoprotein, PON1 phenotype

P-10-584-2

Preparation of new melatonin-horse radish peroxidase conjugate

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Melatonin (N-acetyl-5 methoxytryptamine) measurement in biological fluids is important and several methods such as immunoassays, HPLC, etc. have been developed for this purpose. In this study, by combination of two protocols (Periodate oxidation, Mannich reaction) melatonin was conjugated to Horse Radish peroxidase enzyme (HRP) and separated by size exclusion chromatography, used in immunoassay by monoclonal antibodies. Results showed that the method of conjugation is much better than others and this conjugate can be used for immunoassay of melatonin. In this assay detection limit was found to be 10pg/dl.

Keywords: melatonin, HRP, immunoassay

P-11-843-1

Effect of Azadirachta indica (Neem) and glycine max (Soybean) extracts on alloxan-induced male diabetic rabbits

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In the present study antidiabetic potential of Neem and Soybean extracts (aqueous and alcohol) has been evaluated in alloxan-induced diabetic rabbits. Eighteen healthy, male rabbits were divided into

different groups including, Normal control, and Diabetic control, treated with Neem aqueous extract, treated with Neem alcohol extract, treated with Soybean aqueous extract and Soybean alcohol extract, with 3 rabbits in each group. The extracts were given orally for 21 days, 50 ml extract (1%) per rabbit per day. At every 3rd day blood sample was collected, serum separated and glycemic level, total cholesterol level was determined by kit method. The data obtained revealed that neem aqueous and soybean alcohol extracts reduced the glycemic level, 46.81% and 49.78% respectively. The treatment with these extracts also reduced the cholesterol level significantly when compared with normal and diabetic groups. Hence, it is concluded that the neem and soybean possess significant antidiabetic activity.

Keywords: alloxan, rabbits, soybean aqueous extract, neem alcohol extract, glucose, cholesterol

P-10-709-1

Effect of diet induced hyperhomocysteinemia on fibrinolysis parameters in rabbits

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Some epidemiological studies indicated that a higher level of plasma homocysteine interferes with fibrinolytic process. The aim of this study was to evaluate the effects of diet induced hyperhomocysteinemia on fibrinolysis parameters in an animal model. Hyperhomocysteinemia was induced by methionine diet supplement in 6 New Zealand white rabbits for 12 weeks and other 6 rabbits received normal diet as control group. Fasting citrated plasma was obtained before and after intervention from all animals. Calcium chloride and streptokinase were used for induction of plasma clotting and dissolution respectively. Clot dissolution process was monitored by decreased light absorbance in one minute interval for 30 minutes. Kinetic curves of time versus absorbance were plotted and fibrinolysis parameters including lag time for initiation of clot lysis, maximal rate lysis (V_{max}) and time needed for 50% clot dissolution were calculated. After intervention, plasma levels of homocysteine in experimental animals were higher than control group ($19.68 \pm 8.13 \mu\text{mol/L}$ vs. 9.65 ± 2.03 , $PV=0.02$). There were significant differences in fibrinolytic parameters between experimental and control animals before intervention, while after intervention, the mean lag time and time of 50% lysis were significantly increased (respectively, $PV=0.0001$, $PV=0.0001$), but the mean of V_{max} was significantly decreased ($PV=0.03$) in experimental animals than control group. These results indicate that diet induced hyperhomocysteinemia in rabbit could interfere with the process of in vitro fibrinolysis and prolong the clot dissolution time. Hyperhomocysteinemia may partially inhibit in vivo fibrinolysis and induce thrombosis. This may be an acceptable explanation for higher risk of cardiovascular disease in hyperhomocysteinemic patients.

Keywords: fibrinolysis, homocysteine, rabbit

P-10-266-1

Determination of prooxidant-antioxidant balance after acute coronary syndrome using a rapid assay: A pilot study

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The purpose of the current study was to investigate a novel measure of oxidative stress in patients with acute coronary syndrome using a prooxidant-antioxidant balance (PAB) assay which is simple and rapid. Blood samples were taken from 94 patients admitted with acute coronary syndrome (ACS). PAB values were determined in the first and second 12 h after the onset of symptoms and compared with values for 81 age- and sex-matched controls. The mean PAB values in the first and second 12 h samples of patients were both significantly higher than that of controls ($p < 0.001$). Among patients, PAB values were also significantly higher in the second samples compared with the first samples ($p < 0.001$). These findings indicate a heightened state of oxidative stress following ACS and suggest that the PAB value may be considered as a cardiovascular risk predictor to estimate the extent of oxidative stress.

Keywords: acute coronary syndrome, coronary artery disease, cardiovascular risk factor, oxidative stress, prooxidant-antioxidant balance

O-11-729-3

Plasma concentrations of zinc, magnesium, calcium, iron, lead and cadmium in patients with osteoarthritis

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Osteoarthritis (OA) is a degenerative joint disease in which slow destructive process of the joint affecting millions of peoples worldwide. Although the exact biochemical cause of OA remains unknown, but some hypotheses have focused on the imbalances in blood levels of essential and toxic minerals. This study was comprised of man and women having age groups (40–50 & 51–75 years) were resident of urban area of Sindh, Pakistan. Serum zinc, magnesium, calcium, iron, lead and cadmium concentrations were investigated in fifty (male & female) patients with osteoarthritis disease and fifty healthy subjects. The data revealed that the mean concentration of zinc, magnesium, calcium, iron were found to be decreased in patients (male & female) with osteoarthritis compared to the control group. On the other hand mean value of lead and cadmium were significantly higher in both age groups compared to the control. In addition to various elements, other blood biochemical parameters such as alkaline phosphatase, uric acid, high density lipoproteins, low density lipoproteins and total cholesterol were also assessed to determine whether there was a relationship with OA disease. The data revealed that no significant change was found in other biochemical parameters compared with reference values, while variation in parameters is due to gender.

Keywords: osteoarthritis, zinc, magnesium, calcium, iron, lead, cadmium

P-11-729-4

Purification and characterization of proteases from cotton seeds

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Proteases of cotton seeds were purified by column chromatography using Sephadex G-100. The Fraction-V shows two bands on SDS gel electrophoresis, which was further separated on CM-Sephadex C-50 and two fractions, were obtained (Fraction-V-I and Fraction-V-II). Both V-I and V-II fractions showed homogeneity showing single band on SDS gel electrophoresis. The enzymes of Fraction-V-I and Fraction-V-II were purified by 1.33 and 1.49 fold with yield of 2.08 and 2.57 respectively. While specific activities were found to be 44.7 and 50.2 units /mg protein for Fraction-V-I and Fraction-V-II, respectively. The optimum temperatures for proteases activity of Fractions V-I and V-II were found to be 25°C and optimum pH 4 and 5 respectively. Protease activity for both fractions was found heat stable and retained 65% and 90% of their activity with in 10 minutes at 30°C. Protease activity of both fractions was increased in the presence of COCl₂ and ZnCl₂ but decreased with EDTA and o-phenanthroline. Protease of Fraction V-I and V-II strongly hydrolyses animal peptones than other substrates. The Km, Vmax and energy of activation for both Fractions V-I and V-II are 0.07 and 0.12 mole/L, 6 and 25 μ mol/min, 6.24 and 6.93 KJ/mol, respectively.

Keywords: protease, cotton seed, Sephadex G-100

P-10-713-1

Acetic acid as an anabolic stimulator in xanthomonas campestris b82

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Xanthan gum, a microbial biopolymer produced by the *Xanthomonas campestris*, has many applications in food, agro-chemistry, pharmaceutical, and chemical and cosmetic industries. Xanthan is known as being biocompatible compound allowing its use in various medical applications such as implantation and or controlled release devices. Moreover, xanthan is considered as a biodegradable as well as bioadhesive compound. It promotes wound-healing effects. In medical and pharmaceutical applications, xanthan is also used as a component in hydrogels. Here, we studied the effect of acetic acids on xanthan production. It was found that addition of acetic acid in to culture broth in late exponential growth phase increased xanthan production. In this study we evaluated energy balance of bacterial cells during xanthan production. For this purpose, ATP content of bacterial cells was measured after exponential phase of bacterial growth. We estimated whole ATP of bacterial cells during growth by reaction between luciferin and luciferase. The results indicated that in sublethal concentrations of acetic acid amount of ATP in cells of *Xanthomonas campestris* decreased and concomitantly xanthan production increased. In mid-phase of the fermentation process, ATP concentration in the

cells of *Xanthomonas campestris* was calculated after addition of acetic acid in different concentrations. ATP concentration was measured using a luminometer apparatus. Various counts (45300, 34000, 22600 RLU/s) and gum production (9, 10, 12gr/l) resulted from different concentrations of the acid (6.25, 3.12, 1.56mM).

Keywords: ATP concentration, *Xanthomonas campestris*, acetic acid

P-10-709-2

Purified human lipoprotein(a) interfere in-vitro rabbit fibrinolysis.

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Higher levels of plasma lipoprotein(a) [Lp(a)] is a novel cardiovascular risk factor. There are some evidences for interfere of this lipoprotein in fibrinolysis system. The aim of this study was to evaluate the effects of purified human Lp(a) on in-vitro plasma clot dissolution from rabbit. In this experimental study, citrated plasma was obtained from 6 New Zealand white rabbits and the purified human Lp(a) was added to each plasma samples from rabbits in three concentrations (3.75, 7.5, 15 mg/dl) and one sample was prepared without Lp(a). Then, Calcium chloride and streptokinase were used for induction of plasma clotting and dissolution respectively. Clot dissolution process was monitored by decreased light absorbance in one minute interval for 30 minutes. Kinetic curves of time versus absorbance were plotted and fibrinolysis parameters including lag time for initiation lysis of clot, maximal rate lysis (Vmax) and time needed for 50% clot dissolution were calculated. In concentration of 15 mg/dl Lp(a), the mean time of 50% lysis was significantly increased (p value=0.0001), but there were no significant differences in the mean of lag time and Vmax compared to the sample without Lp(a) condition. Our results indicate that purified human Lp(a) could interfere in streptokinase induced fibrin clot dissolution in a dose dependent profile and prolong the fibrinolysis time. This may be an acceptable explanation for thrombogenic properties of this lipoprotein.

Keywords: fibrinolysis, lipoprotein(a), rabbit

O-10-709-3

Evaluation of the interaction of moderate hyperhomocysteinemia and lipoprotein(a) on fibrin clot structure in rabbits plasma

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Higher plasma levels of lipoprotein (a) [Lp(a)] and homocysteine are considered as atherothrombotic cardiovascular risk factors. There is some evidence for synergistic effects of these two risk factors. The aim of this study was to evaluate the possible interaction of mild hyperhomocysteinemia on an in-vitro intervention of human Lp(a) on fibrin clot structure in rabbits plasma. In this case and control experimental study, hyperhomocysteinemia was induced in 6 New Zealand white rabbits by high methionine diet for 12 weeks, and 6 rabbits received normal diet in the same condition as control. Fasting citrated plasma from all animals was prepared after intervention.

Purified human Lp(a) were added to each plasma samples from rabbits until three concentrations (3/75, 7/5, 15 mg/dl) were prepared. Then, fibrin clot were induced in the absence and presence of 3 different concentrations of human Lp(a) by addition of calcium chloride in a defined plastic tube. Flow rate of saline solution through each clot was determined and a permeability coefficient (ks) as an index of clot compactness calculated. After intervention, plasma levels of homocysteine in experimental animals were higher than control group (19.68±8.13 µmol/L vs. 9.65±2.03, pvalue=0.02). The mean of difference ks in different levels of Lp(a) and without Lp(a) were significantly increased in the control group than experimental group, respectively (pvalue=0.002, pvalue =0.0001, pvalue =0.0001). Our results indicate that Lp(a) has more effect on increase of clot compactness in the presence of lower concentration of homocysteine. It is suggested that binding sites of homocysteine and Lp(a) to fibrin are similar. Therefore, homocysteine with binding to these sites decreases binding of Lp(a) to fibrin.

Keywords: clot structure, homocysteine, interaction, lipoprotein(a), rabbit

P-10-647-1

Evaluation of relationship between lipoprotein(a) concentration and plasma lipid oxidizability in men

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Elevated levels of lipoprotein (a) [LP (a)] are known as a cardiovascular risk factor whose exact underlying mechanism of action in hearth diseases is not clearly defined. So the aim of this study was to evaluate the direct method for lipid oxidizability in diluted whole plasma and to asses the relationship between oxidation parameter with lipids, lipoproteins and LP(a) in a group of adult men. In 100 healthy adult men (36.8±10.3 years), fasting plasma levels of lipids and lipoproteins were evaluated. Also lipid oxidation was estimated by monitoring the change of conjugated diens in diluted plasma after addition of Cu²⁺; malondialdehyde (MDA) was determined by thiobarbituric acid method. LP (a) with a mean of 18.3±18.7mg/dl has no significant correlation with other variables. Plasma triglyceride (TG) was significantly related to lag time (r=0.3, p=0.01) and T-max (r=0.2, p=0.05). There were found also positive correlation between lag time and High density lipoprotein-Cholesterol (HDL-C) (r=-0.2, p<0.05). This study indicated that plasma samples with higher levels of TG are more resistant to initiation of lipid oxidation. On other hand, plasma with high levels of cholesterol and low density lipoprotein-Cholesterol (LDL-C) are more susceptible to lipid peroxidation. In our study LP (a) had no relationship with lipid oxidation parameter. It is concluded that Cu-induced lipid oxidation on diluted plasma is a reliable and flexible method for evaluation of plasma oxidizability and assessment of the effect of different compound on lipid oxidation.

Keywords: lipoprotein (a), plasma, oxidizability, Cu²⁺, men

P-10-1012-2

Red blood cell antioxidant status and hexokinase activity alter significantly during obstructive cirrhosis

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Many clinical and experimental observations suggest that oxidant stress plays a key role in the progression of acute and chronic cholestatic liver diseases. The aim of this work was to study the erythrocyte antioxidant defense status as well as hexokinase activity in relation to the severity of liver damage using the rat model of acute and chronic cholestasis. The activities of erythrocyte glutathione peroxidase (GPx), superoxide dismutase (SOD) and hexokinase (HK) as well as the concentration of glutathione were measured after 7, 28 and 42 days of surgery on bile duct-ligated (BDL) and sham-operated rats. A group of 7 healthy rats considered as unoperated controls. We also evaluated the total reducing capacity of plasma using FRAP method. GPx activity was significantly reduced in the chronic phases (days 28 and 42) while SOD did not show any noticeable changes due to induction of cholestasis. HK activity indicated a remarkable progressive increase until the day 28 but it returned to the vicinity of control values by the day 48. Total glutathione content was significantly decreased only by the advanced cirrhosis stage (day 48) whereas the FRAP values doubled at the initial stage and began to decrease for the rest of study. The results illustrates that antioxidant ability of erythrocytes only reduces severely in the chronic stages of cholestasis where the liver injury is vast while considering the previous studies, other tissue cells are more susceptible to cholestasis-induced oxidative stress.

Keywords: erythrocyte, cholestasis, antioxidant defense, glutathione peroxidase, superoxide dismutase, hexokinase

O-10-857-1

Detection of suitable prognostic marker in peripheral blood sample of breast cancer patients

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Breast cancer is the most common cause of cancer death. Therefore, there is a great need to identify suitable prognostic and diagnostic markers. In this study, we aimed to examine gene expression of different biomarkers in breast cancer patients using real-time PCR. Markers included apoptotic and anti-apoptotic genes (p53, Fas, Bcl-2), TH1 cytokines (IL-2, IL-12, IFN-gamma), angiogenesis and angiostatic factors (SDF-1/ CXCR4, IP-10/CXCR3) and immuno-modulatory genes (FOXP3, CTLA-4). Gene expressions were evaluated for 50 patients with breast cancer and 40 healthy women using quantitative RT-PCR. This data has shown that Fas, Bcl-2 and p53 transcripts significantly increased in breast cancer patients' peripheral blood samples compared to healthy controls (p=0.0178, 0.0045, 0.0068, respectively). IL-12 and IFN-gamma showed a considerable rise in patients as well (p=0.016, 0.027, respectively). But no significant difference was found in IL-2 transcripts. A significant lower expression

of IP10/CXCR3 was found in breast cancer patients ($p=0.0028$, 0.04 , respectively). However, there was not a significant difference in SDF-1/CXCR-4 transcripts between patients and healthy controls. Patients also showed significantly higher expression in FOXP3 and CTLA4 genes than healthy women ($p=0.0001$ and 0.0003). These apoptotic, anti-apoptotic markers and TH1 cytokines may be used as suitable prognostic biomarkers in breast cancer. In contrast, IP-10/CXCR3 could be appropriate suppressor markers for tumorigenesis. Up-regulation of FOXP3 and CTLA4 displays their essential role in tumor progression as well.

Keywords: breast cancer, prognostic marker, real-time PCR

P-10-832-1

Evaluation of lipid profile in a population of Iranian term newborns

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Cardiovascular diseases are one of the most important causes of death all over the world. Recent studies have shown that a cardiovascular disease starts in childhood so evaluation of newborns lipid profile might be of benefit. In this study we aimed to measure lipid and lipoprotein levels in a representative sample of Iranian newborns. Samples of umbilical cord blood was obtained from 200 full term newborns (89 males and 111 females) to be used to determine lipid profile levels: Total cholesterol (TC), triglycerides (TGs), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and non-HDL cholesterol. Mean TC: 74.58, Mean TGs: 96.59, Mean HDL-C: 28.68, Mean LDL-C: 29.25 and Mean Non-HDL cholesterol: 45.90. Biochemical factors which were studied did not show significant difference between genders. Our findings show that cord blood TGs level in Iranian neonates are higher than other countries that have been studied, no significant difference was observed in others biochemical factors. So Iranian population can be at high risk for cardiovascular diseases and this should be addressed in longitudinal studies.

Keywords: lipid profile, cardiovascular diseases, cord blood

O-10-580-1

Elevated Levels of Anti Heat Shock Protein (HSP) - 27 and C-reactive protein in Stroke Patients and their Relation with Prognosis

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Stroke is an important health issue and early identification of stroke should represent a significant contribution to health improvement so that interventions can be targeted to those most likely to benefit. C-reactive protein, a peripheral marker of inflammation, has consistently been observed to be related to the risk of cerebrovascular and cardiovascular events and is consistently elevated in the circulation of patients after acute ischemic stroke. Furthermore, anti-HSP-antibody

including anti-HSP-27 antibody have also been reported to be associated with vascular events. So, we aimed to assess CRP and anti-HSP27-antibody levels in stroke patients to determine their role in prognosis of these patients. Blood samples were collected from 183 cases in the first 24h after stroke onset. Anti-HSP27 IgG titers were determined using an in-house enzyme-linked immunosorbent assay (ELISA) and compared with values for 83 age- and sex-matched control subjects. CRP was measured by commercial kits. Median anti-HSP27 antibody titers was $0.18(0.02-1.80)$ and $11.43(2.16-16.24)$ absorbency units for CRP levels, both being significantly higher than control group, which was $0.08(0.01-0.78)$ and $3.23(1.06-14.76)$, respectively ($P<0.001$). Anti-HSP27 was significantly correlated with BMI ($P<0.05$), and CRP was correlated with LDL ($P<0.05$). Both CRP and anti-HSP27 antibody titers were not associated with prognosis in these patients ($p>0.05$). Although HSP27 has a protective role in vascular tissue but the higher titers of anti-HSP27 antibody levels in stroke patients reveal the higher risk of vascular events, playing as a novel risk-factor in stroke patients

Keywords: Anti-HSP27 antibody, C-reactive protein, stroke

P-10-346-1

The levels of 8-isoprostane and catalase activity in squamous cell carcinoma of the esophagus

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Lipid peroxidation is an oxidative process which occurs at low levels in all cells and tissues. Under normal conditions a variety of antioxidant mechanisms serve to control this peroxidation process. The aim of present study was to assess levels of 8-isoprostane (8-iso PGF₂α) as a lipid peroxidation biomarker and catalase activity as an antioxidant enzyme in squamous cell carcinoma (SCC) of the esophagus patients as compared to healthy subjects. Serum and urine samples of SCC patients ($n=32$) and healthy subjects ($n=45$) were collected. A competitive enzyme immunoassay kit (Cayman chemical Co) was used to detect the level of 8-iso PGF₂α in urine samples of patients and healthy subjects. So a competitive enzyme immunoassay kit (Cayman chemical Co) was used to detect the catalase activity in serum samples. Levels of 8-iso PGF₂α increased in SCC patients in comparison with healthy subjects. This increase was significant statistically ($P<0.05$). Catalase activity in patients group was increased compared to control group ($P<0.05$). The present study shows that levels of 8-iso PGF₂α and catalase activity were increased in SCC patient in comparison with healthy subjects significantly. In conclusion, increase in 8-iso PGF₂α generated by lipid peroxidation in SCC patients may participate as physiological mediator of squamous cell carcinoma of the esophagus.

Keywords: squamous cell carcinoma, 8-isoprostane, catalase activity

O-10-868-1

Expression of SDF-1/CXCR4 and IP-10/CXCR3 in human cancer cell lines

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Angiogenesis is an essential process for progression of solid tumors such as breast cancer. Chemokine and chemokine receptors closely correlate with this process, for example, SDF-1(Stromal cell derived factor-1)/CXCR4 (CXC chemokine Receptor) axis plays important roles in recruitment of endothelial progenitor cells and angiogenesis. In contrast, IP-10 (Interferon-gamma-induced protein)/CXCR3 (CXC chemokine Receptor) axis has angiostatic effects and might suppress tumor progression. The main aim of this study was to compare the SDF-1/CXCR4 and IP-10/CXCR3 expression profiles of different human cancer cell lines such as Hela, Hep-2, SKOV3, LNCaP and MCF7. SDF-1/CXCR4 and IP-10/CXCR3 mRNA expression profile in cancer cell lines were measured using quantitative real-time PCR. As a result IP-10 and CXCR3 mRNA levels were significantly lower in Hep2 cells (human laryngeal cancer cell line) compared to other cell lines. SDF-1 and CXCR4 showed significantly overexpression in Hep2 cells in comparison to other cell lines. Differentially expressed SDF-1/ CXCR4 axis as angiogenic factor in Hep2 cells showed the important role of this chemokine and chemokine receptor axis in the formation and progression of head and neck cancer. Therefore, this axis can be introduced as one of the candidates for cancer treatment using gene therapy or immunotherapy for head and neck cancer patients.

Keywords: head and neck cancer, IP10, CXCR3, SDF1, CXCR4

P-10-751-1

Pepsinogen I, II, gastrin levels in random adult population Shiraz city southern Iran

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Gastric cancer remains the second greatest cause of cancer death worldwide. Human pepsinogens (PG) are inactive proenzymes of pepsin originating in the gastric mucosa and are classified biochemically and immunochemically into two groups of pepsinogen I (PGI) and pepsinogen II (PGII). Serum PG levels reflect the morphologic and functional status of the gastric mucosa. In a population-based study, 846 subjects were selected by cluster random sampling method based on postal code division of Shiraz, southern Iran. The study included 305 men and 541 women. A gastroenterologist completed the clinical questions of the questionnaire in the clinic. After completing the interview, the basal blood samples were taken after overnight fasting for measurements of PG I, PG II. The serum PGI, PGII, and gastrin were measured by enzyme-linked Immunosorbant assay (ELISA). Their mean age was 50.53 years for both men and women (range: 35-99 years old). In the patients <44 years, the level of PGI and PGI/II ratio were significant ($p<0.05$), and also the level of gastrin and PGI&II ratio were significant ($p<0.05$). Values of gastrin increased significantly in male as compared with female ($p<0.001$). The results indicated the different values of gastrin

in the participants who drank alcohol ($p<0.007$). Therefore, it seemed possible that it was in this period that the function of gastric mucosa altered significantly causing significant changes in the serum PG and gastrin level.

Keywords: pepsinogen I, II, gastrin, population, southern Iran

P-10-839-1

Association between MGMT expression and p53 mutation in Glioblastoma Multiform

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Glioblastoma Multiforme (GBM) is the most aggressive and most common brain tumor with glial origin. The prognosis remains poor despite advances in surgery and adjuvant therapies. p53 and O6-methylguanine-DNA methyltransferase (MGMT) are both tumor suppressor genes, implicated in GBM resistance to radiation and chemotherapy. In order to assess the expression status of these two proteins, we analyzed 50 GBM samples. Demographic and clinical data along with post surgery tumor samples from 50 GBM cases were gathered from pathology archive. MGMT and p53 protein expression was evaluated by immunohistochemistry. Patients' ages ranged from 7 to 80 years and the male-to-female ratio was 3.2. Frontal and temporal lobes (46% and 22%, respectively) were the most common locations. Headache was the first and the most common symptom (64%). The relationship between p53 and MGMT status with tumor localization, gender, and age and disease symptoms was not significant. 52% of cases were p53 positive (mutated gene), predominantly in the nuclei of tumor cells. MGMT immunohistochemistry was negative in 35 (70%) and positive in 15 (30%) of patients. Specimens which were IHC-negative for MGMT expression showed a significantly higher expression of p53 ($P=0.03$). In p53-mutated cells, MGMT expression was significantly lower. These results point to the fact that there is a positive regulatory relation between MGMT and p53, reflected as a tendency of MGMT activity decline with p53 inactivation. However, we cannot deduce from this study which protein is the regulator of the other.

Keywords: glioblastoma multiform (GBM), immunohistochemical, O6-methylguanine methyl transferase (MGMT), p53

P-10-824-3

Measuring of liver, hazelnut and walnut cholesterol to be use in Mycoplasma cultures instead of horse serum (HS)

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Estimation of cholesterol from different animal and plant sources and possibility of replacing various sources of cholesterol instead of HS in Mycoplasma cultures (to produce Mycoplasma vaccines) is of great importance because of limitation of horse keeping centers in Iran and lack of quick access to HS. In this survey the amount of total

cholesterol in liver, hazelnut and walnut was measured according to the methods of Liebermann-Burchard method and Alcyon 300i (abbott) apparatus. We used Thin Layer Chromatography (TLC) to determine the existence of cholesterol in the extracts. Then, separated suspensions of liver, hazelnut and walnut were prepared and used in culture media for mycoplasmas. The cultures were incubated for 48 hours. We had subcultures on PPLO Agar to have colonies. Results of this study showed that the Mycoplasmas had the most proliferation in media with hazelnut, and media with liver and walnut supported less proliferation. But the diameters of colonies in this media were bigger than HS. Finally, a better growth of microorganism can be achieved by creating a balance of the rate of supplement and extracted cholesterol from different sources for Mycoplasma cultures.

Keywords: cholesterol, hazelnut, liver, walnut, Mycoplasma

O-10-866-1

Tyrosine phosphorylation pattern in sperm proteins isolated from normozoospermic and infertile teratozoospermic men

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In mammalian system, spermatozoa are not able to fertilize the oocyte immediately upon ejaculation, thus they undergo a series of biochemical and molecular changes which is termed capacitation. During sperm capacitation, signal transduction pathways are activated which lead to protein tyrosine phosphorylation. In this study, we have characterized tyrosine phosphorylation pattern in sperm proteins isolated from normozoospermic and infertile teratozoospermic men referred to Avicenna Infertility Clinic in Tehran. Semen samples were collected and spermatozoa were isolated using percoll gradient centrifugation. Spermatozoa were then incubated up to 6 h at 37°C in 3% Bovine Serum Albumin- supplemented Ham's F10 for capacitation following a standard protocol. Before and after capacitation, total proteins from spermatozoa were extracted and subjected to SDS-PAGE. To evaluate protein tyrosine phosphorylation pattern, western blotting with specific antibody against phosphorylated tyrosines was performed. The results from western blotting showed that: 1) different sets of proteins in normozoospermic and teratozoospermic groups were detected 2) the phosphorylation pattern between the normozoospermic and teratozoospermic groups were different 3) the intensity of protein phosphorylation appears to have increased during capacitation in the normozoospermic group relative to teratozoospermic group. These results suggest that the differences in types of proteins and diminished tyrosine phosphorylation efficiency of sperm from teratozoospermic men may be responsible for compromised capacitation and low fertilization success in this group.

Keywords: tyrosine phosphorylation, capacitation, normozoospermic men, teratozoospermic men

P-10-498-1

The effects of body acupuncture on lipid profile in Iranian obese and overweight subjects

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Body acupuncture has been reported to reduce levels of the serum total cholesterol, triglyceride, HDL-C and LDL-C in subjects in clinical practice. In the present study we have evaluated the effects of body acupuncture on several biochemical parameters, including levels of the serum total cholesterol, triglyceride, HDL-C and LDL-C in subjects of both genders, divided into 2 groups as follows. Case group (n=90, female=67, male=23) subjects with low-calorie diet and body acupuncture. Subjects were recruited from Nutrition Clinic, Ghaem hospital, Mashhad, Iran. The acupoints on their bodies included: Tianshu(St25), Zusanli(St36), Fenglong(St40), Naiguan(P6), Sanyinjiao(SP6). Control group (n=92, Female=68, Male=24) subjects with low-calorie diet and unreal body acupuncture. The acupoints on their bodies were not real and the needles were just reaching the surface of their skins. Both groups received 3 treatment sessions per week each 20-30 minutes for 6 weeks. Some of biochemical parameters, including TG, TC, HDL-C, LDL-C, were measured twice in all subjects, first at the beginning and second 6 weeks after the treatment. We observed the same significant reduction in LDL-C (p<0.05), total cholesterol (p<0.05), triglycerides (p<0.05), and increased HDL-C (p<0.05) in both the case and control groups. The difference of lipid profile between groups was not statistically significant. It appears that needling, NOT body acupuncture has beneficial effects on lipid profile in obese and overweight subjects.

Keywords: body acupuncture, total cholesterol, triglyceride, HDL-C, LDL-C, obesity, overweight, lipid profile

P-10-499-1

Effects of Simvastatin therapy on prooxidant-antioxidant balance in dyslipidemic subjects

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Simvastatin is an effective statin modulating process involved in atherosclerosis which is used for lipid-lowering therapy. In this study we investigated the effects of simvastatin treatment on prooxidant-antioxidant balance (PAB) in a group of dyslipidemic patients. Eighty patients attending a lipid clinic, and previously not receiving lipid-lowering treatment, were selected and divided into two groups based on randomized, double blind, and placebo controlled & cross-over. One group with low density lipoprotein (LDL)>130mg/dl were treated with simvastatin and another group treated with placebo according to cross over model for 2.5 months. A significant decrease of the PAB value was observed in group that was treated by simvastatin compared to placebo group (p<0.001). This study indicates that the PAB value may be considered as a cardiovascular risk factor and treatment with simvastatin may lead to decrease cholesterol.

Keywords: prooxidant-antioxidant balance, Simvastatin, cross-over, atherosclerosis

P-10-691-1

Evaluation of Superoxide dismutase enzyme in dogs infected with Babesia gibsoni

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Babesiosis is a common tick borne disease of dogs in tropical and subtropical regions of the world caused by different species of Babesia. The present study aimed to examine superoxide dismutase levels in dogs with clinical babesiosis, caused by Babesia gibsoni. The study was on 7 naturally occurring cases of canine babesiosis with the tick infestation, erratic pyrexia, pale mucosa and prolonged illness. Microscopic examination of Giemsa stained blood smears confirmed B. gibsoni infection in the erythrocytes. Six healthy dogs of different age, sex and breeds were used for control group. The result demonstrated that production of superoxide in erythrocytes was significantly higher than in the control group (superoxide dismutase: 0.013 ± 0.0008 units/mg Hb vs. 0.005 ± 0.0008 units/mg Hb). At the conclusion we observed that superoxide dismutase enzyme increased in erythrocytes parasitized with B. gibsoni. And suggest that probably oxidative damage, due to lipid peroxidation, might be caused in host erythrocytes by the parasite.

Keywords: superoxide dismutase, erythrocytes, Babesia gibsoni

P-10-691-2

Lipid peroxidation status in canine babesiosis

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Babesiosis is a common tick borne disease of dogs in tropical and subtropical regions of the world caused by different species of Babesia. This study aimed to determine the erythrocytic lipid peroxidation infected with Babesia gibsoni. The study was on 7 naturally occurring cases of canine babesiosis with the tick infestation, erratic pyrexia, pale mucosa and prolonged illness. Microscopic examination of Giemsa stained blood smears confirmed B. gibsoni infection in the erythrocytes. Six healthy dogs of different age, sex and breeds were used for control group. Levels of Lipid peroxide were significantly ($P < 0.01$) higher in sick dogs than those of cytologically negative dogs (6.02 ± 0.32 nmol MDA/mg Hb vs. 1.88 ± 0.10 nmol MDA/mg Hb). In conclusion, B. gibsoni infection in dog is associated with a parasitic burden-dependent corpuscular oxidative damage as indicated by membrane lipid peroxidation.

Keywords: Lipid peroxidation, Babesia gibsoni, erythrocyte

P-10-241-1

The effect of chronic exposure to noise stress on the level of CPK-MB and troponin-I in adult mail rat

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Sound pollution is the main encounter in the new industrial community. Sound as a nonspecific environmental stress factor can influence the many physiologic and psychological parameters of the living organism and also can induce stress response. The cardiovascular system is the primary system influenced by different types of stress. Creatine Phosphokinase-MB (CPK-MB) and troponin-I are the essential myocardial enzymes released into circulation from myocardium are the most specific indicators available for the diagnosis of myocardial infarction that can be detect in the plasma within 8 hours after MI. The probable effect of chronic exposure to noise stress on the level of CPK-MB and troponin-I are evaluated in this study. 50 adult male wistar rats (250 ± 20 g) were divided in 5 groups each group consisting of 10 rats: 1 control, 1 sham and 3 experimental group. The experimental group was exposed to continuous sound with the intensity of 100 db for 4, 8 and 12 hour respectively. Sham group were transferred to the sound room for 8 hour each day to experience the same condition without sound. At the end of experiment fresh plasma were collected and the levels of CPK-MB and Troponin-I determined by qualified one step immuno-chromatography test. The data were analyzed by means of non-parametric mann-withny U test ($p < 0.05$). Statistical analysis showed no significant difference between control and experimental and sham group. The result shows that noise stress with 100 db causes no serious or significant damage in the myocardial cells in long term exposure. The study of acute exposure to noise stress may be useful for further investigation in this case.

Keywords: noise pollution, CPK-MB, troponin-I, stress, myocardial infarction

P-10-1032-2

The effects of opium addiction on the serum levels of Transforming Growth Factor (TGF)- β and white blood cells counts in male and female rats

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Opium has been used as a therapeutic medicine for thousand of years. This was the reason why opium prescribed by ordinary people and it make an excuse to explaining to use it. However, addiction has caused severe problems for both the abusers and society. There are few studies that demonstrated the effects of opium on the molecular levels. As regulatory T-cells secrete TGF- β , which reduces immune response, we decided to investigate the effect of opium on TGF- β and counts of white blood cells (WBC) in addicted male and female rats. Wistar rats (14 males and 14 females, 220 to 250 gram body weight) were treated with opium solution (as 1st day 30 mg/kg, 2nd day 60 mg/kg, 3rd day 90 mg/kg, 4th day 120 mg/kg and 5th, 6th, 7th, 8th days 150 mg/kg) or physiology serum (as control) through intra peritoneal injection at 8 AM and 8 PM (7 rats in each groups). By 9th day after opium (150 mg/kg) or physiology serum injection, blood was taken and TGF- β and white blood cells were evaluated. $P < 0.05$ is considered as significant

difference. The mean serum levels of TGF- β in addicted male rats were significantly lower than that observed in case group, whereas the mean serum level of TGF- β in addicted female rats was significantly higher as compared to control group. While the total numbers of WBC in addicted female rats were significantly less than female control group, the counts of neutrophils in addicted female rats were significantly higher than female control group. However, in addicted male and female rats the counts of lymphocytes were significantly lower in comparison to control groups. The results of the present study demonstrated that the opium can differentially influence the production of TGF- β and also the total and differential number of white blood cells in male and female rats.

Keywords: opium, diabetes, addiction, TGF- β

P-10-912-1

Study on some biochemical parameters of sheep hydatid cyst concerning of organ and cyst fertility

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Unilocular Hydatid cyst caused by the metacestode stage of *E. granulosus* is a cyclozoonose of economic and public health significance in Iran. The adequate treatment for hydatidosis requires the knowledge of basic aspects related to survival of infectious agents, especially protoscolexes. The aim of this study is to evaluate the viability of protoscolexes in sheep lung and liver hydatid cysts together with some biochemical parameters. A total of 75 samples of hydatid fluids were collected from the lung and liver of sheep from Tabriz abattoir and consequently, viability test was performed, using vital staining method (Eosin 0.1%) and the levels of total protein, glucose, cholesterol and triglycerides were determined by the colorimetric method. The variation of fertility rates of organs cysts (Mean \pm SE: 63.29% \pm 4.31-62.15% \pm 5.17 for lung and liver cysts, respectively) was insignificant ($P > 0.05$). Quantitative variations in the levels of total protein, glucose, cholesterol and triglycerides, were found in lung cysts (Mean \pm SE: 0.54 \pm 0.02 g/dl – 46.3 \pm 4.07 mg/dl – 2.22 \pm 0.73 mg/dl – 6.61 \pm 2.11 mg/dl, respectively), compared to liver cysts (0.5 \pm 0.06 g/dl – 45.4 \pm 3.4 mg/dl – 2.2 \pm 0.52 mg/dl – 2.11 \pm 0.78 mg/dl, respectively), although only triglycerides levels difference was statistically significant ($P < 0.05$). No significant difference was found in studied biochemical parameters between fertile and sterile cysts of lung and liver except to cholesterol level in liver cysts (Mean \pm SE: 2.4 \pm 0.58 mg/dl – 0.75 \pm 0.4 mg/dl, in fertile and sterile cysts, respectively).

Keywords: biochemical parameters, fertility, hydatid fluid, sheep

P-10-496-2

The effects of body acupuncture on level of the serum hscrp in Iranian obese and overweight subjects

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Body acupuncture has been reported to reduce body weights of subjects in clinical practice. Also a few data is available on relationship between obesity and hsCRP levels in Asian populations. hsCRP has been identified as a strong independent risk factor of cardiovascular events. In the present study we have evaluated the effects of body acupuncture on body weight and level of the serum hsCRP and the relationships between obesity and hsCRP levels in subjects of both genders, divided into 2 groups as follows: 1) Case group: (n=90, female=67, male=23) subjects with low-calorie diet and body acupuncture. The acupoints on their bodies included: Tianshu(St25), Zsanli(St36), Fenglong(St40), Naiguan(P6), Sanyinjiao(SP6). 2) Control group: (n=92, female=68, male=24) subjects with low-calorie diet and unreal body acupuncture. The acupoints on their bodies were not real and the needles were just reaching the surface of their skins. Each patient passed three treatment sessions per week each lasting 20-30 minutes for 6 weeks. Body weight and level of the serum hsCRP measured pre and post treatment for all subjects. There was observed a significant reduction in body weight ($p < 0.05$) and level of the serum hsCRP ($p < 0.05$) in both the case and control groups. It appears that the relationship between obesity and hsCRP levels in subjects and needling, NOT body acupuncture, has beneficial effects on body weight and level of the serum hsCRP in obese and overweight subjects.

Keywords: body acupuncture, needling, hsCRP, body weight, obesity, overweight, cardiovascular events

P-10-921-1

Cell free fetal DNA assessment in maternal serum is a new approach for noninvasive prenatal diagnosis

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During the last decade, discovery of cell-free fetal DNA (cffDNA) in the maternal circulation and advances in identification and even clinical application of this DNA has opened a new approach in prenatal diagnosis. In the last five years and in this regard we have been able to assess the cffDNA in maternal serum for sex determination, fetal RhD genotyping and finally B- thalassemia diagnosis. In all of subjects we employed differences in genomic DNA between mother and fetus using different PCR techniques. In sex determination we could detect SRY gene of chromosome Y in maternal serum using Nested PCR. After delivery, results showed that the accuracy of sex determination were 90.62%; in RhD genotyping it was revealed that 37 out of 41 cases of RhD genotyping has been determined appropriately (95.45%). In B- thalassemia diagnosis, 20 couples with fetuses at risk of B- thalassemia were evaluated, we used RFLP analysis in 8 sites and determined which paternal allele (normal or mutant) was inherited by the fetus. If

the fetus receives the normal paternal allele, so it won't have major B-thalassemia and CVS analysis is not needed. But if it is determined that the mutant paternal allele has been inherited by fetus, then fetus won't be normal and may have minor or major B- thalassemia. Then by CVS analysis and finding no maternal mutation, the probability of major B-thalassemia will be zero.

Keywords: Cff DNA, maternal serum, RhD genotyping, prenatal diagnosis, sex determination

O-10-916-1
Development of an immunoaffinity method for purification of streptokinase

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Streptokinase (SK) is a potent activator of plasminogen to plasmin, the enzyme that can solubilize the fibrin network in blood clots. SK is currently used in clinical medicine as a therapeutic agent in treatment of thromboembolic blockages, including coronary thrombosis. It is naturally secreted by β -hemolytic streptococci. There are a few methods for purification of SK, but all of them have drawbacks. We have developed an immunoaffinity chromatography method that can purify the SK in a single step with high yield and stability. At the first stage a CNBr-Activated sepharose 4B-Lysine column was made and the human blood plasma was purified on that. The purified plasminogen was coupled with sepharose 4B. The SK product of the bacterial culture was preliminary extracted by salt precipitation and then purified by affinity chromatography on plasminogen substituted sepharose-4B. The rabbit was immunized with the purified SK and the anti-SK(IgG) purified on another SK substituted sepharose -4B. The immunoaffinity column was developed by coupling the purified anti-SK(IgG) to sepharose 6MB-Protein A. This column could purify the SK of the bacterial culture in a single step, with high yield and stability. The purity of SK was confirmed by SDS-PAGE and its biological activity determined in a specific SK assay. This method of SK purification is superior to the previous conventional methods as it does not require the addition of a tag to the recombinant SK or any chemical modification on the ligand.

Keywords: streptokinase, blood clot, purification, immunoaffinity

P-10-691-3
Assessment of hematological and biochemical parameters in fish with experimental spinal deformities

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Morpho-anatomical abnormalities in fish have been discussed in many studies as a frequent and important problem for aquaculture. Abnormalities of the neuromuscular system and of calcium metabolism, and certain growth, genetic, and mechanical factors may all play roles in the pathogenesis of the disorder. In addition to spinal deformities such as lordosis, scoliosis and kyphosis, which increased with prolonged deficiency, experimentally induced vitamin C deficiency for 300 days retarded growth and caused mortality. Hematological studies represented anemia accompanied by anisocytosis. Hematopoietic

studies on erythropoiesis showed increased reticulocytes. CBC revealed leucopenia accompanied by neutrophilia. Biochemical findings didn't show any significant changes.

Keywords: aquaculture, fish, vitamin C deficiency, hematology

P-10-413-1
 β -caseins inhibit the formation of amyloid fibrils in apo-forms of yeast alcohol dehydrogenase

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β -casein is a milk protein having a chaperone activity. One of the states in protein aggregation is formation of amyloid fibrils that are β sheet-rich structures. Nowadays, over 25 disorders such as Alzheimer's disease have been identified that are created by amyloid fibrils. We investigated the effects of bovine and camel β -caseins on prevention of amyloid formation in apo-forms (apo-I and apo-II) of yeast alcohol dehydrogenase (YADH, EC.1.1.1.1). To induce amyloid formation in apo-YADHs, 60% methanol solution was used. Obtained results of UV-Vis spectrophotometer by using Congo Red (CR) reagent and fluorescence spectrofluorimetry by Thioflavin T (ThT) demonstrated that both β -caseins (bovine and camel) have ability to prevent in vitro amyloid formation and they can decrease the formation of amyloid fibrils in apo-forms of YADH. Besides, bovine β -casein revealed more anti-amyloid activity than camel β -casein. Consequently, β -caseins (bovine and camel) have anti-amyloid properties. Our results can provide a good opportunity to design the effective therapeutic methods against amyloid diseases.

Keywords: amyloid fibrils, apo-forms, β -caseins, YADH

P-10-628-1
Study of relationship between Beta-2-microglobulin and inflammatory factors in hemodialysis patients

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Beta-2-microglobulin (β 2M) is a medium molecular weight uremic toxin which causes amyloidosis in patients on long-term dialysis. In this article the relation between β 2M and inflammatory factors like as C-reactive protein (CRP), albumin and high density lipoprotein (HDL) in hemodialysis patients was studied. We selected 52 hemodialysis patients and measured β 2M, CRP, albumin and HDL in patients' sera, before and after dialysis. We observed a significant indirect relationship between β 2M and albumin whereas there was no relationship between β 2M with CRP or HDL. We concluded that increasing β 2M in association with inflammation in patients undergoing hemodialysis causes a decrease in serum albumin concentration.

Keywords: albumin, beta-2-microglobulin, hemodialysis, inflammation

P-10-960-1

Comparison of toxic effects of caffeine administration on C57BL/6J mice during pregnancy in the presence of phenobarbital

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Caffeine is a natural alkaloid compound, which is metabolized by cytochrome-P450 enzyme complex in the liver. Since high consumption of caffeine in pregnant women may induce fetal side effects we compared the effects of route of caffeine administration in pregnant C57BL/6J mice with and without induction of maternal metabolism by phenobarbital. In the present study 60 pregnant mice C57BL/6J were randomly divided in two groups: Oral caffeine (oral control, phenobarbital-oral caffeine treated, and oral caffeine treated groups) and the other interperitoneal (IP) caffeine (IP control, phenobarbital-IP caffeine treated, and IP caffeine treated groups). Phenobarbital was administered IP to control and Phenobarbital groups, and added distilled water to caffeine groups at 80 mg/kg on day 9 gestation. Caffeine at 200 mg/kg were administered IP to phenobarbital-caffeine and caffeine groups 11th and 12th day of gestation, and D.W was injected IP to control groups 11th and 12th day of gestation, all mice were sacrificed on day 17 gestation, fetuses were removed and weighed. Results showed no significant difference between controls and Phenobarbital - (oral and, IP) caffeine groups, but on the other hand revealed significant different between phenobarbital-oral caffeine and oral caffeine groups, phenobarbital-IP caffeine and IP caffeine groups and also between oral caffeine and IP caffeine groups ($p < 0/05$). The result of this study which Phenobarbital at least dosage of 80 mg/kg caused a decrease of toxic effects of caffeine on fetuses' weights.

Keywords: caffeine, phenobarbital, pregnancy, fetal, C57BL/6J

P-10-938-1

Isolation of human growth hormone receptor from HepG2 cells and immuno receptor assay study

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Many different methods such as cell proliferation assay, Radio-Receptor Assay (RRA) and bioassay were used for evaluation of the biological activity and receptor binding potential of recombinant proteins, hormones and cytokines. Long timetable course and application of radioactive hormones are the disadvantages and limitations of these methods. Research for other useful methods for bioassay of recombinant proteins and hormones are under study. The aim of this research was to study the application and effectiveness of Immuno-Receptor Assay (IRA) for the receptor binding potential of hormones in vitro. For this purpose recombinant human Growth Hormone (rhGH) and growth hormone receptors from HepG2 cells were used as the model protein. First ELISA micro-titer plates were coated by partially purified growth hormone receptors. Then rhGH was interacted with coated receptors. Finally the fraction of bound complex was determined by classic ELISA detection system by using

colorimetric substrates. Our result indicated that the profile of hormone-receptor binding depended to hormone concentration and dose manner. The profile of IRA hormone-receptor diagram is comparable with RRA ones.

Keywords: human growth hormone receptor, HepG2, Immuno-Receptor Assay

P-10-984-1

Effect of *Androctonus crassicauda* (Arachnida: scorpiones) toxin on biochemical parameters of Albino mice blood

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Scorpion *Androctonus crassicauda* is second dangerous scorpion in khozestan province (southwestern Iran) and its venom is used in the production of polyvalent antisera. After night hunting of scorpions their venom was collected by electrical stimulation. The venom was lyophilized and a dose of 200µg/kg was made from it. The venom dilution was injected to twenty 18-20 gram albino mice and 5 mice were kept without injection as control. After 2 hours blood samples were taken by cutting tails, serum was separated for determination of blood glucose, urea, creatinine, cholesterol, triglyceride and total protein. The results showed a significant increase in all parameters except blood glucose which showed a slight decrease two hours after envenomation.

Keywords: scorpion, venom, glucose, urea, triglyceride, creatinine, cholesterol, total protein

P-10-723-1

Dual nucleotide specific G6PD purified from *Streptomyces aureofaciens*

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Glucose 6-phosphate dehydrogenase (G6PD) catalyses the first reaction of pentose phosphate pathway providing NADPH required for anabolic reactions. G6PD from *S. aureofaciens* utilizes both NAD⁺ and NADP⁺ as coenzyme, the binding mechanism of which was investigated in this study. Proliferation of these bacteria has been done in liquid sporulation culture. The enzyme was purified using ammonium sulfate (60%) and chromatography on the DEAE-cellulose and Sephadex G-100 by 150 fold reaching S.A. of 2.1 U/mg protein. It was shown that the purified enzyme was stable for 60 days at -20°C after which the activity declined rapidly. Electrophoresis studies with activity staining showed 3 active bands. The main one belonged to dimeric form of the enzyme with molecular weight of 107000 D. Denaturation by urea (4M) and renaturation in the presence of either NAD⁺ or NADP⁺ showed different conformational changes upon coenzyme binding as monitored by fluorescence studies. Km and Vmax obtained from kinetic studies showed that NADP⁺ stimulates reactivation two fold more than NAD⁺. The reactivation process was also monitored by measuring the accessibility of Histidine residues toward diethylpicrocarbonate modification. As the reactivation proceeded, fewer Histidine residues were able to be modified. It is concluded that NAD⁺

and NADP⁺ bind to different conformational structures of this enzyme and a single enzyme molecule can be shifted from one conformation to the other depending on the concentration of coenzyme.

Keywords: G6PD, dual coenzyme

P-10-657-1

Changes in enzyme activity of human tears due to menstrual period

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Human tear represents blood serum in many aspects of biochemical compositions and contains many biomarkers including metabolic enzymes and proteins. As tear sampling is an easy non-invasive method, it can be used as a medium for studying variations in many biochemical factors during different physiological conditions. In this research we used routine laboratory procedures for assessment of some important enzymes in tear samples during various stages of menstrual period in a group of healthy volunteers aged 18-22 years old. Their stimulated tear samples were collected using sterile capillary test tubes (4th day of period and after period for the control samples). Peroxidase activity was measured using hydrogen peroxide and 4-aminoantipyrin as substrates. The end-point method was used to determine the activity of α -amylase in the presence of starch and dinitrosulfosalicylic acid at 520 nm. Finally, the biological activity of tyrosinase was measured using L-3,4-dihydroxyphenylalanine (L-dopa), 3-methylbenzthiazolinone-2-hydrazone (MBTH) and formaldehyde. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue G-250 staining was also used for detection of proteins in samples. The results showed increased tyrosinase and peroxidase and decreased α -amylase activity in tear during period. It should be emphasized that presence of tyrosinase in human tears was detected for the first time and the increase in its activity together with rise in the activity of peroxidase is related to antioxidant capacity of tears during menstrual period.

Keywords: menstrual change, human tear, peroxidase, tyrosinase, amylase

P-10-493-1

D3 – D5 dopamine receptors expression in paranoid schizophrenia patients

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Schizophrenia, commonly developed in adolescents and young adults, is one of the most common mental disorder, but the pathophysiology and etiology of schizophrenia is still obscure. Numerous studies on dopamine and schizophrenia have suggested that the change in the dopamine system is related to schizophrenia, but there is little direct evidence for "dopamine hypothesis in schizophrenia". The purposes of study were to examine if the mRNA of the peripheral dopamine receptor is statically or dynamically changed in schizophrenia, and whether or not these receptors have value as a potential peripheral markers reflecting one in schizophrenia. 34 drug-medicated

schizophrenics and 29 healthy people were enrolled. Sequential reverse transcription and quantitative polymerase chain reaction of the mRNA were used to investigate the expression of dopamine receptors and it was compared in each group. In drug-medicated schizophrenics D3 and D5 dopamine receptor mRNA expression of the peripheral lymphocytes was not significantly different compared with that of controls. If we want to use dopamine receptors as a biomarker in schizophrenic patients, we must test patients before using any antipsychotic drug. This study also, reveals that the dopamine receptors on peripheral lymphocytes can be use in pharmacodynamic studies as a marker.

Keywords: schizophrenia, RT-PCR, dopamine receptors

P-10-363-1

Adiponectin and development of type 2 diabetes in the Ardabil population

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Adiponectin, also called GBP-28, apM1, AdipoQ and Acrp30, is a novel adipose tissue-specific protein that has structural homology to collagen VIII and X and complement factor C1q, and that circulates in human plasma at high levels. It is one of the physiologically active polypeptides secreted by adipose tissue, whose multiple functions have started to be understood in the last few years. This case-control study was performed on 100 subjects, (50 diabetics) and (50 non diabetics). The subjects were randomly selected and matched for age, sex, and body mass index (BMI). Standing height and body weight were measured and body mass index (BMI) (kg/m²) was obtained. All data are expressed as mean \pm SD. Differences in frequencies were analyzed through the chi-square method. Comparison of the continuous data between the two groups was performed by one-way analysis of variance (ANOVA). We were able to demonstrate a strong and highly significant (P<0.0001) positive correlation between adiponectin and HDL-cholesterol levels (regression analysis: r=0.86). Adiponectin serum levels were correlated to HDL-cholesterol even if factors such as age, gender, BMI and fasting insulin concentration were excluded. Although the total physiological role of adiponectin is as yet unclear, experimental studies have indicated that adiponectin has potential antiatherogenic and anti-inflammatory properties.

Keywords: adiponectin, HdL-C, analysis of variance, antiatherogenic, anti-inflammatory

O-10-146-2

Inhibitory effects of some flavonoids on human serum paraoxonase (PON1) activity

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Human serum paraoxonase (PON1) is an esterase located on high-density lipoprotein (HDL). PON1 is recognized as an antioxidant enzyme

and studies support an antiatherogenic role for the enzyme. Dietary flavonoids exert health benefits through antioxidant mechanisms. Some investigations have shown that flavonoids can induce the PON1 gene expression and thus increase PON1 activity. The current study was undertaken to investigate effects of 11 flavonoids on serum PON1 activity to gain an understanding of flavonoids and PON1 interactions in serum. Paraoxonase activity of PON1 was determined by adding serum to Tris/HCl buffer (100 mM/L, pH 8.0) containing paraoxon (2 mM) and CaCl₂ (2 mM). The rate of hydrolysis of paraoxonase substrate was measured spectrometrically by monitoring p-nitrophenol liberation at 412 nm ($\epsilon = 18,290 \text{ mol}^{-1} \text{ cm}^{-1}$). Also, paraoxonase activity was measured in the presence of flavonoids (10 μM). Flavonoids were added as methanol stocks to a final solvent concentration of <0.2% (v/v). This concentration of methanol has no significant effect on PON1 activity. Among 11 used flavonoids in this study, myricetin, morin and naringenin had the most inhibitory effect on PON1 activity. Percentages of control activity in the presence of myricetin, morin and naringenin were 89 \pm 3, 88 \pm 5, 73 \pm 6, respectively. Kinetic parameters were calculated for naringenin (the most effective flavonoid) by Lineweaver-Burk plot: $K_m=0.9 \text{ mM}$ and $V_{max}=143.6 \text{ nM/min/ml serum}$. The plot shows that naringenin is a competitive human serum paraoxonase inhibitor. The current study is a primary examination and it can provide a background for further investigations. It was concluded that the studied flavonoids particularly naringenin could inhibit PON1 activity in serum. Considering effects of flavonoids on the PON1 gene expression, it is possible that flavonoids increase PON1 activity via effect on the PON1 gene expression no by interaction with the enzyme protein.

Keywords: PON1 activity, flavonoids, naringenin, kinetics

O-10-997-1

Helicobacter pylori infection does not affect the oxidative status in patients with gastroesophageal reflux disease

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Gastroesophageal reflux disease (GERD) is caused by reflux of stomach and duodenal contents into the esophagus. Helicobacter pylori infection may be involved in the pathogenesis of GERD, as any effect of H. pylori infection on the LES or local gastric acid secretion may lead to either the occurrence or disappearance of GERD. The aim of this study was to study the effect of H. pylori infection on oxidative stress status in GERD. 155 patients (68 male and 87 female) with history of GERD were enrolled in this study. From all patients who underwent upper gastrointestinal endoscopy two biopsies from antrum were obtained for H. pylori- urease test. Also, blood samples were drawn for detection of H. pylori infection by ELISA and measurement of malondialdehyde (MDA), total antioxidant capacity (FRAP), superoxide dismutase (SOD) and glutathione peroxidase (GPX). Data was compared between patients in presence and absence of H. pylori infection. The overall prevalence of H. pylori infection in GERD was 75.0% (116/155). There were no significant differences in the levels of MDA (0.94 \pm 0.32 vs. 0.91 \pm 0.35), FRAP (973.7 \pm 218.5 vs. 1049.5 \pm 201.6), SOD (1127.4 \pm 502.1 vs. 1317.1 \pm 414.9), GPX (225.1 \pm 123.7 vs. 222.3 \pm 101.4), BMI (25.8 \pm 4.9 vs. 25.3 \pm 4.1) and age 42.6 \pm 15.1 vs. 41.4 \pm 15.6) in presence and absence of H. pylori infection in patients with GERD ($p>0.05$). We showed that H. pylori infection has no effect on oxidative stress status in GERD. There appears to be no correlation between the H pylori+ and any of the oxidative stress parameters and total antioxidant capacity in GERD patients. In conclusion, probably

GERD masks differences in oxidative status and antioxidant levels on patients with GERD in presence and absence of H. pylori infection.

Keywords: H. pylori, oxidative stress, GERD

O-10-62-1

Relationship between the obesity and overweight with plasma levels of leptin and small dense LDL among adults

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Obesity is a chronic condition with increased body fat due to a complication in appetite and energy metabolism. The main aim of this study was to investigate the effect of BMI regardless of other risk factors in amount of sdLDL and leptin and insulin. In a cross-sectional study apparently healthy people over 20years old were recruited. BMI, insulin, leptin, sdLDL, FBS and lipid profile were investigated. Our results showed that sdLDL, leptin, insulin, TG, cholesterol and FBS but not HDL were significantly different between normal and overweight individuals. We found significant correlation between sdLDL and lipids cholesterol in people with BMI<25 and BMI \geq 25 ($p<0.05$). Investigation of the association of these factors with BMI showed that age, sex, TG and waist circumference were only independent factors associated with BMI. Our founding showed that sdLDL is associated only with cholesterol among metabolic syndrome components. Among all the predictive factors only TG, and waist circumference were independent factors associated with obesity which still remained as risk factors after adjustment of sex and age.

Keywords: insulin, leptin obesity, sdLDL

P-10-965-1

Effects of body acupuncture on Hs-CRP and creatinine in Iranian obese and overweight subjects

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Body acupuncture has been reported to reduce Hs-CRP and creatinine in subjects in clinical practice. In the present study, we have evaluated the effects of body acupuncture on Hs-crp and creatinine in subjects of both genders divided into 2 groups as follows. Case Group: 90 (female=67, male=23) subjects with low-calorie diet and body acupuncture. Subjects were recruited from Nutrition Clinic, Ghaem hospital, Mashhad, Iran. The acupoints on their bodies included: Tianshu(St25), Zasanli(St36), Fenglong(St40), Naiguan(P6), Sanyinjiao(SP6). Control group: 92 (female=68, male=24) subjects with low-calorie diet and unreal body acupuncture. The acupoints on their bodies were not real and the needles were just reaching the surface of their skins. Each patient received three treatment sessions per week each 20-30 minutes for 6 weeks. Both groups were investigated for 6 weeks. Hs-crp and creatinine were measured pre- and post- treatment for all subjects. We observed significant reduction

in creatinine ($p < 0.000$) in the pre and post treatment for all subjects. The difference of Hs-crp in the pre and post treatment for all subjects was not statistically significant. It appears that needling on body acupuncture has beneficial effects on creatinine in obese and overweight subjects.

Keywords: body acupuncture, body weight, Hs-crp, creatinine

O-10-1032-1

Differential effects of opium addiction on the serum levels of Transforming Growth Factor (TGF)- β and white blood cells counts in diabetic male and female rats

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Diabetes is one of the metabolic disorders with high frequency. Some people in Iran think, opium reduces diabetic damages and this rumor is the main reason for its consuming. On the other hand, immune system in diabetes plays an important role. Nevertheless, regulatory T-cells secrete TGF- β and this manner reduces immune response, we decided to investigate the effect of opium on TGF- β and white blood cells (WBC) in addicted diabetic male and female rats. Wistar rats (14 males and 14 females, 220 to 250 gram body weight) were treated with streptozocine (STZ) subcutaneously (50 mg/kg). After 8 days diabetes confirmed and then opium solution (as 1st day 30 mg/kg, 2nd day 60 mg/kg, 3rd day 90 mg/kg, 4th day 120 mg/kg and 5th, 6th, 7th, 8th days 150 mg/kg) or physiology serum (as control) were injected intraperitoneally at 8 AM and 8 PM (7 rats in each groups). By 9th day after opium injection (150 mg/kg) or physiology serum blood were taken and TGF- β and counts of WBC were evaluated. $P < 0.05$ was considered as significant difference. While the mean serum levels of TGF- β in addicted diabetic male rats was significantly lower than that observed in only diabetic male group, the mean serum levels of TGF- β in addicted diabetic female rats was significantly higher as compared to only diabetic female group. The total numbers of WBC and the counts of neutrophils in addicted diabetic female rats were significantly higher than those observed in diabetic female control group. However, in addicted diabetic female group the counts of lymphocytes were significantly lower in comparison to diabetic female rats. Surprisingly, in addicted diabetic male rats the counts of lymphocytes was significantly higher in comparison to diabetic male groups. No significant difference was observed between addicted diabetic and diabetic male rats with respect to the total numbers of WBC and the counts of neutrophils. The results of the present study demonstrated that the opium differentially influences the production of TGF- β and also the total and differential number of WBC in diabetic male and female rats.

Keywords: opium, diabetes, addiction, TGF- β

O-10-1021-1

Anti-protease and anti-metastatic effect of *Salvia officinalis* hexane fraction

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The most common cause of cancer death in humans is metastasis of the primary tumor to distant sites. The expansion of a tumor needs blood vessels, which provide structural basis for cancer metastasis. Matrix metalloproteinases (MMPs) are key players in the growth of tumor vessels. Many natural health products inhibit cancer metastasis. Sage (*Salvia officinalis* L. laminaceae) is reported to have a wide range of biological activities, such as anti-bacterial, fungistatic, virustatic, astringent and anti-hydrotic effects. Herein, *S. officinalis* aerial parts were extracted in ethanol and its successive hexane fraction was evaluated for anti proteinase effect on MMPs expression and activity by gelatinized zymography and gel electrophoresis using human umbilical vein endothelial cells (HUVEC). At near confluence, the HUVEC cell cultures were tested with different concentrations of hexane fraction of sage. Hexane fraction of sage significantly inhibited the expression and activity of MMP-2 in a dose-dependent manner (100-200 μ g/ml) with virtual total inhibition at a 300 μ g/ml concentration. This finding provides additional pharmacological information on the therapeutic efficacy of sage and it would be considered as a novel starting point for the development of a new anti-metastatic drug to limit tumor progression. However, further investigations are required to ascertain the potential beneficial role of sage in an in vivo system.

Keywords: *Salvia officinalis*, anti-metastatic, HUVEC

P-10-1037-1

Study of recombinant erythropoietin effects on serum paraoxonase activity, total antioxidant and lipid peroxidation levels in Male Rats

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Effects of recombinant human erythropoietin (rh-EPO) on blood HDL-associated paraoxonase 1 (PON1) and oxidant/antioxidant status were investigated in normal rats which are divided into three groups of control, low dose and high dose of rh-EPO received. After one week of interperitoneal injection of rh-EPO in high dose group, and after four weeks time in low dose group, parameters including paraoxonase arylesterase activity, oxidant (malonyldialdehyde [MDA]) and antioxidant (total antioxidant status [TAS]) levels were measured. Serum paraoxonase arylesterase activity increased after the rh-EPO use. Blood TAS values were found increased and MDA levels decreased during this episode. We conclude that rh-EPO increases paraoxonase arylesterase activity and strengthens blood antioxidant potential. It also leads to a significant decrease in the level of oxidation product (MDA) in the blood samples, which demonstrates reduced oxidation reactions in the body.

Keywords: arylesterase, malonyldialdehyde, paraoxonase, recombinant human-erythropoietin, total antioxidant

P-10-679-1

Matrix metalloproteinase-9 functional polymorphism and serum activity of matrix metalloproteinase-9 in patients with early and late-onset coronary artery disease

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Coronary heart disease (CAD) is a multifactorial disorder that has both genetic and nongenetic causes. The genetic etiology of early-onset CAD has been attributed in many studies to common polymorphisms in genes that predispose to subtle changes in lipid level or those that accelerates atherosclerotic progression and destabilize vulnerable plaque. Matrix metalloproteinases (MMPs) have proteolytic activity against connective tissue proteins. MMP-9 is one of the MMPs that is required for the degradation of extracellular matrix, and is enzymatically active in atherosclerotic plaques. This enzyme is known to be associated with the progression and development of atherosclerotic lesion. The CT promoter polymorphism at position -1562 of the gene affects its transcription level. To determine the association between MMP-9 genotypes and its enzymatic activity and whether these genotypes and enzyme activities influence the onset of CAD, we examined the frequency of MMP-9 genotypes among patients with early-onset CAD (n=53, <50 years old) versus late-onset CAD (n=50 >70 years old) by molecular analysis. We also examined the activity of MMP-9 in both group by SDS-PAGE zymography. The highest MMP-9 genotype frequency in late-onset CAD belonged to CC, and the frequency of the alleles "C" and "T" were 76.7% and 23.3%, respectively. The mean value of MMP-9 enzyme activity in early-onset CAD was significantly higher than the late-onset CAD (P<0.05). comparison of MMP-9 activity in the two groups (CC and CT+TT) showed that MMP-9 serum activity in CT+TT group was significantly higher than CC group (p<0.001) and the analysis showed that a unit increase in MMP9 concentration increases the odds of disease 1.040 times.

Keywords: matrix metalloproteinase, coronary artery disease

P-10-1052-1

Chemiluminescence study of scavenging activity of Deferoxamine on reactive oxygen species

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Inhibition of iron-mediated generation of reactive oxygen species (ROS) or free radicals by some molecules might give some clues for treatment of many diseases in humans as well as animals. Deferoxamine (DFO) would be one of the drugs of choice for this purpose; as a siderophor DFO is naturally produced by *Streptomyces pilosus*, and is used as a drug of choice to treat patients with iron overload. In this study free radicals scavenging effect of DFO against ROS such as hydroxyl radical (OH[·]), superoxide anion and hydrogen peroxide (H₂O₂) was investigated by chemiluminescence (CL) assays. The results from this study showed a significant dose dependent

inhibitory effect of DFO on ROS. The scavenging activity and the half-inhibition concentration (IC₅₀) for this compound were calculated; the results showed that 10⁻⁴M of DFO can scavenge more than 95% of H₂O₂ and 2×10⁻³M of DFO has the ability of scavenging more than 85% of OH[·] in this study system. Also our preliminary results from the inhibitory effect of DFO on the OH[·]-induced oxidative damage of DNA were remarkable. Further studies are in progress in our laboratories to explain the molecular mechanisms of DFO on cells and DNA.

Keywords: antioxidant, chemiluminescence, deferoxamine, reactive oxygen species, scavenging activity

P-10-1055-1

Effect of dietary fat type and vitamin E on some of biochemical blood characters of broiler breeders and their chicks

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In two experiments the effects of fat type and different levels of vitamin E on some of biochemical blood characters of broiler breeding hens and their chicks were studied. In the first experiment, 90 broiler breeder hens (Ross 308 strain) at 27 week of age were fed used in a 2×3 factorial trial in a completely randomized design (4% canola oil and 4% tallow with different levels of vitamin E) for the period of 8 weeks. Results showed, interaction between fat type and vitamin levels in glucose (GLU), malondialdehyde (MDA), uric acid and antioxidant were significant (P<0.01) and the effects on total protein (TP), HDL and LDL were significant (P<0.05) but the effects on triglyceride (TG), cholesterol (CHO), and albumin (ALB) were not significant. In the second experiment for biochemical of serum chicks, TG and MDA of serum were significant (P<0.05) with fat type and GLU, CHO, TP, ALB, HDL, LDL, Uric acid and Antioxidant no significant in fat type were absorbed. These data suggested that supplementation of 4 % Canola oil with 150 mg/kg of vitamin E to diets based on corn-soybean meal can influence antioxidant and MDA and some of blood characters for better chick production.

Keywords: fat type, vitamin E, biochemical character, broiler breeder and chick

P-10-1056-1

Effects of anticoagulant on plasma biochemistry of sheep and comparison with serum

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Limited information exists about the effects of various types of anticoagulants on plasma biochemistry in sheep. The aim of this study is to evaluate the effect of EDTA, heparin and citrate on plasma biochemistry in sheep. Fifteen healthy Sangesari breed of sheep were blood sampled in different tube containing anticoagulants and in plain tube to harvest serum. The concentration of glucose, albumin, triglyceride, cholesterol, urea, calcium, magnesium, phosphorus, bilirubin, total protein, AST and creatinine were measured. Glucose concentration was significantly higher in EDTA tube in comparison with serum. With the exception of phosphorus, cholesterol and bilirubin, other measured parameters were significantly lower in EDTA in

comparison with serum ($p < 0.05$). Concentration of albumin and total bilirubin were significantly higher in heparin tubes in comparison with serum, while the amounts of urea was significantly lower in heparin tubes ($p < 0.05$). All the parameters except albumin were significantly lower in citrated tube in comparison with serum ($p < 0.05$). In order to eliminate the dilution effect of citrate on results, dilution corrected citrated plasma were calculated with the correction index. Except for creatinine, calcium, and albumin, the other parameters strongly correlated with serum after dilution corrected citrated plasma. The results of this study showed that heparin tube is recommended except for measuring albumin, bilirubin and urea. EDTA is not recommended. Correction dilution effect of the parameter's values obtained from citrated tubes should be performed.

Keywords: plasma, sheep, heparin, citrate, EDTA

O-10-1012-1

RBC membrane proteins undergo severe modifications during progressive obstructive cirrhosis

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Several studies have shown that erythrocyte membrane composition changes in patients with cholestasis. The alterations are supposed to reflect the status of hepatocyte membrane. We investigated how the membrane proteins modify after bile duct ligation and the overall goal was to correlate these modifications with the severity of the liver damage. We assessed the protein composition of erythrocyte membrane (protein sulfhydryls and carbonyls) after 7, 28 and 42 days of surgery in bile duct-ligated (BDL) and sham-operated animals. Also, a group of unoperated healthy rats were analyzed at the beginning of the study as the basic status of the mentioned markers. There was no considerable change of protein sulfhydryls content in 7-day BDL rats but after 28 and 42 days of obstruction, it showed a significant progressive decrease (32.99 ± 6.49 and 24.62 ± 4.91 nmol mg⁻¹ prot) compared with the healthy subjects (46.45 ± 2.53 nmol mg⁻¹ prot). In contrast, protein carbonyls were remarkably increased after 7 days of surgery as compared to those of controls (3.38 ± 0.45 vs. 1.29 ± 0.23 nmol mg⁻¹ prot) and remained at high levels for the rest of the study (3.35 ± 0.50 and 3.89 ± 0.36 nmol mg⁻¹ prot for days 28 and 42, respectively). This study clearly demonstrates that major alterations in protein status of erythrocyte membrane occur subsequent to cholestatic liver injury and the changes become more noticeable by the progression of disease. Also, our results suggest that protein carbonyls content is an earlier indicator compared to that of protein sulfhydryls although the latter correlates better to the extent of liver damage.

Keywords: cholestasis, bile duct ligation, erythrocyte membrane, protein sulfhydryls, protein carbonyls

O-10-914-1

Induction of anti EGFR antibodies using synthetic mimotope of a therapeutically applied antibody

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Epidermal Growth Factor Receptor (EGFR) is a transmembrane glycoprotein that has been considered as a target for cancer therapy. One of the most promising therapies developed against EGFR are monoclonal antibodies (MAbs) which inhibit binding of the ligand(s) to the receptor. Immunization against the receptor can be a suitable substitute for MAbs. Using phage display technique, the epitopes of ICR62 (a rat MAb against EGFR which is in the preclinical phase) was determined. Alignment of peptides found on PIII of M13 phage performed with EGFR sequence showed some nonlinear homology, so considered as mimotopes (epitope mimics). The most frequent sequence was synthesized chemically and coupled to BSA. Two groups of BALB/C mice were injected with peptide-BSA conjugate and BSA. The antisera were collected and the titer of raised antibody was measured. The EGFR was purified from A431 cell line by affinity chromatography and purification from polyacrylamide gel. Using antisera against BSA as nonspecific in an ELISA assay, the antisera against peptide-BSA (the specific binding antibody) showed high affinity (up to 1:2000 dilution) towards the purified EGFR. The presence of reactive 170 kD protein was also confirmed by Western blotting. The synthetic peptide is able to induce antibodies against EGFR.

Keywords: cancer, EGFR, ICR62, mimotope, vaccine

P-10-814-1

MGMT promoter hypermethylation is associated with P53 mutation in Iranian Glioblastoma Multiforme patients

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Glioblastoma Multiform (GBM) is the most common brain tumour in adults with the worst prognosis among others. Its standard treatment entails surgery, radiotherapy and chemotherapy. Alkylating agents are the mainstay of chemotherapy, causing DNA damage by adding a methyl group to guanine residues in DNA. Repairing enzyme which removes the methyl group, leads to tumour cell resistance to chemotherapy. O6-methylguanine-DNA methyltransferase is a DNA repair enzyme that transfers the mutagenic and cytotoxic alkyl group from the O6 position of guanine. MGMT is transcriptionally silenced by promoter hypermethylation in several human neoplasias. We used Methylation-specific PCR (MSP) to analyze the MGMT promoter methylation status of 50 glioblastoma tumours. Hypermethylation was detected in 24 of 50 (48%) samples. We also analyzed mutant p53 expression by immunohistochemical analysis of glioblastoma tissue samples. A significant association was found between MGMT methylation and p53 mutation status ($P < 0.05$). These results

suggested that epigenetic inactivation of MGMT plays an important role in the survival of glioblastoma patients and this inactivated gene is involved in p53 mutation.

Keywords: hypermethylation, glioblastoma, O6-methylguanine-DNA methyltransferase, p53

O-10-4-18

Free to total prostatic specific antigen ratio used for detecting prostatic cancer: An investigation on suitable cut-off point for the proportion of cancer in Iranian population

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Although total serum prostatic specific antigen (PSA) has emerged as the most predictive test for prostatic carcinoma but it lacks specificity. The free to total PSA (f/tPSA) ratio provides important enhancement in specificity. Since a proper cut-off value for the f/tPSA is a prerequisite for the incidence rate estimation in any population, this study was carried out to define proper cut-off value for f/tPSA in Iranian men with serum level between 4-20ng/ml. 332 male patients with tPSA between 4-20ng/ml underwent ultrasound guided transrectal biopsies. Serum tPSA and fPSA were measured by Elecsys 2010. Relationship between f/tPSA and prostate cancer was determined. Cancer was detected in 49 patients. Incidence of prostatic cancer for tPSA level <10ng/ml was 17 and for tPSA level of 4 to 20ng/ml was 32. Mean f/tPSA values were significantly lower in prostate cancer group (0.12) than in benign histology group; BHG (0.16). Among patients with PSA level 4 to 10 ng/ml mean f/ tPSA in BHG was 0.16 and in cancer group was 0.13 (p<0.05). For PSA level 10-20ng/ml, mean f/tPSA in BHG was 0.16 and in cancer group was 0.12 (p<0.05). Using cut-off value of 0.13 produced 76% sensitivity and 77.5% specificity, whereas cut-off value of 0.14 maintained 83.5% sensitivity and 61% specificity. Measurement of f/tPSA improves specificity of prostate cancer detection. This study recommends a cut-off value of 0.14 for Iranian men.

Keywords: prostate, cancer detection, biopsy, cut-off value, benign histology

P-10-15-1

Prevalence rate of the metabolic syndrome and dyslipidemia in adult population of south Iran

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The metabolic syndrome (MS) is a clustering of some cardiovascular risk factors, including hypertriglyceridemia, abdominal obesity, insulin resistance, glucose intolerance and hypertension. Limited information

is available about the prevalence of MS in south Iranian population. The aim of this study was to investigate the prevalence of the metabolic syndrome and dyslipidemia among 452 subjects (222 males and 230 females) aged 20-75 years in urban population of south Iran. The metabolic syndrome was defined by National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) and World Health Organization (WHO) diagnostic criteria. Insulin resistance was estimated by homeostasis model assessment (HOMA-IR). 60.2% males and 51.8% females had at least one abnormal serum lipid concentration. The prevalence increased with age in both genders. The age-adjusted prevalence of the metabolic syndrome was 23% (95% CI 19–26%) by the ATP III definition and 28% (24–32%) by the WHO definition. With ATP III, the age-specific rates were similar for men and women aged 20–49 years but were significantly higher for women aged 50 years. With WHO, rates were higher for men than women aged 20–49 years and similar for those aged 50 years. The most common component of the metabolic syndrome in men and women was low HDL cholesterol with the ATP III and the presence of glucose intolerance and HOMA-IR with the WHO. The metabolic syndrome is common among adult population of south Iran and is related to modifiable risk factors.

Keywords: dyslipidemia, insulin resistance, Iran, metabolic syndrome

P-11-1014-1

Anti tumor potential of *Eucheuma cottonii* L. in breast carcinoma model

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Breast cancer affects 10-25% of women globally. The findings of new method to prevent or treat this kind of malignancy have been the subject of many studies in the last decades. The present study was conducted to assess and identify the therapeutic effects of red seaweed, *Eucheuma cottonii* ethanol extract (ECE) on mammary gland tumor. Solid mammary tumors were developed in 6-8 weeks female Sprague-Dawley rats by injection of CRL 2283 cell line. Tumor development was monitored by weekly palpation. Also the level of lipid peroxidation was measured in plasma before and after treatment. Oral administration of ECE at a dose of 100 mg/kg body weight for four weeks inhibited the growth of tumors and lipid peroxidation was decreased significantly (P<0.05). The findings indicate that ECE has anti tumor effect in experimental mammary carcinoma in vivo and it may be used as an alternative antitumor drug.

Keywords: breast cancer, CRL 2283 cell line, *Eucheuma cottonii* L., lipid peroxidation, Sprague-Dawley rats

P-10-1060-2

Effect of oxidative stress caused by acute exercise on levels of some serum metabolites and activity of enzymes in professional Wrestlers and Taekwondo players

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Regular exercise practice has a protective role on coronary heart disease and promotes antioxidant defense system, whereas acute exercise induces oxidative stress. In the present study we evaluated the level of lipid peroxidation, various lipid parameters such as cholesterol, HDL-cholesterol, triglycerides (TG) and activity of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) in serum of 10 wrestlers, 10 Taekwondo players at pre- and post-training conditions. ANOVA and independent t-test indicated that, post-training MDA levels were significantly higher than pre-training MDA levels in the wrestlers ($p < 0.05$), whereas there was no significant difference in Taekwondo players group. TG in post-training samples were found to be significantly lower than in pre-training Taekwondo ($p < 0.01$) and wrestlers ($p < 0.001$). HDL-cholesterol levels in all of the training groups did not show any significant variations. Activity of serum CPK in post-training state (315.20 ± 116.86 U•L⁻¹) and (301.66 ± 156.87 U•L⁻¹) were significantly ($p < 0.001$) increased as compared to pre-training state; (80.20 ± 38.52 U•L⁻¹) and (43.66 ± 10.76 U•L⁻¹) for wrestlers and taekwondo players, respectively. There were no significant differences between LDH activities in both of training groups after exercise. There was no significant difference between the two groups in the total thiol markers. Our results demonstrated that strenuous exercises in professional wrestlers and Taekwondo players led to significant increase in CPK activity and can stimulate over-production of serum lipid peroxidation and reduction in TG levels.

Keywords: CPK, HD-cholesterol, LDH, oxidative stress, TG, wrestlers

P-10-937-1

A survey about vitamins B1 and B6 effects on hyperglycemia cats blood glucose

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Due to the important role of vitamins in nutrients metabolism especially carbohydrates, we conducted the following study. 30 hale cats were divided in to 3 groups. After keeping them kept for 10 days, the 1st group continued to receive a regular diet, the 2nd group was fed additional 50gr sucrose daily for each cat, and 30gr sucrose, 300 mg vitB1 and 100 mg B6 was added to the regular diet of the third group for each cat daily. After 10 days, blood samples were taken and checked out for blood glucose levels. The results showed that in the 1st group the average blood glucose was 120 mg/dl (minimum 98, maximum 132); in the 2nd group the average blood glucose was 490 mg/dl (minimum 320, maximum 680); and in the third group the average blood glucose was 280 mg/dl (minimum 200, maximum 430). According to the results we find out the important role of B vitamin on glucose metabolism. The 3rd group had sucrose in its diet like the 2nd

group but the difference between the results (210 mg/dl) show the role of vitamins B on blood glucose metabolism.

Keywords: vitamin B1, vitamin B6, hyperglycemia, cat, glucose

O-10-850-2

Correlation between symptomatic reactivation of HSV infection and the serum levels of TNF-α and IL-10

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Herpes simplex virus infection is a recurrent infection which often becomes apparent from latent infection established after acute infection. The role of immune factors and cytokines in the control of recurrent HSV lesions after viral reactivation is complex. However, the exact role of cytokines remains unclear. In this study we investigated the role of tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) as the two important T-helper-1 and T-helper 2 cytokines in HSV recurrence. Thirty seven individuals with recurrent herpes infection and thirty two age and sex matched individuals with no recurrences as the control subjects participated in this study. The HSV DNA isolated from symptomatic patients was confirmed by PCR. The level of serum IgG and IgM against HSV serum, IL-0 and TNF-α were also measured using ELISA method. Our data revealed that in symptomatic recurrent infection, the levels of anti-HSV IgG and IL-10 are enhanced, however, the level of TNF-α decreased as compared to that of asymptomatic non-recurrent infection. This study confirmed the controlling effect of TNF-α as a Th1 cytokine in HSV recurrence; however increased IgG response and IL-0 levels seem to correlate with recurrent symptomatic HSV infections.

Keywords: TNF-α, IL-10, HSV

P-10-1078-1

Production and characterization of three different monoclonal antibodies against tissue plasminogen activator

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Tissue-type plasminogen activator (t-PA) is a serine protease that converts the zymogen plasminogen into the active serine protease plasmin, the primary enzyme responsible for the removal of fibrin deposits. Some diseases are accompanied by a decrease or increase in t-PA concentration. On the other hand, t-pa is one of the first drugs used to treat stroke cases. But it has some complications. In a low percentage of patients treated with this drug hemorrhagic strokes will happen. In this study we have investigated production of different monoclonal antibodies against different epitopes of t-PA by using hybridoma technique. These molecules can be applicable in designing ELISA tests in order to measure this molecule's concentration in different biological fluids and also they may be used to inhibit the side effects of t-pa by neutralizing that.

Keywords: antibody, hybridoma, stroke, t-PA

P-10-498-2

The effects of body acupuncture on body composition in Iranian obese and overweight subjects

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Body acupuncture has been reported to reduce body weight, BMI, body and trunk fat mass in subjects in clinical practice. In the present study, we have evaluated the effects of body acupuncture on body composition including body weight, BMI, body and trunk fat mass in subjects of both genders divided into 2 groups as follows; Case Group: (n=90, female=67, male=23) subjects with low-calorie diet and body acupuncture. Subjects were recruited from Nutrition Clinic, Ghaem hospital, Mashhad, Iran. The acupoints on their bodies included: Tianshu(St25), Zusanli(St36), Fenglong(St40), Naiguan(P6), Sanyinjiao(SP6). Control group: (n=92, female=68, male=24) subjects with low-calorie diet and unreal body acupuncture. The acupoints on their bodies were not real and the needles were just reaching the surface of their skins. Each patient received three treatment sessions per week each 20-30 minutes for 6 weeks. Both groups were investigated for 6 weeks. Body weight, BMI, body fat mass, trunk fat mass, percent of body and trunk fat were measured before and after treatment in all subjects. Significant reduction was observed in body weight (p<0.05), BMI (p<0.05), body fat mass (p<0.05), trunk fat mass (p<0.05), body and trunk fat percentage (p<0.05) in both the case and control groups. It appears that needling not body acupuncture has beneficial effects on body composition in obese and overweight subjects.

Keywords: body acupuncture, body weight, BMI, body fat mass, trunk fat mass, percent of body fat, percent of trunk fat

P-10-923-1

Purification of streptokinase by chemical modification and separation of the contaminating proteins

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Streptokinase is used clinically as an intravenous thrombolytic agent for the treatment of acute myocardial infarction and is commonly prepared from cultures of *S. equisimilis* strain H46A. Streptokinase, unlike the contaminating proteins which make up the impurities, does not contain the amino acids cysteine or cystine. This structural difference may be employed to provide a more effective method for the purification of streptokinase from the fermentation broth. The H46A strain of group C streptococcus was grown in Brain Heart Infusion (BHI) in a BioFlo 110 fermentor. The pH was maintained between 6.9 and 7.1 by neutralization with NaOH and temperature was adjusted to 37° C for 5 hr, the fermentation was stopped by rapid

cooling to 4° C and by the addition of hexyl resorcinol. After centrifugation and sterilizing filtration of the supernatant, the filtrate was concentrated by ultrafiltration. The pH of the concentrate was adjusted, cooled, and the fraction precipitating by cold methanol was harvested. This was dissolved in water at neutrality. Protein solution was reduced with dithiothreitol (DTT) and incubated at 30° C. Aldrithiol-2 was then added and the solution incubated with agitation. The solution was then cooled and centrifuged. The supernatant was then decanted from the precipitate. The purity of streptokinase was confirmed by SDS-PAGE and its biological activity determined in a specific streptokinase assay. Numerous methods of purifying streptokinase have been described often result in unacceptable losses of streptokinase or inadequate removal of impurities and employ expensive harsh or flammable reagents, but the mentioned method can give high yield of streptokinase and is easy to perform.

Keywords: streptokinase, thrombolytic, purification, chemical modification

P-10-1099-2

Characterization of *S. aureus* enterotoxin in an allergic patient with osteomyelitis

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A 42 Years-old atopic woman with allergic rhinitis and osteomyelitis received flucloxacillin sodium (FS) 1 gram qds for 2 weeks, for the treatment of a flucloxacillin – resistant *s. aureus* (FRSA) osteomyelitis. *S. aureus* enterotoxin was isolated by biologic methods. Resistant *s. aureus* (FRSA) was recovered from fistula swab. Sodium fucidate (SF) 500 mg qds was introduced in the 14 Day. The patient became afebrile and the culture became sterile after 12 day with FS and SF treatment. The isolates were tested by agar dilution, and agar screening for enterotoxin. An isolate representative of the hospital epidemic FRSA clone which was isolated before, were subcultured several times in the presence of FS Quality control with *s. aureus* ATCC 25923 and purity control was performed with each test. Culture showed 3 strain of coagulase negative *s.aureus*. Several FRSA isolates collected from patient grew in the FC of 0.6-8 mcg/ml before induction with FC. After 5 subcultures with FS, FRSA isolates showed a FS, MIC of 3.2mcg/ml and had a correlation with enterotoxin. We studied a case of Allergic patient with osteomyelitis due to a *s. aureus* strain successfully controlled with oral SF plus, SF, the fact they resistance could be induced in hospital epidemic FRSA strain.

Keywords: enterotoxin, *S. aureus*, flucloxacillin, allergic patients

P-10-481-1

Fatty acid distribution in low density lipoprotein in diabetes in Sari, North of Iran

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Atherosclerosis is commonly found in diabetes. There is an association between low density lipoprotein (LDL) phenotype, which is more

prevalent in the diabetic state, and atherosclerosis. LDL is more easily oxidized and it is possible that fatty acid compositional changes, particularly an increase in polyunsaturated fatty acids, could underlie this association. However, there is little information about fatty acids in the different LDL phenotypes in the literature. Our study examined LDL subfraction composition in 25 non-insulin-dependent diabetic (NIDDM) patients and 15 control subjects. LDL was isolated and fractionated into LDL 1, 2 and 3 by density gradient ultracentrifugation. NIDDM patients had significantly more fatty acids in all LDL subfractions than control subjects ($P < 0.01$). Palmitic and linoleic acids were significantly greater in all subfractions in the diabetic patients compared to control subjects ($P < 0.01$) and palmitoleic and oleic acids were also greater in LDL1 and LDL2 in diabetic patients ($P < 0.01$). We conclude that in NIDDM fatty acids are increased in all LDL subfractions and this may be the reason for the increased atherosclerosis in diabetes irrespective of phenotype.

Keywords: NIDDM, low density lipoprotein, Sari, fatty acid

P-10-1166-1

Application of somatic proteins for diagnosis of *Fasciola hepatica*, *Fasciola gigantica* and *Dicrocoelium dendriticum*

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Dicrocoelium and *Fasciola* are duct parasites of different animals that are reported from cattle, buffalo, camel, sheep, goats, wild rabbit and hogs in Iran and many other parts of world. Studies show that these parasites become pathogens in the livers of final hosts. The new technologies in the field of molecular biology have caused basic changes in the classic researches and indistinguishing parasites. One of these methods is the somatic proteins identification of the parasites resulted from SDS-PAGE. The results of present study show that *D. dendriticum* protein bands are in 14.4-116 KDa range and their number is 15. *F. hepatica* protein bands are in 18-63 KDa and include 7 bands, while *F. gigantica* in the same range has 9 bands. According to the results, this can be introduced as a new method for diagnosis of trematodes.

Keywords: clinical diagnosis, *Fasciola hepatica*, *Fasciola gigantica*, *Dicrocoelium dendriticum*, protein bands, SDS-PAGE

P-10-433-1

The effect of ASA on the respiratory chain complexes of type I diabetic rats

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Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of pancreas, leading to a deficiency of insulin. This type of diabetes can be further classified as immune-mediated or idiopathic. Aspirin (acetylsalicylic acid or ASA) have been used as an analgesic to reduce fever, to treat cardiovascular diseases, cataract, some cancers, etc., with the unknown mechanisms. We investigated the effect of high-dose and long-term ASA therapy on the

streptozotocin (STZ)-induced diabetic rats, as a model of type I diabetes. In this study, rats were divided into four groups, two normal and two diabetic groups and then type I diabetes was induced in diabetic groups by i.p. injection of STZ (50 mg/kg). One normal and one of the diabetic groups received 100 mg/kg/day of ASA in drinking water for five months up to 5 months. Our results indicated the beneficial effect of ASA on type I diabetic in comparison with the untreated diabetic rats. ASA treatment improved blood glucose, insulin secretion, weight, antioxidant system, lipid profile, liver enzymes and respiratory chain complexes (I, II and III). We hypothesized that it has a chemical chaperone like activity and affects the protein folding thus its beneficial effect is not restricted to one organ and system. The mechanism(s) involved in these beneficial effects are investigating in our studies.

Keywords: acetylsalicylic acid, aspirin, rat, type I diabetes

O-10-40-1

Effect of IFN- γ and TMP-SMX on neutrophil NO production in CGD patients

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Chronic granulomatous disease (CGD) is an inherited disorder with severe and recurrent bacterial and fungal infections in which phagocytic leukocytes fail to generate superoxide (O_2^-) and antimicrobial oxidants. The prophylactic and therapeutic validity of a long-term use of trimethoprim-sulfamethoxazole (TMP-SMX) and Interferon- γ (IFN- γ) has been well established. The role of nitric oxide (NO) produced by phagocytic cells is unknown, and the mechanism of TMP-SMX is unclear. We have measured NO production in vitro and in vivo by using Griess test in 20 CGD patients (group one: 10 patients treated with cotrimoxazole, group two: 10 patients treated with cotrimoxazole and IFN- γ simultaneously) and control healthy individuals. Serum nitrite level was $24.9 \pm 8.4 \mu\text{mol/l}$ in the control group, $24.3 \pm 5.7 \mu\text{mol/l}$ in group 1, and $32.2 \pm 5.6 \mu\text{mol/l}$ in group 2. There was a significant relationship between groups in the serum nitrite level ($P = 0.023$); patients received IFN- γ had higher amount of NO compared to other group. NO production was increased after addition of 100U IFN- γ in vitro in two groups of patients significantly; the amount of NO in group 1 was $20.2 \pm 6.1 \mu\text{mol}/106 \text{ PMNs}$ and increased to $24.3 \pm 8 \mu\text{mol}/106 \text{ PMNs}$ ($P = 0.002$), likewise its amount was $20.6 \pm 8 \mu\text{mol}/106 \text{ PMNs}$ in group 2 and increased to $27.7 \pm 9.2 \mu\text{mol}/106 \text{ PMNs}$ ($P = 0.003$) after adding of in vitro. Our study showed NO production increased after IFN- γ treatment. It can be one of mechanisms of IFN- γ therapeutic effects on CGD patients. Since NO has bactericidal actions particularly against intracellular pathogens, its application is crucial in CGD patients and can prevent their infections.

Keywords: chronic granulomatous disease, CGD, IFN- γ , sulfamethoxazole, trimethoprim, nitric oxide

O-10-4-3

Inhibition of thermal aggregation of type B yeast hexokinase by small molecules: possible involvement of aromatic interactions

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Protein aggregation is a pathological hallmark of several human disorders, and a central problem in biotechnology, occurring during purification, sterilization, shipping and storage of protein structures. Currently, there is no approved therapeutic agent directed towards inhibition of aggregate formation. Recently, it has been suggested that one important approach in the development of therapeutic agents is the use of small molecules which have been demonstrated to cause inhibition of amyloid core formation via π -stacking interactions. In order to explore the effect of small molecules in amorphous aggregate formation, yeast hexokinase-B, a key enzyme in metabolism, has been chosen for the present study. Thermal aggregation of the enzyme was investigated in 50mM phosphate buffer, pH 7 at 60 °C and the extent of aggregation was measured by monitoring the increase in absorbance at 350 nm versus time. Possible anti-aggregation effects of a variety of artificial small molecules including indole, carbinol, phenol, tryptophan, tryptophol, indoxyl acetate and indo-methacin were explored. Turbidity of the protein solutions was found to be diminished by the presence of these small molecules in the above condition, with the highest and the lowest effects being exerted by indo-methacin and indole, respectively. Dynamic light scattering and HPLC confirmed that indo-methacin had the highest anti-aggregation effect. The present investigation therefore has demonstrated that involvement of π -stacking forces between amino acids which has been reported to occur in relation to formation of amyloid structures is also involved in the case of amorphous aggregate formation

Keywords: yeast hexokinase B, protein aggregation, small molecule, π -stacking interactions

P-10-4-21

Association between Angiotensin II type 1 receptor A/C 1166 polymorphism and the presence of angiographically-defined coronary artery disease in an Iranian population

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Association studies have shown a relationship between a polymorphism of the angiotensin II type 1 receptor (AT1R, A/C 1166) gene and

coronary artery disease (CAD), hypertension, and myocardial infarction in Caucasian and Japanese populations. We have investigated the frequency of the A1166C polymorphism in Iranian patients with angiographically-defined CAD and compared them with healthy subjects. Patients with CAD (n=315) were identified by angiography (>50% stenosis of any major coronary artery) (Angio+). Angiography negative (Angio-) subjects (n=98) were defined as having lesions with <50% stenosis. The angiography positive group comprised individuals with single, two vessels, or three vessel disease. Controls (n=135) were drawn from the same homogeneous Iranian population (Mashhad, Northeastern of Iran). The AT1R polymorphism was assessed using a polymerase chain reaction restriction fragment length polymorphism based method. Of the Angio+ group 397 had the A/A genotype, 114 were of the A/C genotype and 37 had a C/C genotype. There were significant differences in both allele and genotype frequencies between Angio+ and controls ($p < 0.001$, and between Angio+ and Angio- groups ($p < 0.001$). There was also a relationship between genotype and the number of coronary arteries affected. The A/C1166 polymorphism of AT1R gene may be an important risk marker for CAD in this Iranian population.

Keywords: Coronary artery disease, Angiotensin II type 1 receptor A1166C gene polymorphism, vessels disease