Vitamins and nutrition

Cod: 1574

VITAMIN D STATUS IN A TURKISH POPULATION: A RETROSPECTIVE STUDY

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BACKGROUND: Recent studies have reported lower vitamin D levels than expected in different sex and age groups in developing countries. In the present study, we investigated vitamin D status and relationship between age and sex groups among a Turkish population.

METHODS: Serum 25-OH Vitamin D3 [25(OH)D3] levels measured in 13429 individuals (4641 males and 8788 females) aged between 1-95 with an ion-exchange HPLC method with UV dedector. Data were collected retrospectively from outpatients via laboratory information system of Gulhane Military Medical Academy (Ankara, Turkey), between January 2007 – December 2011. The values that exceeded ±4 SD were excluded. All variables were tested for normality using the Kolmogorov-Smirnov test. Statistical analysis was performed using Medcalc Ver 9.2.01 (MedCalc Software, Ostend, Belgium).

RESULTS: The mean serum 25(OH)D3 concentrations in summer were 25.9 ± 12.7 and 23.2 ± 13.6 ng/mL for males and females, respectively. They were 23.0 ± 12.1 and 21.5 ± 13. ng/mL in winter for males and females, respectively. Reference intervals in summer were 6.55 – 55.2 and 6.05 – 56.0 ng/mL for males and females, respectively. They were 6.20 – 50.5 and 5.90 – 55.6 ng/mL in winter for males and females, respectively. Our current reference interval (RI) for 25(OH)D3 is 11-70 ng/Ml in our laboratory.

CONCLUSIONS: For all groups, RI limits were lower than current values which were in use in our laboratory. Our study results indicate that vitamin D deficiency is a highly prevalent condition in a Turkish population and could be a matter of concern for public health authorities.

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PLASMA METHYLMALONIC ACID AND HOMOCYSTEINE AS FUNCTIONAL VITAMIN B12 DEFICIENCY MARKERS IN PHENYLKETONURIA AND HEALTHY CHILDREN

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BACKGROUND: Phenylketonuria (PKU) patients are at risk for functional B12 deficiency, based on their diet restricted of natural proteins. Elevated levels of methylmalonic acid (MMA) and/or homocysteine (Hcy), which are more sensitive functional markers of deficiency than B12 in blood are considered as measures of impaired intracellular B12 status. However, the diagnostic value of these parameters are not studied extensively.

METHODS: The aim of this study is to investigate functional B12 deficiency using plasma MMA and Hcy in treated PKU patients and healthy subjects with serum B12 levels in normal range; to compare the diagnostic value of MMA and B12 in B12 deficiency. 31 PKU patients on diet and 26 healthy children aged 5-18 years were included in the study. Serum B12, ferritin, folate, MCV and phenylalanine concentrations were studied with routine laboratory methods. HPLC was used for plasma Hcy assay. Plasma MMA was determined using a stable isotope dilution-multiple reaction monitoring liquid chromatography-tandem mass spectrometry (SID-MRM LC-MS/MS) method.

RESULTS: The analytical performance for MMA assay was as follows: accuracy 100±4%; linearity up to 100 μM; intra- and inter-assay CVs% less than 6.0; LOD, 0.021 μM and LOQ, 0.085 μM; recovery, 86.4%. When PKU and control groups were compared, no significant differences were found in B12, MMA and Hcy levels. 12.9% of patients with PKU were found with low B12 concentrations, whereas 29% and 9.7% of patients with PKU had high MMA and Hcy respectively. In the control group, 30.7% and 11.5% had elevated plasma MMA and Hcy concentrations, respectively. Taking data of PKU and control subjects together, plasma MMA and MCV were negatively related to serum B12 levels. We have also found that, in B12 deficiency plasma MMA measurement is significantly 5.33 times (95% CI=1.62-17.49) more diagnostic than serum B12 assay.

CONCLUSIONS: Our study clearly demonstrated that; even with serum B12 levels within reference ranges, functional B12 deficiency may be seen in both healthy subjects and PKU patients who are at risk for potential B12 deficiency. Additionally, the significantly higher diagnostic value of plasma MMA measurement compared to serum B12 in B12 deficiency, has been shown for the first time in PKU and healthy children.

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FULLY AUTOMATED LC-MS/MS ANALYSIS OF 25-HYDROXYVITAMIN D2 AND 25-HYDROXYVITAMIN D3 WITH ZINMASS-200 CLINICAL LC-MS/MS ANALYZER

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BACKGROUND: The measurement of serum concentrations of 25-hydroxy vitamin D2 and D3 is the most accurate way to determine vitamin D status in human body. Quantitation of these metabolites is very important because of their clinical significance in variety of disorders. The conventional methods based on HPLC-UV and immunoassay has disadvantages for sensitivity, specificity and analysis time. As a result of these conditions, interest in LC-MS/MS methods growing during recent years.

METHODS: 500 ul of serum samples pipetted into sample preparation vials. 400 ul of Reagent 1 including salt solution in appropriate concentration added and mixed serially by vortex unit of automated sample preparation system. 400 uL of deuterated 25-hydroxyvitamin D3 internal standard mixture in organic solvent added and mixed serially by vortex unit of sample preparation system. After centrifugation at 5.000 rpm for 1min, 50 ul of upper phase injected to LC-MS/MS system. Deionized water and methanol were used as mobile phases for isocratic elution. 100x2 mm reversed-phase HPLC column used for chromatographic separation. Analysis run time was 3 minutes. Confirmation and quantification ions for all analytes and internal standards determined in APCI(+) scan mode.

RESULTS: Analysis run time was 3 minutes for 25-hydroxyvitamin D2 and D3. Recovery for analytes were between 97%-105%. Linearity from 2,5 to 400 µg/L for both analyte. Correlation coefficients were 0,9989 and 0,9995. LOD for 25-hydroxyvitamin D2 was determined as 1 µg/L while LOD for 25-hydroxyvitamin D3 was 0.8 µg/L. CV values for 25-hydroxyvitamin D2 and D3 were 2,4 and 2,7% for intra-assay and 3,4-3,6% for inter-assay. No interferences was found for these analytes. The NIST SRM 2972 sample was measured with this automated system and the results were found in median range for both analyte.

CONCLUSIONS: Fully automated, reliable, rapid and sensitive LC-MS/MS method was developed for analysis of 25-hydroxy metabolites of Vitamin D. All sample preparation, data acqusition and calculation steps were made by a ZinMass-200 Series Clinical LC-MS/MS Analyzer without any human control for reducing user errors in sample preparation.

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THIOL STATUS IN VITAMIN B12 DEFICIENCY

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BACKGROUND: Vitamin B12 (B12) is a hematopoietic and essential vitamin for the body and plays an important role in metabolism. Its deficiency causes many hematologic and neurologic symptoms. Thiol (SH) groups exist in enzyme's active sites and serum levels of SH is especially determined by albumin (alb). SH groups are also present in functional and structural non-alb proteins. It is known that homoscyteine (HC) has SH but its effect on serum SH levels is minimal. In this study we aimed to evaluate if B12 affect SH status of metabolism or not.

METHODS: 32 patients with B12 deficiency as pre-treatment group (pre-tx) and 30 healthy volunteers as control group (control) were included. Patients with B12 deficiency were treated with cyanocobalamin 4 times in a month. After treatment patient's samples were taken as post-treatment group (post-tx). Serum B12, alb and HC levels were measured. SH levels were measured by Sedlak J.'s method.

RESULTS: All parameters were expressed as mean±SD. B12, HC, alb and SH levels of pre-tx were 150.29±34.21 pg/mL, 15.60±6.11 µmol/L, 4.64±0.41 g/dL, 219.30±24.90 µmol/L; levels of post-tx were 692.01±329.67 pg/mL, 8.56±2.65 µmol/L, 4.54±0.33 g/dL, 205.13±4.76 µmol/L and levels of control were 374.74±117.91 pg/mL, 12.51±4.35 µmol/L, 5.45±0.46 g/dL, 232.35±4.76 µmol/L; respectively. SH levels of both pre-tx and post-tx groups were significantly lower than level of control ($p<0.05$, $p<0.05$; respectively). HC levels of pre-tx was significantly higher than levels of control and post tx ($p<0.05$, $p<0.001$; respectively). Also post-tx SH and HC levels were significantly lower than pre-tx's levels ($p<0.05$ and $p<0.001$; respectively). Alb level was significantly higher in control than both pre-tx and post-tx ($p<0.001$ and $p<0.001$; respectively) but no difference was found between pre-tx and post-tx.

CONCLUSIONS: It was shown that SH levels decreased in B12 deficiency. This decrease might be explained by alb decline. Since pre-tx and post-tx alb levels were not significantly different, we thought that the reduction of SH levels with one month B12 treatment is due to the non- alb proteins. This decrease in SH levels is considered to be as a results of decrease in HC or increase in intracellular SH related proteins.

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COMPARISON AMOUNT OF VITAMINS OF B1 ,B2 ,B6 IN IRANIAN AND IMPORT RICE BEFORE AND AFTER POLISHING

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BACKGROUND: Rice is the principle food for 50% of the world population, and is also the main foodstuff in the Iranian dietary. Rice contains B-vitamins (especially, B1, B2 and B6) that could provide nutritional needs. The aim of the present study was to evaluate effectiveness of different cooking procedure on B1, B2 and B6 vitamins content in the rice.

METHODS: In an experimental study rice is cooked by direct cooking (Dampokht) and cooking with wash and drain (Abkesh) as described in the common cook books and a stainless steel cookware was used. Homogenized rice's solution heated 70 centigrade for 40 minutes in a bathroom. Rice's solution passed through whatman filter paper and 0.45 micrometer filter consequently and injected to HPLC column to determine vitamins. We used different kinds of rice brand, including; Sadri (Ali Kazemi), Sang Tarem, Tarem dom seyah, Fajr (high product), Tarem Neda (high product), Pakistanian and Indian rice. The amounts of vitamin was measured and compared during and after cooking in different procedure.

RESULTS: Generally, our results showed decreased level of vitamins in cooking with wash and drain (Abkesh) in comparison with direct cooking (Dampokht). Interestingly, the amount of B1–vitamin increased after direct cooking (Dampokht).

CONCLUSIONS: The results indicated that the rice cooking procedure is very important in the maintenance of B-vitamins in the foodstuff and draining of the rice (Abkesh) will lead to lose a lot of water-soluble vitamins.

Vitamins and nutrition

Cod: 1579

EFFECTS OF ARTIFICIAL FOOD COLOURINGS, EXPOSED DURING PRENATAL TERM VIA MOTHERS DIET WITH THE DOSE OF NOAEL, ON LEARNING AND MEMORY

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BACKGROUND: Artificial food colourings (AFC) and additives are among the most used items in today's daily life. Some research studies suggest that exposure to AFC and additives can affect some processes such as behaviour, learning and development in children. In this study, we aimed to investigate the effect of exposure to AFC during intrauterine period on behaviour and memory in adult period.

METHODS: A total of 30 female rats were included in this study. Animals were equally seperated into two groups as follows: Controls (CG) and AFC given group (EG). EG was given a mixture of nine AFC at NOAEL (No Observed Advers Effect Level) dose and CG was administered only tap water in daily basis via oral gavage way beginning one week prior to pregnancy till delivery. When the offsprings have become adults, the possible effect of AFC on learning and memory functions was assessed by using Morris water maze and the behavioral outcomes were evaluated by performing open field and forced swimming tests.

RESULTS: In-group comparison of daily performances of all groups and also EG and CG, there was a significant decrease for the data of latency to escape to the hidden platform beginning from day-2 ($p < 0.01$). Whereas in between-group comparison, there was a statistically prominent decrement in male EG compared to those of female CG and EG in day 1 and in male CG compared to those of female CG in day 2 for the data of latency to escape to the hidden platform ($p < 0,05$). There was also a significant decrease in mean speed values in female EG than female CG when all groups were compared within and in EG than the CG when EG and CG were compared ($p < 0,05$). There was a significant increase in defecation number in EG and in male gender ($p < 0,05$). Male EG has had a significantly lower mobility time than the female CG, whereas they had a prominently higher immobility time ($p < 0,05$).

CONCLUSIONS: As a result, we have concluded that intrauterine exposure to widely used AFC at NOAEL dose level does not necessarily affect to the hippocampus-dependent spatial learning and memory, however it can lead to some negative outcomes in motivation, anxiety and depression tests and this effect may differ according to gender.

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VITAMIN D IN PATIENTS WITH CHRONIC BENIGN AND MALIGNANT PANCREATIC DISEASES: A PILOT STUDY

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BACKGROUND: There are no extensive data on the comparative evaluation of 25-hydroxyvitamin D (VIT D) serum levels in patients with chronic benign and malignant pancreatic diseases. We evaluated serum VIT D levels in patients with chronic pancreatitis (CP), pancreatic adenocarcinoma (PA) and in healthy subjects (HS).

METHODS: Sixty-nine consecutive subjects were studied: 20 patients with a firm diagnosis of CP (12 M, 8 F; mean age 56.0 yrs, range 25-74; 14 were alcohol drinkers and 12 were smokers; 12 had calcifications and 5 diabetes mellitus; 6 were studied during a flare of CP), 20 histologically confirmed PA (11 M, 9 F; age 63.5 yrs, range 47-80; 5 patients were alcohol drinkers, 3 were smokers 11 had diabetes; all patients had pancreatic pain); 29 HS (13 M, 16 F; mean age 59.5 yrs, range 44-75). Serum VIT D levels were measured using chemiluminescence assay (CLIA) (DiaSorin, Italy). VIT D levels were normally distributed and ANOVA test was applied to analyse the data. In addition, fecal pancreatic elastase-1 (FE), as marker of exocrine pancreatic insufficiency (ScheBo Biotech AG, Germany), was also measured in patients with pancreatic diseases.

RESULTS: The 3 groups of subjects were comparable for sex (P=0.554) and age (P=0.107); exocrine pancreatic insufficiency (FE <200 μg/g) was present in 14 patients with CP and in 1 patient with PA (P<0.001). Serum VIT D was significantly different among the 3 groups of patients studied (mean±SD; 10.9±8.1 ng/mL in CP patients, 12.5±5.6 ng/mL in PA patients and 23.6±8.3 ng/mL in HS; P<0.001). CP and PA patients had similar VIT D serum levels (P=0.258) and these levels were significantly lower than those of HS (P<0.001). Taking into consideration clinical parameters such as the disease (CP, PA, HS), sex, exocrine pancreatic insufficiency, alcohol, smoking habit, pancreatic calcifications, pain and diabetes mellitus, only the type of the disease (i.e. CP or PA) at univariate analysis was significantly associated with low VIT D levels (P=0.036).

CONCLUSIONS: Low VIT D levels are associated with chronic diseases of the pancreas, but the mechanism should be elucidated because these low levels are not associated with sex, exocrine pancreatic insufficiency, alcohol and smoking habit, pancreatic calcifications, pain and diabetes mellitus.

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Cod: 1581

SEPARATION AND QUANTIFICATION OF VITAMINS B1 AND B6 ACTIVE VITAMERS BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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BACKGROUND: Deficiencies in vitamins B1 and B6 are a noteworthy problem in cases of malnutrition, often affecting elderly populations or individuals with a history of alcoholism. As well, cases of malabsorption, as in gastrointestinal compromise due to disease or surgical intervention are also of interest. Since the active metabolites of these B-vitamins have key roles in energy metabolism and nerve function, reliable quantification of their levels in blood is desirable. However, the highly polar and labile nature of these active metabolites is a significant analytical challenge for effective separation by reversed-phase HPLC systems.

METHODS: We describe the application of a hydrophilic interaction liquid chromatography (HILIC) approach with mass spectrometry detection using a Zwitterionic HILIC column.

RESULTS: Using this mode of chromatography, we achieved concurrent and robust separation enabling accurate quantitation of both B1 vitamers, thiamine pyrophosphate (TPP) and thiamine monophosphate (TMP), as well as B6 vitamers, pyridoxal-5-phosphate (PLP), pyridoxamine-5-phosphate (PMP), and pyridoxic acid (PA) within a single twenty-minute run.

CONCLUSIONS: The advantages of this method include: (1) simplified sample preparation which does not require chemical derviatization with fluorescent labels; (2) improved electrospray ionization sensitivity due to the high proportion of volatile organic solvent in the mobile phase used in HILIC; and (3) minimization of ion suppression in MS quantification as the B1/B6 vitamers are effectively retained by HILIC and separated from other small hydrophilic molecules and ions.

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DOES VITAMIN D DEFICIENCY EFFECT PLASMA TOTAL ANTIOXIDANT STATUS LEVELS?

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BACKGROUND: The aim of this study is to determine plasma total antioxidant status levels in individuals whose plasma 25-OH-vitamin D (25-OH-Vit D) levels were measured and to investigate the possible relationship between vitamin D and plasma total antioxidant status levels and whether 25-OH-Vit D deficiency has an effect on antioxidant status.

METHODS: Eighty one individuals with no history of any systemic disease whose 25-OH-Vit D levels measured by high-performance liquid chromatography were included to the study. Individuals were divided into 3 groups as deficiency, insufficiency and sufficiency, according to their 25-OH-Vit D levels. Plasma total antioxidant status levels were measured colorimetrically.

RESULTS: Age was significantly lower in deficiency group than sufficiency group. When compared to 25-OH-Vit D sufficiency and insufficiency groups, lymphocyte count was significantly higher and erythrocyte sedimantation rate was significantly lower in deficiency group. In 25-OH-Vit D deficiency, insufficiency and sufficiency groups, positive moderate [(rs = 0,477; p= 0,001), (r= 0,489; p= 0,040), (r= 0,452; p= 0,039)] and good [(r= 0,513; P< 0,001), (rs= 0,535; p= 0,022), (r= 0,532; p= 0,013)] correlations was found between hemoglobin+hematocrit and total antioxidant status, respectively. In 25-OH-VitD sufficiency group negatively moderate (r= -0,450; p= 0,041) correlation was found between C-reactive protein and total antioxidant status levels. No significant correlation was found between plasma total antioxidant status and 25-OH-Vit D levels.

CONCLUSIONS: Any significant relationship between plasma 25-OH-Vit D and total antioxidant status levels wasn't found.
Key Words: Total Antioxidant Status, 25-OH-Vitamin D

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COMPARASION OF HOLOTRANSCOBALAMINE WITH OTHER MARKERS OF VITAMIN B12 DEFICIENCY IN MYELOPROLIFERATIVE DISORDERS

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BACKGROUND: Myeloproliferative disorders (MPD) is a heterogenous group of diseases. Vitamin B12 levels are measured falsely elevated with conventional methods because of increased carrier protein synthesis in MPD. The measurement of total plasma vitamin B12, has limitations of sensitivity and specificity. Our objectives were to compare effectiveness of Holotranscobalamine (activeB12) with other markers of vitamin B12 in detecting a true vitaminB12 deficiency in MPD.

METHODS: Vitamin B12 levels in patients followed up at our hospital hematology clinic for myeloproliferative disorders were measured by both electrochemical immunoassay and HoloTranscobalamine methods, and the results were compared. Also methylmalonicacid(MMA), folate, homocystein, AST, ALT, kidney function tests and CBC& differentials were obtained. The exclusion criteria were patients with chronic gastrointestinal disorders, prior gastric/ileal resection, concurrent metformin use, being on an absolute vegetarian diet.

RESULTS: By using electrochemical immunoassay total vitamin B12 of 7 patients(14%) were elevated. Only 2 of the patients(%4) were found to have vitamin B12 deficiency by this method. When we measured B12 levels by HoloTranscobalamine, we found low levels in 14 patients(%18). Active B12 was %36 sensitive and %86 spesific. İn this patients vitamin B12 deficiency was %62 by measuring MMA.

CONCLUSIONS: HoloTranscobalamine assay was reported to be superior to conventional B12 measurements in diagnosis of vitamin B12 deficiency. Vitamin B12 levels are measured falsely elevated with conventional methods because of increased carrier protein synthesis in MPD. Our study showed that HOLO TC is more effective in revealing unrecognized vitamin B12 deficiencies in patients with MPDs.

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IMMUNE COMPLEXES MIGHT EFFECT HOLOTRANSCOBALAMINE ASSAY OF VITAMIN B12 DEFICIENCY IN MYELOPROLIFERATIVE DISORDERS

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BACKGROUND: HoloTranscobalamine method is a first choice assay for detecting true vitamin B12 deficiency in meloproliferative disorders(MPD) made up of polycythemia vera (PV), essential thrombocytosis (ET), chronic myeloid leukemia (CML) and myelofibrosis (MF). İn this group of diseases vitamin B12 levels are measured falsely elevated with conventional methods because of increased carrier protein synthesis in MPD. Our objectives were to determine effects of immuncomplexes on Holotranscobalamine (activeB12) assay.

METHODS: VitaminB12 levels in patients followed up at our hospital hematology clinic for myeloproliferative disorders were measured by both electrochemical immunoassay and HoloTranscobalamine methods. To exclude immune complexes, samples were treated with polyethylene glycol(PEG) and HoloTranscobalamine assays were repeated. Also methylmalonicacid (MMA), folate, homocystein, liver, and kidney function tests were obtained. The exclusion criteria were patients with chronic gastrointestinal disorders, prior gastric/ileal resection, concurrent metformin use, being on an absolute vegetarian diet.

RESULTS: When we measured B12 levels by using HoloTranscobalamine, we found low levels of B12 in 14 patients (18%). Active B12 was 36% sensitive and 86% spesific. After PEG treatment, 25 of patients (50%) were vitamin B12 deficient and the sensitivity, specifity of HoloTranscobalamine methods for vitamin B12 deficiency were 76% for each. İn this patients, vitamin B12 deficiency was 62% by measuring MMA. B12 levels by HoloTranscobalamine assay decreased by 19% in ET, 40% in KML, 30% in MF and 21 in PV patients.

CONCLUSIONS: Our results showed that Immunoglobulin–HoloTranscobalamine complexes may affect holotranscobalamin assays which have been recently reported to have a superior diagnostic accuracy for vitamin B12 deficiency.

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CORD BLOOD BONE MARKERS IN RELATION TO MATERNAL FOLATE AND VITAMIN B12 CONCENTRATION

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BACKGROUND: Maternal folate was suggested to be associated with infant anthropometric measurements and previous studies reported association between bone health and folate status in adults. In the present study effect of maternal folate and vitamin B12 on cord blood bone markers was investigated.

METHODS: Eighty-four pregnant women and their term neonates included in the observational study. A questionnaire composed of 19 items about socio-demographic status, weight gain during pregnancy, diet and nutrition habits and multivitamin supplementation were used to collect data. Maternal and cord blood Ca, P, vitamin D, osteocalcin, calcitonin and maternal serum vitamin B12 and folate were measured. Multivitamin use during pregnancy was recorded.

RESULTS: Cord blood calcium and phosphorus levels were significantly higher than maternal values ($P<0.05$). Maternal and cord blood 25(OH) vitamin D concentrations were not significantly different ($P=0.84$). Maternal folate and vitamin B12 were not significantly correlated with maternal and cord blood osteocalcin and calcitonin. Cord Calcitonin and osteocalcin levels were not significantly different between those taking multivitamin supplementation and those not taking multivitamin supplementation. Cord calcitonin were significantly related with cord calcium ($P=0.006$).

CONCLUSIONS: Maternal folate or vitamin B12 did not show a significant effect on cord blood bone marker concentrations.

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Cod: 1586

EVALUATION OF VITAMIN K DEPENDENT PROTEINS IN ROMANIAN NEWBORNS

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BACKGROUND: In our study we have evaluated in paired samples, mothers and their newborns, known biomarkers reflecting vitamin K status: descarboxyprothrombin (PIVKA-II), uncarboxylated osteocalcin (ucOC), and the ratio (UCR) between uncarboxylated and carboxylated osteocalcin (cOC). A second objective was to evaluate whether vitamin K status depends on prenatal growth.

METHODS: 59 newborns between 32 and 42 weeks of GA and their mothers were included in the study, which had a cross-sectional design. Maternal blood was taken by venipuncture, child blood was obtained from the umbilical vein. Conformation-specific ELISAs based on monoclonal antibodies were used for measuring circulating PIVKA-II, ucOC, cOC using commercial test kits.

RESULTS: A positive correlation between PIVKA-II and ucOC was found in mothers and their offspring. Forty nine newborns had birth weights appropriate for gestational age (AGA), seven were small for gestational age (SGA) and three were large for gestational age (LGA). In SGA newborns ucOC was significantly lower than in AGA and LGA newborns (27.8 ± 9.5 ng/ml versus 63.7 ± 5.4 ng/ml in AGA and 51.1 ± 21.5 ng/ml in LGA), whereas cOC was independent of GA.

CONCLUSIONS: ucOC increased at increasing gestational age. In SGA newborns, ucOC was significantly lower than in AGA and LGA ones. Collectively, these data suggest that rapid growth in the weeks preceding birth exhaust the available vitamin K supplies in the newborn. The effect is more pronounced for bone (ucOC) than for liver (PIVKA-II); a similar trend was observed for the mothers at the time of delivery.

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Cod: 1587

TERMS OF VITAMIN D DEFICIENCY EVALUATION OF VITAMIN D STATUS IN CHILDREN IN AEGEAN REGION

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BACKGROUND: Although vitamin D deficiency have been suggested in children is widespread recently, the data about vitamin D status of Turkish children is limited. The objective of the present study was to determine prevalence of vitamin D deficiency in children in Aegean region and to show effect of age, gender and seasons on vitamin D levels.

METHODS: The data from hospital information system was examined retrospectively. Between January 2011 and February 2013, children admitted to department of paediatrics our hospital for health examination was taken blood for vitamin D measurement. Serum vitamin D levels were determined by electrochemiluminescent method and categorized as < 10; 10-20; 21-29 and 30-70ng/ml.

RESULTS: A total of 2,909 children aged 1 month to 17 years participated in this retrospective study. All the subjects were divided into subgroups according to their age: 0-3 years old, 4-6 years old, 7-12 years old and 13-17 years old representing infancy and toddler, preschool, school age and adolescence stages respectively. The highest mean level of serum vitamin D was found in the 0-3 years old stage and the lowest one was found in 13-17years old stage.

CONCLUSIONS: In Turkish children, the prevalence of vitamin D deficiency and insufficiency (61%) is considerable size. We suggest that the recommendation for vitamin D supplementation after the first year of life could be beneficial especially for school children and adolescents.
Key words: vitamin D deficiency, vitamin D insufficiency, children, season

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Cod: 1588

L-CARNITINE EXHIBITS A SIGNIFICANT CAPABILITY TO BIND FREE CALCIUM, WHICH COULD BE A REASON FOR A NUMBER OF VARYING EFFECTS IN CELLULAR SYSTEMS

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BACKGROUND: L-Carnitine (LC) is a natural molecule found opulently in red meat and dairy products. It is derived from the amino acids lysine and methionine. In live cells, LC increases the production of ATP by facilitating the transport of activated fatty acids from cytosol to the mitochondrial matrix. Investigating the direct link between LC and free calcium is critical to explain a number of vague results obtained from different studies of LC as a supplement. The chemical structure of LC prompted to measure its ability to bind free calcium.

METHODS: The ability of LC to bind free calcium was carried out spectrophotometrically by measuring the decrease in the formation of Ca^{2+}-o-Cresolphthalein complexone (Ca-CPC) in the presence of different doses of LC compared to the control (without LC). The effect of LC was measured as a free entity in solution and when added to the human serum.

RESULTS: The results from this study showed a significant decrease ($P < 0.05$) in the average absorbance of Ca-CPC in the presence of LC compared to the control in serum and when using LC as a free entity in solution.

CONCLUSIONS: LC exhibits effective capability to bind free calcium. This effect may be behind its ability to sluggish the blood clotting process, amplify the effect of other anticoagulants such as warfarin, inhibit platelet aggregation, and decrease the motility of human sperm.

Vitamins and nutrition

Cod: 1589

A METHOD FOR SEPARATION AND PURIFICATION OF BY-PRODUCTS DIETARY PROTEIN AND TESTING THEIR POSSIBLE USE AS MALNOURISHMENT COMPLEMENT

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BACKGROUND: Malnutrition due to deficiency of animal protein resources and the continuous increase in its cost is considered as one of the important problems which faces developing countries.

METHODS: In the present study extraction and isolation of proteins from dehulled cotton seed and protylan (corn glutine by-product of corn remaining after the removal of starch and oil) were undertaken. This work was concerned with:
a. Application of suitable and economical techniques to isolate the test proteins so as to give the highest pure protein yield.
b. Determination of the chemical composition and amino acid contents.
c. Testing the nutritive value of the test proteins.

RESULTS: The data assessed the high quality of the isolated proteins and their amino acids that was coupled with well marked improve in their digestibility. It was also found that the proteins isolated from dehulled cotton seeds were free from gossypol. The nutritive value of the isolated proteins were markedly improved after feeding the test rats with (1:1) mixture of the two dietary proteins than rats fed on diet containing one of either of the proteins isolated from dehulld cotton seeds or protylan with respect to growth weight, serum total proteins and blood hemoglobin .

CONCLUSIONS: Equal mixing of the two test dietary protein – isolates enhances their possible applications as efficient complements for treatment of malnourishment.

Vitamins and nutrition

Cod: 1590

VITAMIN D PLEIOTROPIC EFFECTS IN AN APPARENTLY HEALTHY SOUTH AFRICAN POPULATION

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BACKGROUND: Vitamin D is traditionally known for its role in bone metabolism, but numerous studies have generated evidence to support vitamin D's association with various pleiotropic effects. In this study we investigated the prevalence of vitamin D deficiency and its pleiotropic effects in an apparently healthy South African population.

METHODS: Participants were from the South African reference interval study. Vitamin D status was evaluated in 263 participants by measuring 25-hydroxyvitamin D (25(OH)D). The relationship between Vitamin D age, smoking, diet, sun exposure and allergy, anthropometric measurements (body mass index, blood pressure), skin pigmentation readings and biochemical parameters by means of correlations.

RESULTS: The median age of participants (36%, men) was 33 years. We found a high prevalence of vitamin D deficiency (26%) and vitamin D insufficiency (55%), with only 19% of the population being vitamin D sufficient. Skin pigmentation, ethnicity, sun exposure and season had no effect on vitamin D levels. Vitamin D levels had an inverse relationship with oily fish intake (p=0.039), total cholesterol (p=0.044), prostate specific antigen (p=0.011), anti-thyroglobulin antibodies (p=0.036), white cell count (p=0.004), basophil count (p-0.048), neutrophil count (p=0.013) and lymphocyte count (p=0.009).

CONCLUSIONS: Our findings show that Vitamin D deficiency is associated with skin pigmentation, sun exposure, diet and an array of pleiotropic effects including an impact on cardiovascular risk factors (age, body mass index, smoking, cholesterol and C-reactive protein), autoimmune thyroid disease and markers of infection and inflammation (neutrophil-, basophil-, eosinophil-, monocyte- and lymphocyte count). Vitamin D deficiency is common in South Africans and the its pleiotropic effects are apparent even within a healthy population.

Vitamins and nutrition

Cod: 1591

HIGHLY STANDARDISED, FAST AND EASY DETERMINATION OF 25-HYDROXYVITAMIN D3/D2 BY SUPPORTED LIQUID EXTRACTION AND U/HPLC-MS/MS ANALYSIS

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BACKGROUND: For the survey of vitamin D synthesis and deficiency a fast and reliable quantitation of 25-hydroxyvitamin D3/D2 is highly demanded. In this work a fast forward U/HPLC-MS/MS workflow was developed to reduce lab costs, labour and analysis time being at the same time highly precise, accurate and robust.

METHODS: 50 µl of plasma/serum and IS are loaded on a 96 well SLE-plate. The easy, fast and highly effective sample preparation provides clean extracts ready for LC-MS/MS in only 3-steps. For the method also all the reagents (CAL, IS, QC) and disposables required were developed. The validation included sensitivity, selectivity, matrix effects, inter- and intraday accuracy and precision for two major triple quad manufacturers: AB Sciex and Waters.

RESULTS: The developed workflow enables the analysis of 80 samples within 7 hours by baseline separation of 25-hydroxyvitamin D3/D2 in 3 min run time. Moreover the LC-column is suitable for both HPLC and UHPLC instruments. The developed reagents include 4 CAL, 2 QCs, an isotopic labelled IS and a system suitability test-mix. The performance on different triple quads will be compared and proficiency test results discussed.

CONCLUSIONS: The new developed workflow can analyse 25-hydroxyvitamin D3/D2 in only 50 µl within a 3 min U/HPLC-MS/MS analysis, reducing lab time and costs, being easily automatable and dependable being traceable to the new NIST Standard SRM 972a. The workflow enables a fast forward analysis of 25-hydroxyvitamin D3/D2 for both HPLC and UHPLC - APCI -Triple Quad MS/MS qualitative and quantitative analysis.

Vitamins and nutrition

Cod: 1592

VITAMIN D DETERMINATION USING LC-MS/MS IN HEMODIALYSIS AND END STAGE RENAL DISEASE

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BACKGROUND: Vitamin D deficiency is high (75 %) in end-stage renal disease (ESRD) and in hemodialysis patients (HD). As their renal un-alpha hydroxylase are deficient, HD are currently supplemented with un-alpha vitamin D. Similarly to general population, normal values are 30-60 ng/mL. 25OHD measurement can be performed by immunoassays, HPLCUV or LCMSMS. Recent studies suggest that 25OHD can differ between immunoassays, according to patient status (pregnancy, intensive care, HD), the reference method being LCMSMS. Our aim was to determine if 25OHD serum level was similar using immunoassay and mass spectrometry methods both for general and HD, allowing the use of the same normal values.

METHODS: We compared 25OHD serum levels for 140 patients (50 independently of their pathology, 30 healthy individuals, 30 ESRD and 30 HD). 25OHD was determined by LCMSMS (Chromsystems kit, API 3200 QTRAP), Immunoassay (EIA, Diasorin Liaison), and HPLCUV (Chromsystems kit).

RESULTS: In total population, using Passing Bablok and Bland Altman statistical analysis, 25OHD serum level obtained by LCMSMS and EIA analysis were not significantly different. Suprisingly, for some patients 25OHD levels were higher using LCMSMS than immunoassay (until 36 ng/mL), leading to difference in clinical interpretation using the consensus normal value of 30 ng/mL. Difference between LCMSMS value and immunoassay values were significantly higher for ESRD and HD patients than for healthy individuals ($p<0.05$). Thus, for a normal 25OHD value at 30 ng/mL, whereas only 13% of 25OHD results lead to different interpretation in healthy population, 50 % of patients with ESRD or hemodialysis were deficient or normal in 25OHD using immunoassay but normal or overdosed using LCMSMS. Discrepancies between LC-MSMS and immunoassays are not explained by C3 epimer or by 1alphaD3 interference.

CONCLUSIONS: Although for 25OHD determination chromatographic methods and immunoassays are significantly correlated and not significantly different in general population, for ESRD and HD patients, LCMSMS results are significantly higher than EIA, leading to different clinical interpretation. Difference between these two methods remains to be elucidated.

Vitamins and nutrition

Cod: 1593

VITAMIN D STATUS IN CHILDREN WITH PNEUMONIA

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BACKGROUND: In recent studies, vitamin D has been demonstrated to play a significant role in immune system functioning.

METHODS: This study investigated the serum 25-hydroxyvitamin D levels of children with pneumonia in Erzurum localized at an altitude of nearly 2000 m. 30 children patients with pneumonia and 33 matched healthy controls were enrolled in this study. The levels of C-reactive protein (CRP), serum 25- hydroxyvitamin D, calcium (Ca), phosphate (P), and total alkaline phosphatase (ALP) were measured in both groups.

RESULTS: There were no significant differences between the two groups regarding demographic data. The serum 25 hydroxyvitamin D levels of patients and controls were 15,06±15,36 and 31,63±15,73 ng/mL, respectively. In patients with pneumonia, 25-hydroxyvitamin D values were significant lower than those of the healthy controls ($p<0.001$). Serum Ca, P, and ALP levels were similar in both groups. Serum CRP levels were significant higher in patients than controls ($p<0.001$). There was negatif correlation between 25-hydroxyvitamin D levels and CRP (r: -,527, $p<0.001$).

CONCLUSIONS: Our results suggest that serum 25-hydroxyvitamin D levels are decreased in children with pneumonia.

Vitamins and nutrition

Cod: 1594

THE SURVEY OF IRFI005 FUNCTION ABOUT REMOVING OF INTRA AND EXTRACELLULAR FREE RADICALS

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BACKGROUND: Antioxidants are the molecules which prevent from the function of the free radicals and Vitamin E is one of the most important antioxidants compounds. This vitamin is insoluble in water and this feature lead to limiting its in polar environments. Now, many researches and studies are conducting in order to use from the water soluble analogues from this compound. In the present study in order to survey and study the desirable features and specifications of Vitamin E in the polar environments from IRFI005 which the synthetic and polar analogue of Vitamin E.

METHODS: Studying the function of the Extracellular IRFI005 antioxidants by absorption spectroscopy method at 430nm wavelength and by using from Galvinoxil free radical in different molar concentrations 1:4 and 1:2 (IRFI005:Galvinoxil). The intracellular effect of 5μM IRFI005 by DCF method and by using from colorful probe DCFH2-DA and Kiomen Hydro Peroxide was studied.

RESULTS: IRFI005 in the extra and intra cellular functions leads to prevention from the antioxidant function. Galvinoxyl in 1:2 ratio had lower OD and was removed from the environment completely. Each molecule of IRFI005 can remove two free radical molecules. Inside the cells, IRFI005 can prevent from function of Hydro Peroxide and increasing the intensity of florescence.

CONCLUSIONS: The findings revealed that in the polar environments, IRFI005 is a very strong antioxidant which is dependent on the dose and it is very active, and can properly prevent from oxidant molecules inside and outside the cells.
Keywords: Free radicals, Antioxidant, Vitamin E, IRFI005, DCF Method

Vitamins and nutrition

Cod: 1595

TOTAL VITAMIN D QUANTIFICATION: EQUIMOLAR DETERMINATION OF 25(OH)D2 AND 25(OH)D3 USING A BIOCHIP IMMUNOASSAY SYSTEM

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BACKGROUND: Fat soluble vitamin D exists in two forms: D2 and D3. The quantification of the tightly regulated active metabolite 1α,25(OH)2D does not reflect vitamin D storage concentrations, therefore 25(OH)D serum or plasma concentrations are assessed. The increasing awareness that 25(OH)D deficiency is associated with multiple clinical outcomes has led to an increase in the number of tests requested. A high throughput assay, which accurately quantifies total 25(OH)D [25(OH)D2 and 25(OH)D3] is clinically relevant and this study reports the analytical performance of such an assay on a biochip immunoassay system.

METHODS: A competitive chemiluminescent biochip immunoassay was used: following displacement of serum binding proteins, endogenous 25(OH)D competes with a 25(OH)D conjugate for binding to antibody immobilised on the biochip. After removal of unbound material, chemiluminescent substrate is added; the intensity of the signal is inversely proportional to 25(OH)D concentration in the sample. The assay was applied to the Evidence Investigator analyser

RESULTS: Calibrator values were traceable to LC-MS/MS with an assay range up to 107.4 ng/ml. Human 25(OH)D depleted serum spiked with either 25(OH)D2 or 25(OH)D3 showed similar B/B0 curves and comparison of IC_{50} values indicated cross reactivity >90%. The limit of blank (LoB), limit of detection (LoD) and limit of quantification (LoQ) were 2.81, 5.7 ng/ml and 7.2 ng/ml, respectively. Analysis of unadulterated serum samples covering a clinically relevant range confirmed assay linearity and inter-assay precision (<10%). Comparison of the biochip assay with LC-MS/MS derived data for 39 serum samples encompassing a large range of 25(OH)D concentrations generated a slope of 0.98 and regression coefficient of 0.91. This complied with a vitamin D external quality assessment scheme (DEQAS).

CONCLUSIONS: This study indicates that the total 25(OH)D biochip immunoassay exhibits equimolar recognition of 25(OH)D2 and 25(OH)D3 and compares favourably with LC-MS/MS data in clinically relevant serum samples. The Evidence Investigator system allows the handling of 54 samples at a time (including calibrators), the application to the fully automated Evolution analyser will lead to higher throughput.

Vitamins and nutrition

Cod: 1596

COMPARISON OF FOUR AUTOMATED SERUM VITAMIN B12 ASSAYS WITH SERUM METHYLMALONIC ACID AND HOMOCYSTEINE

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BACKGROUND: Vitamin B12 deficiency is a common health problem and diagnosis of vitamin B12 deficiency is based on measurement of serum vitamin B12 levels. However, there are different immunoassay methods for the serum vitamin B12 assay and serum vitamin B12 levels alone is not enough for the diagnosis that the measurement of serum methylmalonic acid (MMA) and homocysteine combined with serum vitamin B12 is recommended to evaluate the diagnosis of vitamin B12 deficiency. Therefore we compared four serum vitamin B12 immunoassay methods with the measurement of serum methylmalonic acid and serum total homocysteine levels.

METHODS: The study included 69 surplus serum samples with serum vitamin B12 levels were measured and measurements were duplicated by four immunoassay methods based on the competetive protein binding (CPB) assay: Access DxI 800 Unicel (Beckman Coulter, USA), ADVIA Centaur XP (Siemens Diagnostics, Tarrytown, NY), Roche Cobas E601 (Roche Diagnostics,Germany), Architect i2000sr (Abbott Laboratories, Abbott Park, Illinois, U.S.A). Serum methylmalonic acid levels were determined by liquid chromatography–mass spectrometry and serum homocysteine levels were determined by high pressure liquid chromatography assay kit.

RESULTS: The cut of value of MMA was found 0.3 µmol/L. MMA results were negative significantly correlated with the vitamin B12. Methylmalonic acid and ADVIA Centaur comparison showed the highest correlation (r= -0.337) whereas methylmalonic acid and Unicel DxI 800 showed the poorest one (r= -0.275). There is also a negative correlation between the vitamin B12 and homocysteine levels. Samples with low serum B12 levels have elevated homocysteine levels whereas at high serum B12 levels, homocysteine levels were generally within the reference ranges. Homocysteine and ADVIA Centaur comparison showed the highest correlation (r=-0,448) whereas homocysteine and Unicel DxI 800 showed the poorest one (r=-0,341).

CONCLUSIONS: Our study shows that MMA and homocystein levels correlates with serum vitamin B12 levels. But these relations show an alteration depending on the methods of vitamin B12 measurements. The clinicians and laboratory experts knowing this condition is important during the evaluation.

Vitamins and nutrition

Cod: 1597

SERUM BIOTIN CONCENTRATION AMONG CHILDBEARING AGE WOMEN IN NORTHERN PERSIAN GULF:A POPULATION BASED STUDY

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BACKGROUND: Biotin deficiency causes reduced carboxylase activities which show itself in the form of mental disorders, slow growth and skin damage. The main purpose of this study was to evaluate the serum biotin levels by body mass index (BMI) groups and age category of the reproductive age women in northern part of the Persian Gulf.

METHODS: In a cross sectional study, 1218 women with mean age of 29.8±8.9 year, were selected by a multi-stage stratified random cluster sampling technique. Serum biotin were determined by a competitive ELISA technique.

RESULTS: The mean serum concentration of biotin was 541±394 pmol/L. The percentage of serum based biotin lower than 410 pmol/L and anemia among the subjects was 41.1% and 8.7% respectively. Serum biotin levels were not differ among the anemic and normal samples, $p>0.05$. The mean value of serum biotin levels was directly related to age, BMI and hemoglobin, $p< 0.05$.

CONCLUSIONS: We conclude that the remarkable percent of women of reproductive age living in northern part of Persian Gulf have lower limit of serum biotin.

Vitamins and nutrition

Cod: 1598

MONITORING OF SERUM VITAMIN B12 AND FOLATE STATUS IN BULGARIAN UNIVERSITY POPULATION

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BACKGROUND: Recent published data showed relatively high prevalence of inadequate dietary intake of folate and vitamin B12 in the adult population of Europe. Folate nutritional deficiency is most common in young urban Bulgarian women. Dietary intake of folate and vitamin B12 is known to positively correlate with the serum levels. No data have been reported on serum folate and vitamin B12 status in Bulgarian university population. The aim of the present study was to determine and assess the folate and vitamin B12 status in representative sample of healthy well-educated young population.

METHODS: A total of 100 university students (66% females and 34% males, mean age 21,6±1,8 years) participated in the study. Blood samples were collected for 3 days period in November, 2013 year. Serum folate and vitamin B12 concentrations were measured by chemoluminescence analysis. Statistical analyses were performed with SPSS Statistics 19.0 for Windows.

RESULTS: Mean serum folate was 10,2±4,1ng/mL. The mean serum folate was significantly lower in men (8.8±2,9ng/mL) compared to women (10.9±4,4ng/mL, p<0,005). Serum folate levels under 6 ng/mL were observed in 7,6% of female students. The mean serum vitamin B12 was 432,5±136,2 pg/mL (men 402,5±91,4pg/mL; women 448.2±153pg/mL; p>0,1). Low serum vitamin B12 levels were measured in 2% of the students.

CONCLUSIONS: The result showed adequate serum vitamin B12 status in 98% of the university population. It was found that 10% of the medical students had possible folate deficiency.

Vitamins and nutrition

Cod: 1599

ASSESSMENT OF THE USE OF GEL TUBES IN MEASUREMENT OF 25-HYDROXYVITAMIN D3 BY HPLC AND IMMUNOASSAY METHODS

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BACKGROUND: The accuracy of 25-hydroxyvitamin D3 (25OHD3) measurement on specimens collected into serum separator tubes (SST) has been questioned because of possible interference by the gel. This study investigated whether SST tubes interfere with 25OHD3 measurement by comparing its concentrations in serum from specimens collected into plain and SST tubes and assayed using three different methods.

METHODS: Blood specimens were collected simultaneously from 50 normal subjects into plain and SST tubes. Following centrifugation, 25OHD3 was assayed on the serum using HPLC (Chromosystems) and Architect (Abbott) and Liaison (Diasorin) immunoassays.

RESULTS: There were no significant differences between 25OHD3 results (means ± SD, nmol/L) obtained from specimens collected into plain and SST tubes assayed by HPLC (39.0±18.9vs. 39.3±18.9), Liaison (32.9±15.8vs. 32.8±16.6) or Architect (43.1±19.5vs. 43.2±19.4). In specimens collected into plain tubes, 25OHD3 measurements by HPLC correlated significantly with those from the Architect ($r = 0.861$; $p<0.0001$) and Liaison ($r = 0.881$; $p<0.0001$). Similarly, in specimens collected into SST tubes, measurements by HPLC correlated significantly with those from both the Architect ($r = 0.886$; $p<0.0001$) and Liaison ($r = 0.879$; $p<0.0001$).

CONCLUSIONS: The gel in SST tubes does not interfere with the measurement of 25OHD3 by HPLC or common immunoassays.

Vitamins and nutrition

Cod: 1600

THE HIDDEN RATIO OF DEPLETED IRON STORES: NORMAL HAEMOGLOBIN BUT LOW SERUM FERRITIN

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BACKGROUND: In the last fifty years the importance of iron deficiency and anaemia as a public health problem has been increasingly recognized by health authorities and policy makers. There are no current global figures for iron deficiency, but using anaemia as an indirect indicator by WHO it is estimated that 42.3% all women (15-59 years) in non –industrialized countries, and 10.3% in industrilazied countries; 4.3% all men (15-59 years) in non –industrialized countries, and 30% in industrilazied countries are iron deficient. The serum ferritin level is the most spesific biochemical test that correlates with relative total body iron stores. The aim of this study is to clarify the risk of iron deficiency among men and women (15-59 years) in Kayseri, Turkey with normal blood haemoglobin concentration but low serum ferritin levels according to WHO criteria.

METHODS: Total 608 patient, 120 men (15- 59 years) and 488 women (15- 59 years, non pregnant) consulting family physcians in 2013, whose haeamoglobin concentrations are normal, also concurrent serum iron and ferritin concentration analysed ,have been included into study. Any patient with biochemical elevations signing haemolysis (AST, ALT, Bilirubins, potassium, LDH) and infections (CRP, WBC) are not included.

RESULTS: The arithmetic mean of haemoglobin concentration is 15,2±1,21 for men, and 13,2±0,95 for women. The arithmetic mean of ferrtitin concentration is 86,41±67,06 for men and 36,9±51,67 for women. According to WHO criteria (serum ferritin lower than 15 µg/L in males and females) 154 of 488 women and 5 of 120 men have recognised as having depleted iron stores though normal haemoglobin, haemotocrite, MCV and RBC. Also 47 of those 154 women and 1 of those 5 men have low serum iron levels, too.

CONCLUSIONS: Public risk assessment for iron deficiency according to WHO criteria might include both CBC counting and serum ferritin levels; otherwise 31,5% of women and 4,1% of men with depleted iron stores may be eluded observation. And also, as being the highest benefit-to-cost ratio solution, food fortification must be supported by government especially among women to avoid depleted iron stores.

Vitamins and nutrition

Cod: 1601

ASSOCIATION BETWEEN VITAMIN D LEVELS AND BONE PAIN IN PREGNANCY

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BACKGROUND: Chronic back and tooth pain, often encountered in pregnancy, is not only sign of great discomfort but may signal something serious. It has been linked with adverse outcome. Vitamin D plays important role in bone health and its deficiency have been documented in pregnancy. With the aim of studying its contribution in etiology of bone pain, the present study was carried out. High socioeconomic class was selected as they remain in door especially during the time of day when sun shine exposure can synthesize vitamin D in vivo.

METHODS: Fifty pregnant women with complaint of chronic back pain, attending antenatal clinic of tertiary care hospital were studied. A detailed history of pregnancy was taken including backache, dental pain and health. Oral hygiene measures were recorded. Serum 25-OH vitamin D was analyzed by chemiluminescence on Elecsys-411, at their first visit. Patients with history of smoking and addiction were excluded. Vitamin D deficient subjects (levels<30ng/ml), were identified and put on treatment (oral supplementation of 60,000 U/week for 12 weeks). Dental treatment was also provided. Complaint of toothache and backache was reviewed along with Vitamin D levels after 3 months.

RESULTS: Mean age of women was 29±2.6 years, and gestational age was 12±5.2 weeks. 68% of the patients complain of toothache and sensitivity. Inspite of good oral hygiene bleeding gums, gingivitis, jaw pain and periodontitis was noted (4, 11, 11, 2 patients respectively). Mean Vitamin D levels were 10.3 ±5 ng/ml (82% patients had <20 ng/ml). There was significant increase in vitamin D levels (34.7±7.5 vs. 10.3± 5 ng/ml, p< 0.001) after 3 months of treatment, with majority of the patients having >30 ng/ml. There was significant improvement in signs and symptoms of backache (only 16 patients with backache now). Almost 50% relief was noted in jaw pain and sensitivity as well.

CONCLUSIONS: Significant improvement in bone pain after Vitamin D treatment shows its strong association with pregnancy related complaints. Increased calcium requirement in pregnancy can never be fulfilled without vitamin D supplementation especially in this region wherein its deficiency is endemic.

Vitamins and nutrition

Cod: 1602

THE ROLE OF VITAMIN D DEFICIENCY IN ABORTUS IMMINENS

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BACKGROUND: Abortus imminens is defining as abdominal cramping and vaginal bleeding before 20th weeks of gestation. Maternal nutritional status and immunological factors may play a role in the development of abortion. Vitamin D deficiency may constitute a basis for immunological factors. In this study we were aimed to investigate the relationship between abortus imminens and vitamin D deficiency.

METHODS: A total of 50 pregnant women with the complaint of vaginal bleeding during 6-18 gestational weeks and 50 healthy gestational week matched pregnant women were included in the study. Maternal serum calcium (Ca), inorganic phosphate (iP) and alkalen phosphatase (ALP) activity were measured with photometric methods, intact parathyroid hormone (iPTH) with immunochemical method and vitamin D with ESI LC-MS/MS.

RESULTS: Maternal plasma vitamin D and iP results were not significantly different between abortus imminens and normal pregnancy groups. However Ca and iPTH concentrations and ALP activities were significantly different between groups (p=0.024, p=0.012 and p= 0.019, respectively).

CONCLUSIONS: According to our results there was no relationship between maternal vitamin D results and abortus imminens. However the benefits of proper calcium metabolism during pregnancy is indisputable. Our study showed that impaired Ca metabolism may be associated with abortus imminens.

Vitamins and nutrition

Cod: 1603

THE PREVALENCE OF VITAMIN D DEFICIENCY AMONG POSTMENOPAUSAL WOMEN IN MONTENEGRO

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BACKGROUND: Though vitamin D deficiency is revealed to be pandemic throughout the world, data regarding the vitamin D status among postmenopausal women in Montenegro are limited. Therefore, the aim of this study was to evaluate the prevalence of vitamin D deficiency and to examine the association of serum total vitamin D with cardiometabolic risk factors among postmenopausal women.

METHODS: A total of 188 postmenopausal women aged 43-69 years, with no history of diabetes and cardiovascular disease, were included in a period from May to September 2013. Serum Vitamin D, a reliable marker for assessment of vitamin D status is measured by electrochemilluminiscence. Fasting glucose, lipid parameters, and C-reactive protein (CRP) were determined. Blood pressure, body weight, body height, and waist circumference (WC) were measured, and body mass index (BMI) was calculated.

RESULTS: The vitamin D deficiency (< 50 nmol/L), insufficiency (50-75 nmol/L) and sufficiency (>75 nmol/L) was observed in 41.5%, 38.8% and 19.7% of women, respectively. Severe deficiency (<25 nmol/L) was recorded in 10.6% of women. We didn't observe any correlation with cardiometabolic risk factors, except for positive correlation with CRP (r=0.186, p=0.011). However, after multiple regression analysis sunlight exposure was the only independent predictor of vitamin D status.

CONCLUSIONS: Vitamin D deficiency is highly prevalent among postmenopausal women in Montenegro. These results highlight the importance of improving vitamin D status in postmenopausal women for the prevention of adverse health outcomes, and sunlight exposure may be one of the simplest and the most efficient lifestyle intervention model. On the other hand, unexpected positive association of Vitamin D with CRP may be confounded by some other unaccounted factors in this population.

Vitamins and nutrition

Cod: 1604

THE ASSOCIATION BETWEEN SERUM VITAMIN D CONCENTRATIONS AND COLORECTAL CANCER

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BACKGROUND: Colorectal cancer is the most common cancer and the third leading cause of cancer deaths in Taiwan. Colorectal cancer could be detected early through high risk group screening and prevented by polypectomy and risk factors modifications. There is growing evidence that vitamin D deficiency results in many diseases. Vitamin D is also one of modifiable risk factors for colorectal cancer. Previous studies indicate the association between reduced serum 25-hydroxyvitamin D (25(OH)D) concentrations and increased incidence or mortality of colorectal cancer. This study aimed to establish a method using liquid chromatography tandem mass spectrometry (LC-MS/MS) to measure serum $25(OH)D_2$ and $25(OH)D_3$ concentrations. Furthermore, we evaluated the association between serum 25(OH)D concentration and colorectal adenoma and cancer.

METHODS: Blood samples were collected from April, 2012 to December, 2013. Subjects underwent sedative colonoscopy (N=341) were recruited with non-adenoma group(N=140), adenoma group(N=140), and cancer group(N=61). Serum samples were deproteinized by methanol: isopropanol mixture, and then extracted by n-heptane. The extracted supernatant was injected into LC-MS/MS (API 5000 system). The chromatographic separation was performed on a Symmetry C18 column, 2.1 mm x 50 mm, 3.5 µm with mobile phase methanol.

RESULTS: The linearity of the established method was up to 100 ng/mL with r^2>0.99. The mean recoveries were 103.1% and 100.1% for $25(OH)D_2$ and $25(OH)D_3$, respectively. The intra-run imprecision (CV%) were 6.4% (mean 23.3 ng/mL) and 5.2% (31.7 ng/mL); inter-run CV% were 13.2% (6.4 ng/mL) and 6.3% (38.3 ng/mL) for $25(OH)D_2$ and $25(OH)D_3$, respectively. No significant difference in 25(OH)D concentration was found between adenoma and non-adenoma group (32.9 ± 9.4 ng/mL v.s 31.7 ± 8.4 ng/mL, p=0.3). However, significantly decreased 25(OH)D concentration was found in colorectal cancer group (23.3±6.0 ng/mL), compared to adenoma group (p<0.05).

CONCLUSIONS: We established a LC-MS/MS method to measure 25(OH)D concentrations. The decreased 25(OH)D concentrations may contribute to the pathogenesis of colorectal cancer.

Vitamins and nutrition

Cod: 1605

THE EFFECT OF NERIUM OLEANDER ON OXIDATIVE DNA DAMAGE IN RATS FED WITH IN HIGH FAT DIETS

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BACKGROUND: DNA is the cellular target of oxidative damage induced ROS. Free radicals damage as disturbing their normal function and therefore may contribute variety of diseases. Studies with Nerium oleander (NO) have reported its cardiotonic, antibacterial, anticancer and anti-platelet aggregation, antioxidant activities and its depression in the central nervous system. Recently, it is shown that NO administration reduced lipid and glucose levels. Nowadays, effect of administration of NO on oxidative DNA damage has not been investigated. Seen from this aspect, the purpose of the present study is to evaluate the effect of Nerium-oleander on DNA damage, and SOD (superoxide dismutase) levels in rats fed with high fat diets.

METHODS: Twenty-one Spraque-Dawley male rats (6-8 weeks) were equally divided into three groups: Group 1 (n=7), a control group fed with normal diet for two weeks and administrated with 0.5 ml distillated water via gavage for 90 days. Group 2 (n=7), a study group fed with high fat diet for two weeks and administrated with 0.5 ml distillated water via gavage for 90 days. Group 3 (n=7), a study group fed with high fat diet for two weeks and administrated with 0.5 ml of distillate original lyophilized material oleander, via gavage for 90 days. Blood samples were collected via intracardiac with general anesthesia. All the bloods collected for analyzing the levels of 8-hydrox-2-deoxyguanosine (8-OHdG) and superoxide dismutase (SOD).

RESULTS: 8-OHdG levels were respectively 11.9±1.05, 118.0±7.27 and 25.7±2.09 ng/ml in control group and group 2 and 3. The differences between group 2 and 3 were important ($p<0.05$). SOD levels were 27.8±0.88, 21.7±1.39 and 21.7±1.07 U/ml respectively, in control group and group 2 and 3. An important differences between control and group 2, 3 were not determined ($p>0.05$).

CONCLUSIONS: We implied that NO reduced the level of 8-OHdG in high fat diet fed rats that may mean oxidative DNA damage prevented by NO in dosage of 0,5 ml of distillate original lyophilized material. Furthermore, administration of NO did not have any influence on SOD levels in comparing to group 2 and 3. Further studies are needed to elucidate the effect of NO on antioxidant-oxidant system enzymes in high fat diet feeding.

Vitamins and nutrition

Cod: 1606

IS VITAMIN D TOXICITY REALLY "RARE"?

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BACKGROUND: Relationship between vitamin D levels and many diseases, even with overall mortality, leads to a rapidly increased interest on vitamin D. Although various publications state that vitamin D toxicity is a rare condition, nowadays, it is encountered more frequently due to the increased utilization of vitamin D supplements. The aim of this study is to obtain data on the frequency of vitamin D toxicity. Patients: Total of 78,090 vitamin D3 (vitD) test results obtained in our laboratory between January 2011 and December 2013 were retrospectively evaluated. Repeated measurements from the same patient are excluded. Children <12 months are also excluded because of possible C3 epimerase existence. Data of 69,635 patients (age range: 1-108, median age: 47; 77.4% female) were included.

METHODS: VitD measurements were performed with LC-MS/MS, Waters Quattro Premier QE. VitD levels over 200 nmol/L (80 ng/mL) are classified in four different groups as 200-249.9 nmol/L (A), 250-374.9 nmol/L (B), 375-624.9 nmol/L (C) and >625 nmol/L (D).

RESULTS: Among the 69,635 vitD measurements, 1,062 (1.52%) were >200 nmol/L (age range: 1-92, median age: 52, 79.3% female). Among all cases, the highest value was 2,208 nmol/L. Distribution of these patients according to above classification was as follows:
Group A: 544 (0.78%) (age range: 1-90, median age: 53, 80.7% female)
Group B: 385 (0.55%) (age range: 1-92, median age: 52, 80.5% female)
Group C: 97 (0.14%) (age range: 1-81, median age: 44, 75.3% female)
Group D: 36 (0.05%) (age range: 1-78, median age: 27, 55.6% female)

CONCLUSIONS: Even though the population in this study is mostly composed of primarily vitamin D deficient patients receiving treatment, our data indicates that vitD toxicity is more than "rare". Therefore, clinicians should frequently monitor the vitD levels of patients, especially those receiving parenteral vitD treatment. Patients should also be guided with possible vitD toxicity, especially for oral utilization of high dose vitD. Also, clinical laboratories should establish policies for high vitD levels such as informing clinicians and measurement of serum calcium as reflex test.

Vitamins and nutrition

Cod: 1607

THE EFFECT OF SERUM 25 HYDROXY VITAMIN D3 LEVELS ON ASTHMA

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BACKGROUND: Asthma and other lung diseases have increased over the past decades in the world. A recent studies notes a significant association between vitamin D deficiency and decreased pulmonary function tests. The association between vitamin D and asthma remains uncertain. Our aim in this study is to analyze the relationship between serum 25-Dihydroxy vitamin D3 (vitamin D) levels and level of asthma control test as well as other asthmatic parameters in asthmatic patients.

METHODS: 41 asthma patients (27 women, 14 men), and 29 healthy volunteers (19 women, 10 men) as control group were involved in the study. Vitamin D and PTH were measured with ELISA method, and other biochemical parameters were measured with autoanalyzer.

RESULTS: Vitamin D and calcium levels in asthma patients were found to be significantly low ($p<0.009$, $p<0.003$, respectively), whereas; PTH and albumin levels were significantly higher ($p<0.009$, $p<0,0001$ respectively) compared to control group. There were no associations between vitamin D level and FVC, FEV1, FEV1/FVC, FEF 25/75 ($p>0,05$). Vitamin D was positively correlated with asthma control test ($r=0,438$ $p=0,004$) while, PTH negatively correleted with asthma control test ($r=-0,409$ $p=0,008$).

CONCLUSIONS: Recent studies have shown that vitamin D insufficiency is a potential risk factor for cardiovascular and lung diseases and its severity. Our results showed that serum vitamin D levels were inversely associated with asthma and its severity. Vitamin D deficiency and insufficiency can play a role in pathogenesis of asthma. This study needs to be supported further researches.

Vitamins and nutrition

Cod: 1608

25-HYDROXYVITAMIN D LEVEL IN COMMERCIAL LABORATORY TESTING IN POLAND

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BACKGROUND: Vitamin D is a steroid hormone precursor produced in skin tissue during sunlight exposure, or obtained from diet. Many studies suggest that the prevalence of vitamin D deficiency is increasing health problems worldwide. The blood concentration of 25-hydroxyvitamin D (25(OH)D) is considered the best indicator of vitamin D status, but the full scale of its deficiency is still unknown. The reference values for 25(OH)D are not exactly determined, however concentration above 30 ng/ml is considered optimal. The aim of this study is to evaluate 25(OH)D level in samples taken from patients of commercial medical laboratories in Poland and compare them to the optimal values.

METHODS: The study included 1568 blood samples routinely measured for 25(OH) D level, taken from patients at the age between 7 and 67. Tested specimen were not frozen, serum samples were collected from patients during fall-winter season 2013 in Synevo laboratories.
25(OH)D level was estimated in human serum with automated Roche Cobas 6000 electroimmunoassay.

RESULTS: The average serum concentration of 25(OH)D was 25,1±11,9 ng/ml (mean age 47±20, n=1568). 72% of patients had significantly lower Vit. D level than recommended optimal cut-off value (30 ng/ml). Vitamin D deficiency (<20 ng/ml) was found in 20% cases. However, no significant differences were observed between close vitamin D status in male and female subgroups. Mean concentration of 25(0H)D in winter season was lower than in summer with concentration values respectively 24,3±11,2 vs 26,2±11,4; $p<0,05$. Kuiavian patients had significantly lower average 25(OH)D value than residents of Mazovia (22,3±11,7 vs 25,9±11,8; $p<0,05$).

CONCLUSIONS: Our study confirmed high prevalence of vitamin D deficiency in female and male commercial laboratory patients in Poland. 20% cases show severe vitamin D deficiency, and may have clinical symptoms. Our study can suggest that production of vitamin D in skin is proportional to sunlight exposition, so it varies according to the season and latitude. Our data could indicate the need of routine vitamin D supplementation in adults in Poland. Unpublished data show that using LC-MS/MS method results in even lower 25(OH)D concentration.

Vitamins and nutrition

Cod: 1609

EARLY MARKERS OF OCCULT MEGALOBLASTOSIS IN PATIENTS OF ISCHAEMIC STROKE: PREVENTIVE APPROACH FOR PRIMARY HEALTH CARE

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BACKGROUND: Hyperhomocysteinemia (Hhcys) is an independent risk factor for thromboembolic events; incidence of ischaemic stroke being higher than that of haemorrhagic stroke. Hhcys is also seen in megaloblastic conditions.In some developing countries, occult megaloblastosis is found to exist in the general population.Lowering of total homocysteine level considerably reduces the incidence of thrombotic events.Supplementation of cobalamin and folic acid helps to reduce the level of homocysteine.Early supplementation of the relevant vitamins in detected cases would help in reducing the level of homocysteine, and thus help prevent an impending attack of ischaemic stroke. However, the tests used for the detection of vitamin deficiencies require automated instruments,the principal disadvantages of which, at the primary healthcare level, include difficulty in assay standardization, sample processing, storage and high cost.

METHODS: 100 patients of ischaemic stroke with haemoglobin level of >11.5 gm/dl were included. Complete blood counts were done by electronic counters and peripheral blood films were reviewed for the detection of hypersegmented neutrophils. Cobalamin, folate and homocysteine levels were assayed by chemiluminescent immunoassay techniques.

RESULTS: 70 of the100 patients had a high homocysteine level (>15 µmol/ l) (GroupA); 30 had normal homocysteine level (GroupB). In GroupA, 34.5% patients had low cobalamin, 20% had low folate, while 12.8% had a combined deficiency of both vitamins. At 5% level of significance, the levels of cobalamin and folate were significantly lower,while the number of hypersegmented neutrophils (>5% of the neutrophils having 5 or more lobes or 1 neutrophil with at least 6 lobes)on the peripheral smear was higher in patients of GroupA, compared to GroupB. Forty five (64.2%) out of the seventy patients in GroupA showed hypersegmentation of neutrophils.

CONCLUSIONS: Detection of hypersegmented neutrophils on the peripheral smear can be used as an economical and easily available alternative for diagnosis of occult megaloblastic conditions at the level of primary healthcare in developing countries, so that relevant deficient vitamins can be supplemented early and an impending attack of hyperhomocysteinemia induced ischaemic attack can be averted.

Vitamins and nutrition

Cod: 1610

SERUM HOLOTRANSCOBALAMIN AND VITAMIN B12 LEVELS IN OLDER PEOPLE WITH DEMANTIA

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BACKGROUND: Dementia is an acquired, generalized, and progressive impairment of cognitive function. Vitamin B12 and folate are necessary for metabolic paths. The optimal status of vitamin B12 (cobalamin, Cbl) is not only dependent on dietary intake but more critically on effective absorption which diminishes with age. vitamin B12 measurement is complicated by the lack of a gold standard assay. Plasma holotranscobalamin (biologically active fraction of vitamin B12) have been considered to be the earliest marker of Cbl deficiency. HoloTC, homocysteine (tHcy) and methylmalonic acid (MMA) are believed to be more reliable indicators of intracellular vitamin B12 status.

METHODS: Levels of vitamin B12, folate, holoTC, tHcy and MMA were investigated in 38 patients (22 female, 16 male aged over 60) with demantia and in 30 age-matched control subjects. Vitamin B12 and folate were analysed by automated immunassay system (DxI 800, Beckman and Coulter Diagnostics). HoloTC levels were evaluated by an automated immunassay system (Abbott Diagnostics). tHcy analyses were done by an automated immunassay system (Immulite 1000, DPC). MMA levels were detected by LC/MS/MS system (API 3200, AB SCIEX). Cognitive impairment was defined as a Mini-Mental State Examination (MMSE) score<23/30. Student's t-test and Pearson correlation analysis were used for statistical analyses.

RESULTS: HoloTC (29±15.90 pmol/L in patients, 27.89±8.83 pmol/L in controls) and vitamin B12 levels (167.10±77.94 pg/mL in patients, 192.40±52.43 pg/mL in controls) showed no significant differences between groups (p=0.734 and p=0.132 respectively). MMA (0.42±0.21 μmol/L in patients, 0.31±0.15 μmol/L in controls) and tHcy (19.79±10.04 μmol/L in patients, 14.84±5.66 μmol/L in controls) levels were significantly higher in patients (p=0.022 and p=0.019 respectively). Folate levels (5.59±2.44 ng/mL in patients, 7.01±2.44 ng/mL in controls) were in reference range in both groups, although patients had significantly lower levels (p=0.021). A positive correlation was found between holoTC and vitamin B12 (p<0.01, r=0.505).

CONCLUSIONS: Using MMA levels were recommended rather than holoTC levels for screening functional vitamin B12 deficiency. Further studies are needed to evaluate predictive values of holoTC and MMA.

Vitamins and nutrition

Cod: 1611

STATUS OF VITAMIN 'D' IN PATIENTS DIAGNOSED WITH DEPRESSION

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BACKGROUND: Depression is a common illness worldwide with an estimated 350 million people affected. It is the leading cause of disability worldwide and is a major contributor to the global burden of disease. Vitamin D is produced locally in the skin by sun exposure. To a minor extent, humans also acquire vitamin D from the diet, in particular, fatty fish and from supplements. It is a hormone with a multitude of diverse function. Vitamin D receptors have been mapped throughout the brain suggesting a role for vitamin D in psychosomatic disorders. Results from previous epidemiological studies on relation between vitamin D status and depression are equivocal and is poorly understood. Also, limited information is available relating vitamin D status with depression in Nepalese population

METHODS: The cross-sectional study was conducted in TU Teaching Hospital. 85 Depressive patients and 85 age sex matched controls were enrolled. Depression was defined as per International Classification of Disease-10- Diagnostic Criteria for Research (ICD-10-DCR) guidelines by consultant Psychiatrist. The level of depression and presence of any suicidal tendency was also found out by consultant psychiatrist using ICD-10 DCR guideline and American psychiatric association (APA) guideline for suicidal tendency. Vitamin D was assessed in biochemistry laboratory. Fasting blood samples was collected to analyze serum vitamin D level.

RESULTS: The level of vitamin D in patient with depression was found to be significantly lower than that in normal/control individual. Likewise the level of vitamin D was found to vary with severity of depression; with severe depression the level of vitamin D being the lowest. Similarly the level of vitamin D was found to be lower in subjects having suicidal tendency.

CONCLUSIONS: Depressive symptoms are associated with decreased vitamin D level. The level of serum vitamin D varies with the severity of depression. Serum vitamin D level is low in subjects having suicidal tendency.

Vitamins and nutrition

Cod: 1612

AGE- AND GENDER-RELATED MEAN VALUE OF BLOOD TOTAL VITAMIN D AMONG SAUDI ADULTS

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BACKGROUND: The availability of Vitamin D3 and D2 (total Vitamin D) in the adult population upon many factors including diet, latitude, exposure to sunshine, and possibly is dependent genetic factor. Therefore, the necessity of establishing the reference intervals of total Vitamin D can not be re-emphasized in order to monitor and prevent the vitamin D deficiency in our population. In this study we determined the mean values for total Vitamin D level among the out-patient's clinics of Security Forces Hospital in Riyadh, Saudi Arabia.

METHODS: The results of vitamin D level in blood were reviewed retrospectively from 12954 adult subjects of 18 years or older who were screened for vitamin D during their visit in the out-patients clinics between July 2011 and November 2011. Total vitamin D level was analyzed in our lab by Cobas 6000 from Roach (Basel, SWZ). The mean and standard deviation (SD) were calculated. The significant of P value were set at less than 0.05 using two tailed t-test.

RESULTS: There were 10,174 (79%) female. A deficient of vitamin D was found in 3828 (30%) of all population. The mean and SD of total vit D values were found to be 44.6 SD 38.4 and 43.2 SD 35.6 nmol/L for female and male at age group of 18 to 20 years respectively (P=0.451); 42.8 SD 34.9 and 40.7 SD 29.1 nmol/L at age group 21 to 30 years old respectively (P=0.519); 47.1 SD 35.8 and 45.9 SD 31.8 nmol/L at age group 31 to 40 years old respectively (P=0.910); 50.0 SD 33.9 and 48.4 SD 31.5 nmol/L at age group 41 to 50 years old respectively (P=0.608); 53.2 SD 34.4 and 48.9 SD 27.6 nmol/L at age group 51 to 60 years old respectively (P=0.072); 54.6 SD 34.5 and 51.0 SD 29.1 nmol/L at age group 61 to 70 years old respectively (P=0.015); 54.9 SD 34.5 and 52.0 SD 27.1 nmol/L at age group 71 to 80 years old respectively (P=0.075); and 46.6 SD 23.4 and 54.1 SD 35.3 nmol/L at age group 81 to 90 years old respectively (P=0.061).

CONCLUSIONS: There was a gradual increase in the mean of Total Vitamin D levels in the middle and elderly age of both genders possibly due to increase of public awareness. A round 30% of adult population has Vitamin D deficiency.

Vitamins and nutrition

Cod: 1613

A NEW CERTIFIED REFERENCE MATERIAL OF 25-(OH)-D2 AND 25-(OH)-D3

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BACKGROUND: Vitamin D, discovered as an essential steroid hormone plays a vital role in human metabolism due to the fact that in the last decay has been associated with various diseases such as muscle strength [1], cancer risk mortality [2-4] and insulin resistance [5]. All these mentioned reasons make vitamin D popular in the field of clinical analysis.

METHODS: A rapid, specific, and simple LC-MS/MS-based bioanalytical method was developed for the fully separation of vitamins 25(OH)D2 and 25(OH)D3 from their metabolites 3-epi-25(OH)D2 and 3-epi-25(OH)D3. This method was validated for vitamin 25(OH)D2 and 25(OH)D3 in serum whereas the metabolites were fully separated from their metabolites using the optimized chromatographic parameters. Chromatographic separation of these analytes was achieved at room temperature on a reversed phase Phenomenex LUNA PFP(2) column (150 mm x 2.0 mm, 3.0 μm) using isocratic elution. The isocratic elution was performed with 88%-12% of MeOH-H2O and 0.1% formic acid. The analytes were monitored by atmospheric pressure chemical ionization (APCI) in positive ion multiple reaction monitoring (MRM) mode.

RESULTS: The mean recoveries for Vitamin D2 and Vitamin D3 were 96.41% and 94.71%, respectively. The limit of detection (LOD) for Vitamin D2 and Vitamin D3 was 1.06 and 1.48 ng/mL, respectively. The limit of quantitation (LOQ) for Vitamin D2 and Vitamin D3 was 3.50 and 4.90 ng/mL, respectively. The coefficient of variation (CV) of intra-day samples for Vitamin D2 and Vitamin D3 were 4.60% and 2.16%, respectively. The precision of inter-day (3 days) for Vitamin D2 and Vitamin D3 samples ranged from 3.34% to 4.46% and 1.68% to 2.26%, respectively. The production and certification of the reference material (RM) was done according to ISO Guide 34. The homogeneity and the stability tests of the candidate CRM showed that both of the analytes Vitamin D2 and Vitamin D3 were found to be homogeneous and stable to proceed with the characterization of the material.

CONCLUSIONS: The developed LC-MS/MS-based bioanalytical method is fast, simple and robust for the measurement of both vitamins 25(OH)D2 and 25(OH)D3 in human serum and in addition, this method guarantees the separation of 25(OH)D2 and 25(OH)D3 from their most common metabolites 3-epi-25(OH)D2 and 3-epi-25(OH)D3.

Vitamins and nutrition

Cod: 1614

REFERENCE RANGE OF ELECSYS® VITAMIN D (25-OH) TOTAL IN HEALTHY THAI MEN POPULATION

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BACKGROUND: Vitamin D is a fat soluble vitamin that functions as hormone in calcium and phosphate homeostasis. Plasma total vitamin D level between 21-29 mg/dL is considered vitamin D insufficiency while plasma total vitamin D less than 20 mg/dL is defined as vitamin D deficiency. Vitamin D deficiency is associated with many clinical conditions such as osteoporosis, chronic renal failure, and some cancers etc. Therefore, measuring vitamin D in plasma is importance in diagnosis and monitoring of many diseases. Nowadays, there are many methods for analysis of vitamin D including mass spectophotometry, high performance liquid chromatography and immunoassay. This study aims to defined reference range of new method in total vitamin D measuring in young healthy Thai population.

METHODS: One hundred and fifty eight recruits in Phayao Province, Northern Thailand, with the age between 20-21 years, who participated in standard ten week soldier training, were included in this study. Total vitamin D was determined by sandwich electrochemiluminescence immunoassay method on an e411 analyzer (Roche Diagnostics, Thailand). Fasting blood glucose, blood urea nitrogen, creatinine, triglyceride, total cholesterol, high density lipoprotein cholesterol, liver function tests, total calcium, albumin, phosphorus, and PTH were also tested in all samples.

RESULTS: The plasma total vitamin D showed normal distribution, and the levels measured were 22.29-49.49 ng/mL. The mean value was 35.89 ng/mL. Thirty six recruits (22.8%) had total vitamin D levels below 30 ng/mL, and four of them had PTH above 65 pg/mL. There was no soldier who had blood total calcium below normal.

CONCLUSIONS: The distribution of vitamin D levels and the prevalence of vitamin D insufficiency in these South-East Asia young men was similar to that observed in other Asian countries.