Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Nutrition/Trace Metals/Vitamins

A-447

Assessment Of Oxidative Stress And Antioxidant Status; And Their Correlation With Glycated Hemoglobin In Diabetics As Well As In Healthy Controls

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Background: Several studies have indicated lopsided redox balance due to pro oxidant environment as one of the important etiological factors for diabetes. Some recent researches also indicate a causal relationship with oxidative stress. So far, no detailed study has been undertaken on this aspect in Nepali populations. We, therefore, aimed this maiden study to compare the magnitude of oxidative stress as well as antioxidant capacity and correlate them with the glycated hemoglobin - a marker of hyperglycemia in type two diabetes mellitus (T2DM) and in healthy controls.

Methods: Cross sectional study involving 125 subjects with T2DM and 125 healthy controls. Plasma total peroxide (TP) and Total antioxidant capacity (TAC) were measured to evaluate the oxidative stress and antioxidant status respectively. Oxidative stress index (OSI) was calculated as the ratio of TP to TAC. Fasting blood sugar (FBS), Glycated haemoglobin (GHb), and post prandrial blood sugar (PPBS) were also measured. Statistical analysis was performed using SPSS 17.0.

Results: Medians of FBS, PPBS, GHb, HbA1c, mean blood glucose (MBG), plasma TP and OSI levels were significantly higher (P = <0.001) in T2DM patients compared to healthy group whereas plasma TAC levels were significantly lower (P = <0.001) in T2DM group. In case of diabetic group, GHb showed significant positive correlation with TP (ρ = 0.51; P = <0.001) and OSI (ρ =0.54; P = <0.001) whereas with TAC it showed significant negative correlation (ρ = - 0.53; P = <0.001). However, in case of control group, GHb showed weak positive correlation with TP (ρ = 0.02; P = 0.904), OSI (ρ = 0.05; P = 0.727) as well as with TAC (ρ = 0.01; P = 0.951) which were statistically insignificant.

Conclusion: The study showed an increase in oxidative stress and decrease in antioxidant capacity in diabetes and also indicated a positive correlation between the degree of hyperglycemia and oxidative stress. So, evaluation of oxidative status and choosing the appropriate treatment may help to support antioxidant defense in diabetic patients.

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Oral Administration Of Cyanocobalamin Causes Higher Increase In Circulating Holotranscobalamin Than Hydroxocobalamin: An Indo Danish Study With Different Doses Of Cobalamin

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Background: Most studies on cobalamin absorption are carried out with cyanocobalamin, but little is known as to whether such studies reflect the absorption of the natural forms of the vitamin present in food items, such as hydroxocobalamin. **Objective:** Here we investigate the uptake of oral intake of cyanocobalamin and hydroxocobalamin in an Indian (low cobalamin status) and Danish (high cobalamin status) population.

Methods: The CobaSorb test was used to estimate cobalamin absorption by measurement of circulating holotranscobalamin before and after three daily doses of cobalamin for two days. In the deplete population (n = 59, divided into six groups), the test was performed twice on each participant using a cross-over design with three different doses (1.5 µg, 3 µg, and 6 µg) of cyanocobalamin and hydroxocobalamin. In the replete population (n = 42), the test was performed three times with doses of (3 µg, 6 µg, and 9 µg) cyanocobalamin in 28 individuals, and twice with doses of 9 µg cyanocobalamin and 9 µg hydroxocobalamin in 14 individuals. Holotranscobalamin was measured by an in-house ELISA.

Results: In the cobalamin-deplete population, doses of 6 µg cyanocobalamin and hydroxocobalamin showed higher increase in holotranscobalamin than doses of 1.5 µg and 3 µg with no difference between 1.5 µg and 3 µg. Cyanocobalamin showed a 2-3 times higher increase in holotranscobalamin than hydroxocobalamin for all three doses (1.5µg:p<0.0001; 3 µg: p = 0.0002; 6µg: p<0.0001). In the cobalamin-replete population, doses of 3 µg cyanocobalamin showed a lower increase in holotranscobalamin than doses of 6 µg (p = 0.03) and 9 µg (p = 0.005) with no difference between 6 µg and 9 µg (p = 0.89). Cyanocobalamin (9 µg) showed a twofold increase in holotranscobalamin than hydroxocobalamin (9 µg) (p < 0.0001).

Conclusions: We show that administration of cyanocobalamin result in a twofold increase in circulating holotranscobalamin than administration of hydroxocobalamin independent of cobalamin dose or cobalamin status. In addition, our data suggests that the maximal uptake capacity is reached only by doses of above 3 µg cobalamin administered three times per day in both cobalamin replete and deplete individuals. Our results underscore the importance of using equimolar doses of the same form of cobalamin while comparing the uptake of free as compared to e.g. food-bound cobalamin.

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Comparative Study of Iodine Status between Community School Children and Adult Population in South-Western, Nepal

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Background: The present study was designed to assess and compare the iodine status between the community school children and community dwelling adult population residing in south-western Lumbini zone, Nepal. The thyroid status was also assessed based on hormonal level and presence of goiter in that community and correlated with median urinary iodine (MUI) concentration.

Methods: A cross sectional comparative study was conducted on 170 primary school age children of 8-12 years and 151 adult populations of 20-50 years. Spot urine samples iodine was estimated by Ammonium Persulphate Digestion, WHO method and thyroid hormones in sera were analyzed by spectrophotometric ELISA method. Household salt iodine content was estimated by Iodometric titration method. At low, medium and high urinary iodine, intra assay CVs were 5.99%, 4.9% and 4.93% and inter assay CVs were 13.8%, 8.41% and 6.35%.

Results: Our results showed overall population had frequency of 26(15.29%) school children and 13(8.6%) adult had iodine deficiency. Out of which 8(4.7%) children/0(0%) adult had severe, 6(3.5 %) children/ 3(1.98%) adult had moderate and 12(7.1%) children/10(6.62%) adult had mild iodine deficiency. Though the maximum population had optimal iodine level, but 50(29.41%) children/73(48.34%) adult had more than adequate requirement iodine and 39(22.94%) children/25(16.55%) adult had excessive iodine intake. The median urinary iodine concentration (MUI) in school children was 204.65 µg/L as compared to adult 252.95 µg/L (p=0.0001). The overall goiter prevalence was 5(2.94%) in children/2(1.32%) in adult among them 3(60%) children had iodine deficiency and no iodine deficiency observed in adult. 20(11.76%)children/57(38%) adult had subclinical hypothyroidism with MUI 206.4 µg/L/224.9 µg/L and 8(4.7%)children/41(27%) adult had overt hypothyroidism with MUI 203.3 µg/L /281.0 µg/L showing more than adequate requirement of iodine status in hypothyroid patients. There was positive correlation MUI with TSH (r=0.269, p=0.0001), negative correlation with fT_3 (r=-0.328, p=0.0001) and negative correlation with grading of goiter (r=-0.198, p=0.01).

Conclusion: Our study showed spectrum of iodine status with iodine deficient, more than adequate requirement to excess iodine nutrition in both community children and adult. The high iodine intake as assessed by MUI may trigger the hypothyroidism in children as well as adult. Universal salt iodization and awareness programs regarding adverse effects of iodine excess should be continued to minimize the risk to the vulnerable age groups.

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Burden of Vitamin D Deficiency and its Association with Insulin Resistance in Ghanaian Type 2 Diabetics

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Background: There is increasing interest in the non-skeletal role of vitamin D. Contemporary data suggests that vitamin D deficiency is related to several infectious and chronic conditions; with a possible influence on glucose homeostasis. There is growing incidence of diabetes mellitus among Ghanaians with a large percentage of the diabetics being overweight. In recent times, there has been attention on modifiable determinants for prevention of diabetes mellitus. Current data suggest vitamin D supplementation as a risk modifier for type 2 diabetes mellitus (T2DM), as it improves insulin secretion and reduce insulin resistance in T2DM. The vitamin D status and the association between vitamin D deficiency and diabetes has not been explored in Ghanaian population. This study provides preliminary information on vitamin D status among Ghanaian type 2 diabetics and assessed its association with glucose homeostasis. **Methods:** Briefly, in this case control study, 118 clinically diagnosed Ghanaian type 2 diabetics patients 25 years and above, of more than six months duration attending Diabetic Clinic at the Nkawie Government Hospital, Kumasi, Ghana, were selected as subjects between October and December 2015. Hundred healthy non-diabetics with fasting blood glucose (FBS) less than 6.4mmol/L living in Nkawie district were were selected as controls. Pregnant women and those with chronic illness or were on vitamin D supplementation were excluded. Structured questionnaires were administered to obtain socio-demographic data. Anthropometric data and venous blood samples were obtained from both subjects and controls to estimate their FBS, Lipid profile spectrophotometrically and intact parathyroid hormone, Insulin, vitamin D by using commercial ELISA kits. Statistical analyses were performed using SPSS v20.0 Statistics. **Results:** The average age of the cases was 58.81years and 57.79 years for the controls, more females were diabetic compared to males (n = 93/25). The indices of obesity (BMI, WC, HC, BAI) were significantly higher in the cases compared to the controls. There was vitamin D deficiency of 92.4% among the cases and 60.2% among the non-diabetic controls. Vitamin D deficiency did not significantly associate with **HOMA-β** [T2DM: $r^2=0.0209$, $p=0.1338$ and Control: $r^2=0.0213$, $p=0.2703$] and **HOMA-**IR [T2DM: $r^2=0.0233$, $p=0.1132$ and Control: $r^2=0.0214$, $p=0.2690$] in both the controls and the subjects**. Conclusion**: Vitamin D deficiency is prevalent in both T2DM and non-diabetics. There is no association between vitamin D deficiency and insulin resistance or beta cell function in our study population. Vitamin D supplementation among type 2 diabetics is recommended.

KEYWORDS: vitamin D, diabetes, HOMA-IR, HOMA- β, lipids, obesity.

A-451

Analysis of serum uric acid level and the prevalence of hypouricemia based on a multicenter study in Chinese population

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Context:

Hypouricemia may lead to nephrolithiasis or acute renal failure, however, it did not get enough attention as hyperuricemia and often ignored by clinicians because of the low incidence.The total prevalence rate of hypouricemia(≤119μmol/L) varied from 0.61% to 0.97% in western countries and 0.15% to 0.34% in Japanese outpatients and healthy individuals. No data about the prevalence of hypouricemia in China based on large population was reported until now.

Objective:

The objective of this study was to examine the prevalence of hypouricemia based on a representative multicenter population in China.

Methods:

Data came from the Chinese Physiological Constant and Health Condition (CPCHC). Participants included 34724 participants (16440 males and 18284 females) aged from 8 to 98 years old, which were recruited from six representative provinces (20 cities) of China during October to November 2011. A physical examination was performed and fasting blood was collected for biochemical tests.Uric acid was measured with a Beckman AU Series Automatic Biochemical Analyzer, using Beckman AU reagents. Hypouricemia was defined as a serum uric acid concentration less or equal than 119μmol/l(2.0mg/dl).

Results:

The prevalence of hypouricemia in this population was 0.58% with a range of 2~119μmol/l. Of the total subjects, females had significant higher rate of hypouricemia than males (0.83% vs 0.31%, P<0.01), participants from Ningxia Hui Autonomous Region had significant higher rate of hypouricemia than other volunteers(P<0.01).The mean serum uric acid concentration of the total population was 304.24±85.37μmol/l. Females had significant lower uric acid concentration than males (268.29±68.85 vs 343±68.85μmol/l, P<0.01). Volunteers aged from 8 to 18 had higher uric acid level than that of individuals aged from 19 to 59 years old. Participants came from Sichuan and Yunnan province had significant higher uric acid concentration than those from other provinces. Higher uric acid levels were observed in participants with the increase of BMI.

Conclusion:

Hypouricemia in chinese population is not rare compared with other countries. However, less attention paid to hypouricemia in our country.

Sex, age, region and BMI had significant effect on the level of uric acid.

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Impact of paper mill effluent discharge on the physico-chemical and microbiological qualities of Imo River at Owerrinta, Abia State, Nigeria.

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Background: Pollution of aquatic environment from industrial processes is one of industrial problems in Nigeria. Most industries discharge their effluents into water bodies. Such effluents may contain dissolved substances and microorganisms. This improper waste disposal leads to pollution of surface and ground water, hence unsafe to users and increases water-borne diseases. Water pollution affects the aesthetic status of water, the health of aquatic organisms and humans. Toxic pollutants can also alter the genetic makeup of an organism leading to their death or deformities. Consequently this research set out to assess the impact of paper mill effluent discharges on the physico-chemical and microbiological status of Imo River at Owerrinta.

Methods: River water samples from several locations and effluent sample from discharge point of the paper mill industry were aseptically collected and investigated physicochemically by Hach's and Atomic Absorption Spectrophotometer (AAS) techniques and microbiologicall by pour plate method on nutrient, MacConkey and Sabouraud agar.

Results: Study revealed that the river water at 30meters before the entry of effluents had a higher value of dissolved oxygen (5.90mg/l), pH range value, 6.5, Low values of suspended solids, alkalinity, total hardness, sulphate, nitrate, calcium, chemical oxygen demand, and biochemical oxygen demand. At the point of entry of the effluent, there was a decline in dissolved oxygen content (2.62mg/l) and increase in other factors. At about 150meters from the point of discharge of effluent, quantitatively, most of the parameters fall within the two earlier stations. The total heterotrophic aerobic bacteria counts ranged from $175.0x10^6$ (cfu)/ml and $125x10^4$(cfu)/ml to $8.75X10^6$ and $1.8X10^3$ (cfu)/ml. The fungal counts for all the samples ranged from $0.65X10^6$ (cfu)/ml to $3.5x10^6$ (cfu)/ml. Identified and predominant microorganisms are *Klebsiella species, Pseudomonas spp., Streptococcus faecalis, Bacillus species and Chromobacterium violaceum for* bacteria**,** and *Candida spp. and Aspergillus fumigatus* for the fungal isolates.

Conclusion: Physicochemical analysis showed that the effluent samples had the highest values of most of the parameters assessed, followed by the samples of the region of effluent discharge and lastly by the samples at the downstream. Analyses of the result revealed that a relationship exist between biochemical oxygen demand (BOD) and nitrate, whereas there is no relationship between total alkalinity and total hardness. The presence of microorganisms of public health and economic importance is indication of risks to users.

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Impact of a single oral dose of 100,000 IU vitamin D3 on profiles of serum 25(OH)D3 and its metabolites 24,25$(OH)_2$D3, 3-epi-25(OH)D3, and 1,25$(OH)_2$D3 in adults with vitamin D insufficiency

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Background: We investigate the effect of a high dose of vitamin D3 on circulating concentrations of 25(OH)D3 and its metabolites 24,25$(OH)_2$D3, 3-epi-25(OH)D3, and 1,25$(OH)_2$D3 in healthy individuals with self-perceived fatigue and vitamin D insufficiency (25(OH)D3 <50 nmol/L). **Methods:** 107 study participants (age 20-50 years) were randomized to receive a single 100,000 IU dose of vitamin D3 (n= 52) or placebo (n= 55). Vitamin D metabolite concentrations in serum were measured before, and 4 weeks after, supplementation. **Results:** Overall, 52% of participants receiving vitamin D3 attained a serum 25(OH)D3 level >75 nmol/L. Among individuals who received vitamin D3, there were significant increases in serum concentrations of 25(OH)D3 and its metabolites 24,25$(OH)_2$D3, 3-epi-25(OH)D3, and 1,25$(OH)_2$D3 at 4 weeks; however, inter-individual variability in these changes was substantial. Positive correlations between serum 25(OH)D3 and 24,25$(OH)_2$D3 and 3-epi-25(OH) D3, and a significant negative correlation between serum 1,25$(OH)_2$D3 and 3-epi-25(OH)D3, were found 4 weeks after supplementation. The 24,25$(OH)_2$D3/25(OH) D3 and 24,25$(OH)_2$D3/1,25$(OH)_2$D3 ratios were significantly increased, compared with baseline, in participants receiving vitamin D3. Baseline 25(OH)D3 concentration was the only factor predictive of the change in 25(OH)D3 after supplementation. **Conclusions:** Administration of a single high dose of vitamin D3 leads to a significant increase in concentrations of 25(OH)D3, 24,25$(OH)_2$D3, 3-epi-25(OH)D3 and 1,25$(OH)_2$D3; induction of the catabolic pathway predominates over the production of 1,25$(OH)_2$D3. Due to the high inter-individual variation in the 25(OH)D3 response to supplementation, any given dose of vitamin D is unlikely to achieve optimal vitamin D status in all treated individuals.

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Vitamin D nutritional status and bone turnover biomarkers in acute lymphoblastic leukemia survivors

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Objective: The remarkable progress in the treatment of childhood acute lymphoblastic leukemia (ALL) has lead to an ever-increasing survival rate. This success story is unfortunately linked to increased risks of impaired accumulation of skeletal mass during childhood and adolescence, predisposing patients to impaired bone mass in early adulthood. This study aims at characterizing the vitamin D status and bone metabolism biomarkers in a large cohort of childhood ALL survivors.

Methods: The study population consists of 251 patients [median age at entry: 21.9 years] of French-Canadian origin with an established genetic founder effect, and diagnosed when younger than 19 years with ALL. They were all treated with the Dana Farber Cancer Institute protocols without hematopoietic stem cell transplantation. The median (2.5-97.5%^{ile}) post-treatment time was 13.0 (4.4-23.8) years. The Institutional Review Board of Sainte-Justine Hospital approved the study and the investigations were carried out in accordance with the principles of the Declaration of Helsinki.

Patients' dietary intakes were evaluated after filling a food frequency questionnaire using the Canadian Nutrient File Database. Serum 25-hydroxyvitamin D_3 ($25OHD_3$), for assessing the vitamin D nutritional status, was measured by a QTOF-MS method. Total calcium (Ca_t), inorganic phosphate (P_i) and alkaline phosphatase (Alk Phos) were measured on an Abbott's Architect *c*System and intact Parathyroid hormone (iPTH) on an Immulite 2000.

Serum bone resorption [Carboxy-terminal collagen type-1 telopeptide (CTx,)] and formation [Pro-collagen type-1 amino-terminal pro-peptide (P1NP)] biomarkers were measured by an automated chemiluminescent immunoassay (IDS Immunodiagnostics). Bone mineral density was measured by dual X-ray absorptiometry (DXA) using the Lunar Prodigy absorptiometer.

Results: The food frequency questionnaires revealed that the total vitamin D intake varied greatly (44-2132 IU/d), that only 16.8% of the participants consumed vitamin D supplements, and that 74% were below the RDI (400 IU/d). For those who took supplement (n = 42), the median (2.5 – 97.5$^{\%iles}$) intake was 600 IU/d (21.2-1972 IU/d). Only 14 patients had daily calcium intakes below the RDI (800 mg/d). Out of the 248 patients for whom $25OHD_3$ was measured, 16 were vitamin D deficient (<30 nM) and 66 insufficient (≥30 - <50 nM). Although the mean total body or lumbar (L1-L4) BMD Z-scores were with the normal range, 3 male participants and 2 female participants had Z-scores ≤ 2.5 SD, and 36 male participants and 21 female participants had Z-scores between ≤1.0 and <2.5 SD, classifying them respectively as osteoporotic and osteopenic. No correlation was observed between serum 25OHD concentration and serum bone turnover concentrations.

Conclusions: These data demonstrate that this group of ALL young adult survivors were not at higher risk of vitamin D deficiency or insufficiency than the general Canadian population. However a fair number exhibited osteopenia and a small percentage osteoporosis despite their young age. **(449 words)**

A-455

The relation between vitamin D deficiency in the first rimester of pregnancy and Bacterial vaginosis in Egypt

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Background: Adequate vitamin D intake is essential for maternal and fetal health during pregnancy, Epidemiological data indicates that many pregnant women have sub-optimal vitamin D levels. Notably, vitamin D deficiency correlates with preeclampsia, gestational diabetes mellitus, and bacterial vaginosis, and an increased risk for C-section delivery. Recent work emphasizes the importance of non classical roles of vitamin D in pregnancy and the placenta. The aim of the present study was to evaluate the association between vitamin D deficiency and bacterial vaginosis in the first trimester of pregnancy. **Methods**: a cross sectional study was conducted on 100 pregnant women attending Elshatby Maternity University Hospital during their first antenatal visit between April- December 2014 (6 months). Women were subjected to a pelvic speculum examination at the initial obstetrical visit to ascertain for the presence of BV and vaginal swab was taken to be evaluated by gram's stain for presence of bacterial vaginosis. Plasma 25-OH-D concentration, the major circulating form of vitamin D, was assayed using a commercially available ELISA kit. **Results**: the age of the included pregnant woman ranged from 20-32 with a mean of 24.9±2.91 years, vitamin D deficiency was considered if the serum level <10 IU. insufficient level between 10-30 IU and sufficient level with 30-100 IU in serum. Most of the studied women were less than or equal 25 years (60.0%). The gestational age ranged from 8.0-12.0 with a mean of 9.93±1.59 weeks. Most of the studied women had a BMI more than 25 kg/cm2 (95.0%). 82 cases were positive for bacterial vaginosis, while the other 18 cases(18%) were negative for bacterial vaginosis. The majority of the patients had vitamin D deficiency (53.0% of the patients), while 35.0% of them had insufficient vitamin D. Only 12 cases (12.0%) had sufficient amount of vitamin D. The level of vit D ranged from 1.54-40.0 IU with a mean of 13.97±9.57 IU. There was a significant relation between the incidences of vit. D deficiency and the presence of bacterial vaginosis. **Conclusions:** There was association between vitamin D deficiency in the first trimester of pregnancy and bacterial vaginosis, all subjects with sufficient serum vit .D were free from bacterial vaginosis.(66.7%). All patients with deficient serum vitamin D level have bacterial vaginosis (64.6% of the patients).

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Association of 25 OH-Vitamin D and hsCRP in adults with Essential Hypertension

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Background: Essential hypertension is a typical example of complex, multifactorial trait and a well studied risk factor for cardiovascular disease. hs-CRP is well known marker of inflammation resulting in atherosclerosis and cardiovascular disease. Recent studies showed that 25-OH-vitamin-D has an anti-inflammatory role and decreased levels may increase the cardiovascular risk in subjects with essential hypertension.

Methods: Thirty adults Both male and female with essential hypertension (Systolic blood pressure ≥140mmHg and Diastolic blood pressure ≥ 90mmHg) 30 age and sex matched healthy controls both male and female between 24 -60 years of age were recruited from General Medicine department. 25-OH-Vitamin D levels were assessed by High Performance Liquid Chromatography and hs-CRP was estimated by Turbidometric method.

Results: In subjects with essential hypertension 25-OH-vitD levels (mean ± SD 28.35± 2.05 $p<0.05$) ng/mL were significantly low when compared to control subjects and hs-CRP levels (mean ± SD 9.32 ± 1.04 $p<0.05$) mg/L were significantly increased when compared to control subjects . Our study also showed a significant negative correlation between 25-OH-vitD and hs CRP levels (r = -0.4) ($p<0.01$).

Conclusion: Our study concludes 25 OH-Vitamin D levels are significantly decreased and hs-CRP levels are increased in subjects with essential hypertensives. Negative correlation between 25-OH Vitamin D and hsCRP levels which leads to cardiovascular risk in subjects with essential hypertension. The effects of Vitamin D supplementation on morbidity and mortality of large group of population with essential hypertension.

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Seminal plasma total antioxidant capacity (TAC), magnesium and calcium levels of infertile men

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Background: Poor quality semen of infertile men may be attributable to testicular production of abnormal spermatozoa, post testicular damage in the epididymis or ejaculate from abnormal accessory reproductive gland secretions. The secretions are central to the reproductive potential of semen, especially in acrosome reaction and capacitation processes during fertilization. This study aimed at the assessment of the levels of seminal plasma total antioxidant capacity (TAC), magnesium (Mg) and Calcium (Ca) and their influences on sperm quality of infertile men. **Methods**: Seventy one infertile men attending clinics in General Hospital Calabar, further classified into oligospermic (n=33), asthenozoospermic (n=30) and azoospermic (n=8) based on the WHO classification and forty nine apparently healthy fertile men who served as control were recruited for this study. Informed consents were obtained from all the participants of this study. Semen were obtained from participants following 3-5 days of abstinence from sexual intercourse by self-help into wide mouth bottles held close with the body; delivered within 30 minutes to the laboratory for analyses. Semen quality was evaluated and seminal plasma harvested after centrifugation. Seminal plasma TAC, Mg and Ca levels were determined by colorimetry. Data were analyzed using SPSS statistical package, variations among groups were determined by ANOVA, differences between groups by Student's t-test and association between parameters by Pearson's correlation. Results expressed as mean± SD, significant at $p<0.05$. **Results**: The percentage motility, sperm count, TAC, Mg and Ca levels of the fertile group (69.80±7.29%, ±84.4065.08 $x10^6$/ml, 0.56±0.20mmolTrolox equiv./L, 0.55±0.12mmol/L and 3.17±2.07mmol/L respectively) were significantly higher ($p<0.05$) than (28.38±15.44%, 7.03±5.66$x10^6$/ml, 0.29±0.18mmolTrolox equiv./L, 0.21±0.15mmol/L and 1.86±1.19mmol/L respectively) of the infertile group. Comparison of the normospermic, asthenozoospermic, oligospermic and azoospermic groups, shows that in all the parameters measured the values for the normospermic were significantly higher ($p<0.05$) than those of the various infertile groups. Percentage motility, sperm count and volume vary significantly among the infertile groups, while TAC, Ca and Mg levels did not vary significantly. Azoospermic group had the highest level of Mg (0.25±0.21mmol/L) and lowest level of Ca (1.26±0.55mmol/L). Asthenozoospermic and oligospermic did not differ significantly in all the parameter measured. In the fertile group, Mg and Ca correlated negatively, (r=-0.322, p=0.024). Motility and sperm count correlated positively in both oligospermic (r=0.478, p=0.005) and asthenozoospermics (r=0.605, p=0.001). TAC and motility correlated positively in oligospermic group (r=0.377, p=0.031). **Conclusion**: Spermatozoa travelling the male reproductive tract, are kept viable in fluids secreted by the various accessory glands. Magnesium and calcium from the prostate gland may play important roles in acrosome reaction and capacitation necessary for fertilization of the oocyte, through mechanisms which appear to involve modification of intracellular calcium and other ions, lipid transfer/remodeling in sperm plasma membrane. While low seminal plasma Ca and Mg may be responsible for loss of motility, failed acrosome and capacitation reactions, supraphysiological levels may propagate chemical sterilization. Seminal plasma TAC represents the net oxidative system balance in semen, which prevents spermatozoa death that may occur at oxidative stress level. The study shows that abnormal seminal TAC, Calcium and Magnesium may affect sperm qualities and characteristics associated with fertility

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Performance Evaluation of the Atellica™ Vitamin B12 Assay*

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Introduction: The ADVIA Centaur® Vitamin B12 (VB12) assay (Siemens Healthcare Diagnostics Inc.) is an in vitro diagnostic immunoassay for the quantitative detection of vitamin B12 in serum or plasma. Clinical and laboratory findings for B12 deficiency include neurological abnormalities, decreased serum B12 levels, and increased excretion of methylmalonic acid. The primary objective of this study was to demonstrate the analytical performance of a similar VB12 assay on the Atellica™ Immunoassay (IM) Analyzer** a high-throughput analyzer in development by Siemens Heathineers.

Methods: The Atellica™ VB12 Assay uses the same reagents as the ADVIA Centaur VB12 assay. The ADVIA Centaur® VB12 Assay is a "competitive" immunoassay using direct chemiluminescent technology. Vitamin B12 from the patient sample competes with vitamin B12 labeled with acridinium ester in the Lite Reagent, for a limited amount of purified intrinsic factor, which is covalently coupled to paramagnetic particles in the Solid Phase. The Atellica VB12 Assay requires an ancillary reagent that contains a release agent (sodium hydroxide) and DTT to release vitamin B12 from the endogenous proteins in the sample. Precision of the Atellica VB12 Assay was evaluated according to CLSI protocol EP05-A3. Method comparison of the ADVIA Centaur VB12 Assay on the Centaur XP and the Atellica VB12 Assay followed CLSI protocol EP09-A3. Limit of blank (LoB) and limit of detection (LoD) were evaluated according to CLSI protocol EP17-A2.

Results: Observed repeatability for the Atellica VB12 Assay ranged from 4.1 to 1.3% CV and within-lab precision ranged from 5.9 to 2.4% CV over sample result ranges of 151 to 1708 pg/mL. Quantitative comparison of the ADVIA Centaur VB12 Assay on the Centaur XP and the Atellica VB12 Assay yielded the following regression equation: Atellica VB12 Assay = 0.997(ADVIA Centaur VB12 Assay) + 7.054 pg/mL, with 139 serum samples ranging from 47.58 to 1935.78 pg/mL; r = 0.994. The LoB and LoD were determined to be 37.60 and 53.51 pg/mL, respectively, with two reagent lots tested.

Conclusion: The Atellica VB12 Assay has demonstrated analytical performance capable of measuring vitamin B12 with accuracy and precision for use in the detection of B12 deficiency.

*Under development. Not available for sale. Future availability cannot be guaranteed.

**Not available for sale. Not CE Marked.

HOOD05162002576680

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Biotin Interference in Automated Immunoassay Methods: A Perfect Storm

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Background: Streptavidin/biotin and biotin/anti-biotin reactions are present in many FDA-cleared immunoassays (IA's) that are now being used for patient care. A marked increase in the use of biotin supplements over the past three years and an increase in the size of the doses that are being taken have led to a steady increase in reports of falsely increased or decreased test results owing to biotin interference in subjects taking OTC biotin supplements in doses of >1000 mcg /day. Currently, the most popular doses used in the US are 5000 or 10000 mcg capsules taken 1 to 3 times/day. 100,000 mcg capsules are now available with suggested doses of 1 to 3/day! This study was undertaken to gain a better understanding of the risk of erroneous test results in patients taking biotin supplements. **Methods:** In June of 2016 we reviewed the current package inserts (IFU's) for 417 methods performed on eight immunoassay platforms(Roche Elecsys®, Ortho Vitros®, Siemens Dimension®, Siemens Centaur®, Beckman Coulter Access®/DXI®, Abbott Architect i2000®, Siemens Immulite 2000®, Diasorin Liaison XL®) to determine which methods were potentially vulnerable to biotin interference and to identify the manufacturer-reported interference thresholds(IFT's) above which exogenous biotin caused a significant (> +/- 10%) difference in the test result. A method was considered to be potentially vulnerable to biotin interference when it utilized a streptavidin/biotin reaction, an anti-biotin/biotin reaction, or a pre-bound avidin/streptavidin or biotin/anti-biotin reagent in the analysis.

Results: Our review showed that the methods performed by 2 of the platforms were not vulnerable to biotin interference, but that 173 of the 319 methods (54 %) performed by the other six platforms were. The distribution of the IFT's among the vulnerable methods was (number of methods, IFT(nmol/L): 31,< 50 nmol/L; 36, 15-150 nmol/L; 33, 150-250 nmol/L; 46, 250-500 nmol/L; and 7, > 2000 nmol/L. The IFU's for 20 of the vulnerable methods neither presented an IFT nor mentioned that biotin interference was an analytical limitation of the test! Previously published data from single-dose pharmacokinetic studies and reports of analytical interference in patients receiving 10000 mcg biotin/day for the treatment of genetic disorders of biotin metabolism, predict that methods with IFT's of <200 nmol/L are at risk for generating inaccurate results in subjects who take biotin supplements at doses of 1000, 5000, and 10,000 mcg/day. Subjects that take doses of >100,000 mcg/day are at high risk for markedly inaccurate results and should be tested using interference-free methods.**Conclusion:** The emergent problem of biotin interference has yet to be widely recognized and fully appreciated by the clinical laboratories that perform the testing, or the healthcare providers that order lab tests and interpret test results. The combination of increased biotin supplement use by and the design limitations of many of the currently available clinical immunoassays is a "perfect storm" that has the potential to have a significant, negative impact on patient care. This source of analytical interference needs be promptly and effectively addressed by laboratories and clinical diagnostics manufacturers.

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Prevalence of hyperuricemia and its relevant risk factors in healthy adolescents in China

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Background: Hyperuricemia (HUA) is a risk factor for metabolic disorders, such as gout. In this study, we investigated the serum uric acid (SUA) level and HUA rate and relevant risk factors in healthy adolescents in six provinces/regions of China.

Methods: A total of 14,022 healthy adolescents aged 8 to 18 years were randomly selected from six provinces in China, and biochemical markers such as SUA, blood glucose, blood lipids, liver function, and kidney function were determined. Moreover, high UA levels, hypertension, and obesity were defined and stratified according to applicable international guidelines.

Results: A significant difference was observed in the SUA levels of healthy adolescents from different provinces with different genders, places of residence, and ethnicities. The overall HUA rate was 15.5%. The HUA rate was higher in healthy adolescent boys than that in girls (17.5% vs 13.5%, $P < 0.001$). Moreover, the HUA rate increased with age in boys and peaked at the age of 15 to 16years, whereas it decreased with age in girls and peaked at the age of 11 to 12 years. The HUA rate was highest among healthy adolescents in Yunnan (21.1%) and was higher in urban centers than in other areas (21.1%). In addition, HUA rate was highest in Korean Chinese (21.2%) and lowest in Mongolian Chinese (7.6%) and Hui Chinese (6.4%) adolescents, with significant differences from the rates of Han Chinese adolescents. The levels of biochemical markers such as alanine aminotransferase (ALT), total protein (TP), albumin (ALB), γ-glutamyl transferase (GGT), creatinine (CR), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), calcium (CA), creatine kinase (CK), amylase (AMY), and Urea were significantly higher in adolescents with HUA than in those without HUA. We used a logistic stepwise regression model to select six risk factors for HUA, including gender, place of residence, ethnicity, obesity, exercise, and hypertension. After controlling for these factors, the odds ratio (OR) of HUA was 1.41-fold higher in boys than in girls; 1.52-, 1.33-, and 1.79-fold higher in urban centers than in the suburbs of large cities, small-to-medium cities, and agricultural and pastoral areas; and 1.55 (1.02-2.35)-, 0.44 (0.32-0.59)-, and 0.29 (0.20-0.43)-fold higher in Tujia, Mongolian, and Hui Chinese than in Han Chinese adolescents.

Conclusion: The HUA rate in healthy adolescents in China is related to economic development and lifestyle. Thus, improving quality of life and implementing HUA prevention measures will help to reduce the risk of HUA.

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Survey of Vitamin D status and the relationship with routine biomarkers in apparently healthy younger adults in Beijing

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Background: vitamin D deficiency was prevalent globally. However, data of vitamin D deficiency status among younger adults of Han nationality in China is still not thoroughly analyzed. In this study, we analyzed the vitamin D status among apparently healthy younger adults of Han nationality in Beijing and the relationship with routine clinical biomarkers to provide reliable data for preventing vitamin D associated diseases in the process of aging.

Methods: This is an observational study. Participants included 287 apparently healthy young adults(143 males and 144 females) with an average of 32.2±6.9 years old(19~44 years). We measured 25-hydroxyvitamin D (25OHD) using liquid chromatography tandem mass spectrometry method, vitamin D with deficiency, insufficiency and sufficiency was categorized as 25OHD <20 ng/ml, 20-30 ng/ml, ≥30 ng/ml, respectively. ALT, Ca, P, Cr, Glu, TG, TC, iPTH was analyzed using automatic analyzers.Statistical analysis was performed using SPSS17.0.

Results: The median 25OHD level in the total studied younger adults was 16.0(2.5%~97.5%: 6.1~29.0) ng/ml while in males that was 17.9(8.3~32.3) ng/ml and in females that was 14.4(5.4~26.4) ng/ml. Males had significant higher level of 25OHD than females ($P<0.01$). Of the total subjects, the rate of vitamin D with deficiency (<20 ng/ml), insufficiency (20-30 ng/ml) and sufficiency (≥30 ng/ml) was 72.8%,25.1%,2.1%, respectively, while that of males was 65.0%,30.8%,4.2%, respectively, and that of females was 80.6%,19.4%,0%, respectively. Females had significantly higher rate of 25OHD deficiency ($P<0.01$). With adjusting sex, age and BMI, serum iPTH and Glu was significantly negatively correlated with 25OHD while Cr showed significantly positively correlation with 25OHD.

Conclusion: Vitamin D deficiency is prevalent in younger adults of Han nationality in Beijing, especially in females.

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Quantitation of Vitamin B1 in Whole Blood Using a Simple HPLC Method with Internal Standard

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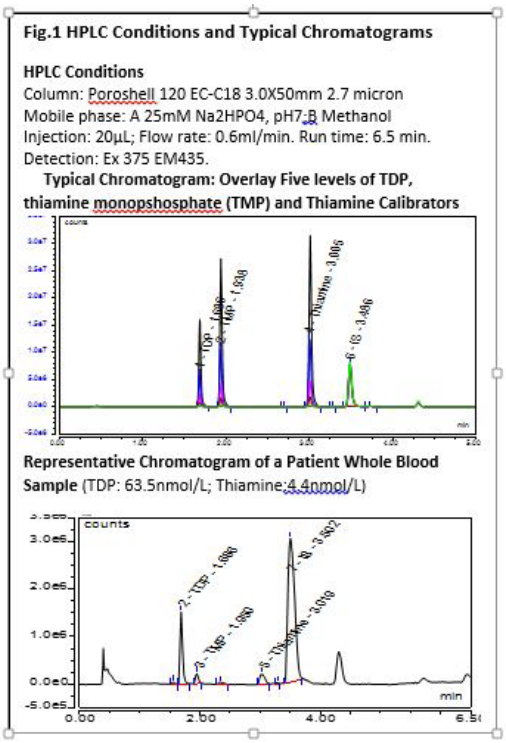
Background: Measurement of whole blood thiamine diphosphate, the bioactive form of vitamin B1, has been considered the most effective way of detecting vitamin B1 deficiency. Free thiamine is the predominant form present in human plasma, and reflects recent dietary intake of vitamin B1. Current HPLC-MS/MS methods usually detect total thiamine after a lengthy enzyme reaction to convert TDP to thiamine using acid phosphatase. The published HPLC methods lack a suitable internal standard (IS) and often include the use of ion pairing reagents in separation or a methyl-tert-butyl ether wash in sample preparation. Our objective was to develop a simple HPLC method that simultaneously measures TDP and thiamine in whole blood with an IS to improve reproducibility.

Methods: Three chemicals (pyrithiamine, amprolium, actylaneurine) that share some structural similarity to thiamine were evaluated for suitability as an internal standard. For sample preparation, 250ul whole blood after freeze and thaw was treated with 750ul of 6.7% trichloroacetic acid (TCA) to precipitate protein. After centrifugation and filtration, the supernatant was derivatized with 0.04% alkaline potassium ferricyanide solution. The derivatized samples were analyzed using Vanquish UHPLC (Thermo Fisher Scientific) with fluorescence detection.

Results: Our data showed that pre-derivatized amprolium was a suitable IS. It generated strong fluorescence at the same wavelength as TDP and thiamine, and does not exist in human blood. The intra- and inter assay precision was: TDP 2.3-2.5% and 4.0-4.8%; thiamine 3.4-5.0% and 2.1-6.5% respectively. The analytical measurement range was 1.7-442.3 nmol/L (TDP) and 1.7-375.4 nmol/L (thiamine). Method comparison of TDP with a reference laboratory HPLC method showed r=0.9625, slope=1.021, intersect=0.982 (n=53). In addition, our data showed that thiamine concentration in whole blood mirrored plasma thiamine levels and can be used to determine recent vitamin B1 uptake.

Conclusion: This is a simple and reliable method for evaluating vitamin B1 nutritional status.



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Reducing utilization of 72-hour fecal fat testing through order restriction and simple alternative tests

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Background: At Calgary Laboratory Services (CLS), 72-hour quantitative fecal fat testing has been available for patients located in Calgary and Southern Alberta for many years. As with other clinical laboratories, this test continued in use despite being made obsolete by the gradual introduction of other tests that could rule in or rule out fat malabsorption. In 2014, a review of clinical appropriateness, utilization and cost was performed at CLS to develop a plan to eliminate and replace the test. This included a research study to evaluate the predictive power of stool specimen characteristics, including weight, qualitative and quantitative (via automated image analysis) measures of stool lipid droplets, for abnormal fat excretion by the 72-hour test. **Methods:** A literature review was performed in consultation with adult and pediatric gastroenterologists to identify the major clinical uses as well as the most appropriate users of the 72-hour test. Laboratory tests and clinical information that provided similar rule-in or rule-out information as the 72-hour test were identified. Ordering restrictions and testing recommendations were proposed with the regional heads of adult and pediatric gastroenterology. Memos were circulated that detailed the agreed-upon changes in advance of go-live. To develop an alternative to the 72-hour test, all historic (2009-2016) 72-hour quantitative fecal fat results (mmol/day) and specimen data were extracted from the CLS laboratory information system, and 100 banked 72-hour stool specimens were examined microscopically for oil-red-o stained neutral fat droplets via (1) qualitative (≥5 droplets / 400X field) manual reading by technologists and (2) quantitative automated image analysis using ImageJ. Logistic regression and ROC-curve analysis were used to identify predictive power of specimen weight and stool lipid droplet measures for abnormal fat excretion (>=21 mmol/day for >=7 years of age; >=7 mmol/day for <7 years of age). **Results:** From 2009-2014, CLS performed ~100 72-hour fecal fat tests per year. On January 1 2015, an ordering restriction preventing adult specimens to be run without approval by clinical biochemist was enacted, reducing workload to 20 tests per year. Tests were cancelled for physicians that did not contact the lab, and contacting physicians were informed of the availability of alternatives, In specimens tested from 2009-2016, fecal weight was a moderately strong predictor of fat malabsorption in patients ≥7 years (Area under curve = 0.74; n = 423) and < 7 years (Area under curve = 0.77, n = 113). In a sample of 91 specimens >=7 years of age, fecal weight alone was a similarly strong predictor (Area under curve = 0.70). Additionally considering either a qualitative (area under curve = 0.76) or quantitative measure of stool fat globules (area under curve = 0.77) improved discrimination over fecal weight alone.

Conclusion: Engagement with clinician stakeholders reduced 72-hour quantitative fecal fat testing, resulting in more appropriate lab utilization and clinical evaluation. Based on the results of our research study, we propose to reflex all specimens submitted for fecal fat analysis for reporting of fecal weight and qualitative stool fat globules.

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Analysis of Vitamin A ordering patterns in a Canadian City

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Background: Vitamins A is a lipophilic vitamin that plays a role in vision. Measurement of Vitamin A is most commonly performed for assessment of deficiency, such as patients who have malabsorptive syndromes. In rare cases, it may be measured for toxicity. Recently, measurement of Vitamin A has become popular as part of the 'wellness' movement and tests are ordered regularly without the usual clinical indications. Over the past few years, the increasing number of requests for Vitamin A at our laboratory has required the addition of a second batched run per week. The objective of this study was to quantify and evaluate increases in Vitamin A ordering at our laboratory. **Methods:** A request for data was submitted to our institutional data team for Vitamin A data between 2010 and 2015 including patient age, sex, test ordered, test result, collection date, and ordering physician. All data was de-identified as per our institutional privacy policy. Analysis was performed using Microsoft Excel 2013.

Results: Vitamin A requests increased from 2431 in 2010 to 4369 in 2015. Of these orders, 89% were from family physicians, 8% from specialists and 3% were from hospital inpatients. When analyzed by ordering physician, it was determined that 10 physicians were responsible for over 73% of all Vitamin A requests. Of these ten physicians, 7 listed integrative or functional medicine as a specialty on their websites. Further analysis of the ordering patterns for these 7 physicians determined that many of their patients had repeat Vitamin A's ordered in less than a year regardless if the previous result had been normal or not. In 2015, the number of Vitamin A's ordered with a previous normal result performed within 12 months was 1253, which accounts for 28% of the 2015 workload. **Conclusion:** Vitamin A is frequently ordered by physicians practicing functional or integrative medicine and may be target for utilization measures at our institution.

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Vitamin B12. Is the normal range a sufficient range?

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Background: Vitamin B12 is a water-soluble compound, not synthesized by the human body and available in foods of animal origin.

Cyanocobalamin deficiency can lead to hematological, neurological and cardiovascular disorders by interfering with homocysteine metabolism and the body's methylation reactions.

Serum dosage of this vitamin has some methodological restrictions of sensitivity and specificity, and clinical symptoms of deficiency may occur even with serum levels into the range of reference values, therefore, the contribution of the laboratory results still uncertain for the clinical follow-up of these patients.

Our work aims critically analyze the distribution of serum vitamin B12 from outpatient dosage in the historical database of our laboratories, since several scientific studies question the reference values or were performed with small population samples.

Methods: This is a retrospective observational study of the laboratory database tests performed between January 2016 and January 2017, in Curitiba, PR, Brazil.

The database is composed of serum dosage results and personal data (gender and date of birth) of individuals who had their exams requested by their attending physicians who are not linked to the laboratory.

In case of serial dosages, only the first result of each patient was considered.

The laboratory analysis of Vitamin B12 was carried out on serum samples by direct chemiluminescence method ADVIA Centaur VB12 Siemens, with sensitivity and

specificity determined by the manufacturer's package insert, suggesting reference range between 211 and 911 pg/ml, detection limit between 45 and 2000pg / ml.

Results: A total of 104,271 patients were evaluated, of which 73,756 (70.73%) were female.

We split the sample by age into 03 groups: Children < 18 years old (12.93%) Adults > 18 and < 60 years (68.16%) and Elderly > 60 elderly (26.91%).

The mean serum level in the evaluated population was 461.87 ± 240.62 pg/mL with a median of 410 pg/mL in a range of 73 to 2000 pg/mL.

Similar results were found when the analysis was performed by subgroups of sex and age.

The quartile assessment suggests a range of normality between 326 (p25) and 524 (p75) pg/mL and in the percentile assessment we found a range between p3 and p97 similar to that suggested by the manufacturer's package insert, from 220 to 977 pg/mL.

Conclusion: The values found in this population were similar to those described in several scientific studies and in the manufacturer's package insert.

Due to the size of the population studied, we believe that the values are adequate for a first analysis, however, tests for this purpose vary widely in sensitivity and specificity.

Thus, the laboratory diagnosis still leaves doubts about its potential contribution in the clinical and therapeutic decisions of patients with results between p3 and p25 of the values found and it is necessary to establish a gold standard interpretation criteria.

We believe that values closer to the median may be more adequate to corroborate with clinical diagnosis, however the correlation with other laboratory dosages may clarify this hypothesis more accurately.

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The association of the vitamin A, vitamin E level and potential biomarkers of mRNA expression with the extent of coronary lesion

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Atherosclerosis is a chronic inflammatory process associated with the majority of cardiovascular diseases (CVD). The oxidative stress is an important event in the pathogenesis of these diseases that are major cause of morbidity and mortality worldwide. Antioxidants, like vitamin A and vitamin E, are substances capable of preventing the deleterious effects of oxidation and recent studies showed that they might have a protective role in CVD. It has been suggested that this substances are associated with decreased risk of CVD and atherosclerosis. 'Omics' analysis can contribute to this field by providing fundamental information to better understand complex biological systems, such as atherosclerosis. Particularly, transcriptomics is a relevant tool for the identification of diagnostic/prognostic biomarkers for CVD. Thus, the objective this study is evaluated the association of the vitamin E, retinol and potential biomarkers of mRNA expression with the extent coronary lesion. The study included adults aged 30-74 years undergoing elective cinecoronariography for the first time. Fasting blood samples have been collected for biochemical analysis. The concentration of vitamin E was determined by high-performance liquid chromatography (HPLC). The atherosclerotic burden was measured through Friesinger score. This score is determined by separately scoring each of the three main coronary arteries within a range of 0 to 15. For this analysis, the FS was divided into three categories: 0-4, 5-9 and 10-15. The gene expression was performed using mRNA of blood peripheral cells follow relative quantification by real-time PCR. The sample consisted of 177 adult patients; 99 patients (55.9%) were male, and 78 patients (44.1%) were female. Regarding to the Friesinger index, the patients were stratified into three groups: 0-4 (n=90), 5-9 (n=50) and 10-15 (n=37). Patients at group 10-15 presented higher age compared to patients at the group 0-4 (p=0.005) and this patients presented less glucose compared to patients at the group 5-9 and group 10-15 (p<0.001 and p=0.015, respectively). Patients in the 5-9 group had higher levels of the vitamin E/cholesterol and vitamin E than patients in the 0-4 group (p=0.035 and p=0.035, respectively). No statistical difference was found in relation to vitamin A. Regarding mRNA relative expression five gene were different mRNA expressed according to the severity of extent of coronary lesion. The *AREG*, *BCL2A1* and *IL18R1* increase the mRNA expression by Friesinger index categorization, higher values were observed in patients 10-15 group than 0-4 (p=0.014; p=0.035 and p=0.017, respectively). Moreover, higher values of *BCL2A1*, *BCL2L1* and *MYL4* mRNA expression was in patients 5-9 group than 0-4 (p=0.012; p=0.004 and p=0.011, respectively). In conclusion, the transcriptional profiling from whole-blood cells presented here suggests a potential use of *AREG*, *BCL2A1*, *BCL2L1*, *IL18R1* and *MYL4* as gene expression biomarkers for stages of atherosclerosis and consequently for CVD. Moreover, the involvement of vitamin E with these genes and the extent of coronary lesion open new possibilities for future studies to evaluated their applicability as biomarkers.

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Changing from solvent-based to serum-based calibration for the CDC serum 25-hydroxyvitamin D LC-MS/MS assay provides quality improvement

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During validation of a 25-hydroxyvitamin D assay, which quantitates serum concentrations of 25-hydroxyvitamin D_3 [$25(OH)D_3$], 25-hydroxyvitamin D_2 [$25(OH)D_2$], and the C3-epimer of 25-hydroxyvitamin D_3 [epi-$25(OH)D_3$] using liquid chromatography-tandem mass spectrometry, solvent and serum were shown to provide equivalent calibration matrices. However, in practice we experienced greater variation in analytical measurements (namely, slope, imprecision, bias) using solvent-based calibration. Thus, we reinvestigated using serum as the calibration matrix.

Solvent-based calibrators for $25(OH)D_3$, $25(OH)D_2$, and epi-$25(OH)D_3$ were prepared in 70% methanol-water; serum-based calibrators were prepared by spiking these analytes in a mixture of sera. The sera were selected because they provided low concentrations of these analytes. Based on 15-19 analytical runs per matrix, the precision for serum-based calibration slopes improved overall but particularly for the epi-$25(OH)D_3$ (CV from 13% to 4%) and $25(OH)D_3$ (CV from 7% to 4%) ($p \leq 0.05$). When comparing the CVs of 3 QC pools used daily, we noted smaller CV differences between the matrices within-run and larger CV differences between the matrices among-runs with serum-based calibration providing better QC precision across runs. NIST Standard Reference Materials were used to assess bias. Average percent bias for $25(OH)D_2$ and $25(OH)D_3$ were smaller for serum-based compared to solvent-based calibration ($p \leq 0.05$).

Quality improvement for this assay was realized by changing the calibration matrix from solvent to serum even though method validation showed that the matrices were interchangeable.

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Obesity and Co-morbidities among School Children in Kumasi, Ghana.

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Introduction and Objective: Obesity in children and the risk of developing diabetes mellitus and comorbidities continue to be on the increase worldwide. Research on obesity in children therefore continues to be of great public health concern, but data on its prevalence and interventionary measures are scanty in Ghana. Our objective is to do a pilot study to assess the situation for further research work. **Methods:** Eighty-five school children aged 5 to 16 years were chosen randomly from two different schools in the Kumasi Metropolis. Parental consent was sought on behalf of all children for the exercise and an experienced medical laboratory scientist and a nurse undertook the measurements. Fasting plasma glucose (FPG) was assayed by glucose oxidase spectrophotometric means. Weight and height were measured using standard equipment and body mass index (BMI) was determined as Weight (kg)/Height ^{2} (m) and calculated as percentiles. Arterial blood pressure (BP) was measured using mercury sphygmomanometer. A structured questionnaire on lifestyle of participants was administered. Analysis was conducted using IBM-SPSS® version 23. **Results:** Eight children (9.4%) were overweight, 3, (3.5%) were obese and 6, (7.1%) were underweight. Fifteen children (17.6%) had Fasting Plasma Glucose between 5.6 and 9.0 mmol/L and therefore classified as having impaired fasting glucose by American Diabetic Association criteria of 5.6mmol/L to 6.9mmol/L, 2, (2.4%) had BPs greater than 140/90 mmHg. Television viewing and lack of outdoor games had significant association on obesity ($p = 0.013$ and 0.015 respectively). There was positive linear relationship between FPG and BMI ($r = 0.23$; $p = 0.003$). **Conclusion:** Majority of the participants were "normal" by the parameters we measured, but the presence of overweight, obesity, in the presence of underweight, impaired fasting glucose, lack of physical activity by the children and absence of health promoting recreation centres for school-going children are important health issues for authorities and other stake holders as well as future researchers need to address.

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Biomarkers of fat-soluble vitamin status from NHANES 2003-2006: covariate analysis

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Monitoring the nutritional status of the U.S. population to inform public health policy is one of the key goals of the National Health and Nutrition Examination Survey (NHANES), an ongoing representative survey of the non-institutionalized, civilian population. Nutritional biomarkers (usually found in blood or urine) impart useful information about a person's recent or long term nutrient intake. Although non-dietary factors can show weak-to-moderate associations with these biomarkers, dietary intake and supplement use are usually the primary determinants of their concentrations.

The fat-soluble vitamins A and E are important to human health. The most commonly used biomarkers for these nutrients are retinol (vitamin A) and α-tocopherol (vitamin E). For each of these biomarkers, particular serum concentrations (medical decision points) are associated with risk for deficiency. Serum concentrations below these cutpoints are associated with visual loss (vitamin A) or neurological injury (vitamin E).

To develop up-to-date population reference intervals and estimates of the prevalence of persons at risk for vitamin deficiencies, biomarker concentrations of fat-soluble vitamins were measured in a representative sample as part of the NHANES conducted in 2003–2006. To further study these data, linear regression models were used to assess the association between biomarkers of fat-soluble nutrient status and sociodemographic, lifestyle, and vitamin intake in adults 20 years and older.

Through systematic modeling advancing from model 1) simple linear regression, to model 2) multiple linear regression using sociodemographic and lifestyle factors, to model 3) adding total intake of specific vitamins, we acquired novel information about the amount of variability in nutritional biomarker concentrations that is explained by these variables. All results to follow were significant at $p \leq 0.05$. For vitamin A, age (r=0.23) and alcohol intake (r=0.20) were modestly correlated with serum retinol concentration whereas for vitamin E, age (r=0.41) and smoking (r=-0.25) were moderately correlated with serum α-tocopherol. Overall, after controlling for socioecomonic and lifestyle variables (model 2), race-ethnicity was the strongest non-intake determinant for vitamin A whereas age was strongest for vitamin E.

Through additional modeling (model 3), we explored the relationships between nutritional biomarkers, diet, and supplements. Race-ethnic differences in serum biomarker concentrations were substantially attenuated after adjusting for total intake of these vitamins. Less so for serum retinol than for α-tocopherol, in the overall subpopulation of adults, total intake of preformed and pro-vitamin A sources and supplements accounted for 4% of the variability in the biomarker, whereas, for vitamin E, intake from supplements (16.8%) and total intake from foods and supplements (23.9%) accounted for substantial variability in serum α-tocopherol. Adjusting for all significant covariables accounted for 17.4% of the variability in serum retinol and 36.2% of the variability in serum α-tocopherol concentrations.

Similar systematic approaches are being applied to water-soluble vitamins and iron status indicators in NHANES 2003-2006.

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Linoleic acid-enriched diet and chronic ethanol exposure activated hepatic NLRP3 inflammasome contributing to liver inflammation and injury in mice

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Background/Aim: Chronic alcohol consumption leads to a spectrum of liver abnormalities, including fatty liver, steatohepatitis, fibrosis and cirrhosis. Fatty liver (steatosis) is the accumulation of triglycerides and other lipids in hepatocytes. Steatohepatitis is characterized by a combination of steatosis and inflammation. Inflammasome activation with subsequent release of IL-1β, a critical pro-inflammatory cytokine, is an important mechanism contributing to alcohol-induced liver inflammation. Both alcohol and dietary factors may affect hepatic inflammasome activity. The aim of the present study was to examine the effects of chronic ethanol administration and different types of dietary fatty acids on hepatic inflammasome activation in mice.

Materials and Methods: C57BL/6N male mice were fed either an unsaturated fat (USF, ω6-PUFA, linoleic acid, enriched) or a saturated fat (SF, medium chain fatty acid enriched) diets. Animals received control (SF or USF) or ethanol containing diets (SF+EtOH or USF+EtOH) for 8 weeks. Liver injury was evaluated by plasma ALT activity. Liver steatosis was assessed by liver tissue histological examination, and biochemical measurement of hepatic triglycerides. Plasma LPS levels were measured as a marker of endotoxemia. Chloroacetate esterase staining was used to evaluate necroinflammatory changes. Macrophage infiltration was determined by F4/80 staining. Hepatic pro-inflammatory cytokine and chemokine expression was assessed by qRT-PCR. Plasma IL-1β (ELISA), and hepatic NLRP3, ASC, caspase-1, and IL-1β mRNA (qRT-PCR) and protein (WB) levels were measured as markers of NLRP3 inflammasome activation.

Results: Compared to SF+EtOH, long term of USF+EtOH feeding resulted in an early stage of alcoholic liver disease characterized by hepatic steatosis with elevated hepatic triglyceride levels (64.23 ± 8.8 *vs* 93.7 ± 7.3 mg/g liver, $p<0.05$), and liver injury with increased plasma ALT levels (27.27 ± 1.9 *vs* 44.91 ± 2.8 U/L, $p<0.05$). USF+EtOH-induced liver steatosis and injury were accompanied by neutrophil and macrophage infiltration, and increased hepatic inflammation with elevated levels of pro-inflammatory cytokines, including TNF-α, MCP-1, MIP-2, PAI-1. USF+EtOH but not SF+EtOH feeding caused endotoxemia assessed by elevated LPS levels. Compared to USF feeding, USF+EtOH administration resulted in up-regulation of hepatic markers of NLRP3 inflammasome, including NLRP3, ASC, caspase-1, and IL-1β mRNA. Mice fed SF+EtOH did not reveal elevated markers of NLRP3 inflammasome activation.

Conclusion: The data demonstrate the differential effects of diverse types of dietary lipids on ethanol-mediated liver injury, and suggest that dietary USF, specifically rich in linoleic acid, may contribute to EtOH-mediated hepatic inflammation via inflammasome activation. The detailed mechanisms need to be further investigated.

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Food Intolerance (IgG-Mediated Food Sensitivity Reaction) Status of Community-based Patients

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Background: IgG-mediated food sensitivity reactions (food intolerance) may have a significant impact on patient health. Unlike the immediate reactive symptoms of food allergy, manifestations of food intolerance often appear hours or days after food consumption. These symptoms can be vague and nonspecific. Severe headache, irritable bowel syndrome, migraine, skin or respiratory conditions have been linked to food intolerance. Food consumption, as a root cause of a patient's ill health, is not always correctly identified. The relative prevalence of population (age and gender) or food-specific intolerances is not widely known.

Objective: Assess the results of food intolerance tests performed by our regional reference laboratory to characterize the patient population receiving this test and their food intolerance status as a potential diagnostic aid.

Methods: Food intolerance testing was performed using a FoodPrint® Microarray 200+ Food IgG assay. This colorimetric microarray-based ELISA quantifies IgG antibodies to *N*=222 foods in patient serum. IgG food antibodies were classified as: positive, >29 U/mL; borderline, 24 to 29 U/mL; and negative, <24 U/mL.

Results: From January 2012 to December 2016, 26479 food intolerance tests were performed. Female and male patients received 72% (*N*=19052, median age 43y, age range 1 to 98y) and 28% (*N*=7427, median age 41y, age range 0 to 105y) of these tests, respectively. Patient age distribution was bimodal with 8y (*N*=182) and 42y (*N*=657) having the highest testing frequencies. From January-December 2016, the relative rates of positive, borderline and negative results were 11.5%, 3.3% and 85.1%, respectively, with no difference noted by gender. There was a negative correlation between patient age and IgG food antibody positivity rates or general food intolerance. Four rates of intolerance were noted for food items: level 1, frequently intolerant foods, were positive in >33% of individuals and consisted of 23 foods, including wheat; egg white; milk (cow); pea; milk (sheep); cola nut; yeast (Brewer's); pistachio; casein; bean (red kidney); barley; milk (goat); agar agar; corn (Maize); bean (white haricot); almond; hazelnut; sunflower seed; cashew nut; gliadin; brazil nut; aloe vera; and peanut; level 2, commonly intolerant foods, were positive in 10-33% of individuals and consisted of 34 foods; level 3, infrequently intolerant foods, were positive in 1-9% of individuals and consisted of 82 foods; and level 4, tolerant foods, were positive in <1% of individuals and consisted of 83 foods. The most intolerant foods were identical for females and males, with positivity rates ranging from 38-93%.

Conclusion:

Food intolerance is widely observed in our patient population. Foods with relatively higher rates of intolerance include: grains, especially those containing higher gluten content; dairy and egg; vegetables with high protein content (pea and beans); nuts and seeds; and plants with high polysaccharide content (agar agar or aloe vera).

Leafy vegetables, fruits, seafood and meats had relatively lower levels of intolerance. Physicians may use this population-based information with the frequency of a patient's food consumption to evaluate potential IgG-mediated food sensitivity reactions.

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Vitamin D metabolite metrology at NIST: Past, present and future

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Vitamin D is a nutrient essential for bone health; however, vitamin D deficiency/insufficiency is estimated to exist within 10% of the US population. Routine blood tests to monitor vitamin D can give inconsistent or even inaccurate results, complicating diagnosis and treatment of vitamin D deficiency. This variability calls into question the ability of vitamin D assays to accurately identify individuals affected by vitamin D deficiency. National Institute of Standards and Technology (NIST), the National Institute of Health Office of Dietary Supplements (NIH-ODS) and the Centers for Disease Control and Prevention (CDC) entered into a collaboration - and as part of a broader Vitamin D Standardization Program (VDSP) - with a goal of standardizing laboratory measurements of vitamin D metabolites to ensure comparability in national health surveys. Standardization ensures accurate and consistent detection and treatment of vitamin D deficiency, regardless of location or laboratory procedure. As a consequence of these efforts by NIST and its partners, confidence in serum-based measurements of vitamin D metabolites has been improved. These accomplishments lower health care costs by reducing the need for retesting, and provide greater certainty in the data collected in national health surveys to support clinical and policy decision making. Since 2010, NIST has provided metrological support for these critical vitamin D metabolite measurements through: 1) the development of Standard Reference Materials (SRMs) for use as primary calibration materials and quality control samples; 2) the development of Joint Committee for Traceability in Laboratory Medicine (JCTLM)-recognized higher-order Reference Measurement Procedures for the measurement of vitamin D metabolites in clinical samples; and 3) administration of a quality assurance program for vitamin D metabolites. Furthermore, team members have provided critical measurements for test samples used in VDSP efforts, and Vitamin D External Quality Assessment Scheme (DEQAS), a PT program administered in the UK, with samples distributed quarterly to 1200 international participants. This effort has explicitly allowed DEQAS to move to an accuracy-based program for vitamin D assessments. The Chemical Sciences Division of NIST has established "Best in the World" capabilities for the determination of vitamin D metabolites in human serum. Much of this service to the clinical community has been delivered in the form of SRMs, intended for use in providing SI traceability and calibration, as well as method validation of these critical measurements. To date, the re-issued SRM 972a Vitamin D Metabolites in Human Serum has sold over 1250 units to hundreds of clinical diagnostics and health monitoring laboratories, both nationally and internationally. Other SRMs include SRM 2972a 25-Hydroxyvitamin D2 and D3 Calibration Solutions, and SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level). New isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC/MS/MS) methods for the determination of emergent metabolites of vitamin D status, such as 24R,25-dihydroxyvitamin D3, have been more recently developed. This measurement capability is being applied to the evaluation of patient samples for DEQAS samples, and to the development of new calibration solution and serum-based SRMs. Candidate ID-LC-MS/MS methods for other metabolites, such as 1,25-dihydroxyvitamin D3, are also currently under investigation.

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Utilization of vitamin B_{12} and folate tests in a major reference laboratory suggests a lack of evidence-based ordering practices by community physicians

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Objective: To assess the evidence-based utilization of vitamin B_{12} and folate testing in a large reference laboratory.

Introduction: Vitamin B_{12} and folate are important enzyme cofactors that play key roles in the utilization of methyl-groups central to DNA synthesis and multiple metabolic reactions. Megaloblastic anemia is a cardinal feature of both vitamin B_{12} and folate deficiency. Clinically, low levels of vitamin B_{12} are associated with decreases in energy and neurological effects that include tingling and numbness of extremities, abnormalities in walking, and cognitive changes. At-risk populations include vegetarians; the elderly; patients with intestinal malabsorption, gastric or intestinal surgery; and those with pernicious anemia. In pregnancy, folate deficiency can lead to neural tube defects and at-risk populations include those with dietary insufficiency and malabsorption syndromes. In our laboratory, tests for vitamin B_{12} and folate are ordered at very high numbers by physicians (>240,000 tests per year), despite the fact that deficiency is extremely rare due to fortification of food and the availability of these vitamins in the diet.

Methods: De-identified patient results from venous blood samples for the period of December 30, 2014 to June 30, 2016 were obtained from the Laboratory Information System. Specific test results queried included those for vitamin B_{12}, folate, and mean corpuscular volume (MCV). Additional information collected included age, collection location, and ordering physician speciality. The data was analyzed in Excel v.14.0.

Results: In the time period, our institution performed 332,644 vitamin B_{12} measurements. Of these, 4.0% were below and 10.1% were above our current reference interval (RI; 155-700pmol/L). 90.5% of all vitamin B_{12} measurements had a matched MCV, of which only 2.8% indicated macrocytosis (MCV >100fL); 0.1 % of vitamin B_{12} measurements below the RI had an associated macrocytosis. During this period, 29,551 serum folate measurements were performed. Of these, all but two (*N*=29,549) had a matched vitamin B_{12} measurement. Of the total folate tests performed, 2.0% were deficient (RI >12.0nmol/L). Analogous to that seen with vitamin B_{12}, 83.7% of folate test orders had a matched order for MCV. Although only 0.36 % of all folate results were associated with a macrocytosis, 18.3 % of patients with deficient levels of folate had a macrocytosis. In our study, 0.3% of patients with vitamin B_{12} results below the RI had an associated deficiency in folate. Only 0.1 % (*N*=7) were deficient in both vitamin B_{12} and folate and had a macrocytosis. Finally, greater than 80% of the above tests were ordered by community physicians.

Conclusions: In our institution, greater than 85% of vitamin B_{12} and folate tests ordered by community physicians are normal and do not add any value to the overall diagnosis and should be eliminated.

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Conundrums of Testing Algorithms for Vitamin B12 Deficiency

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Objective: Testing algorithms are essential tools for test utilization management to reduce unnecessary testing and provide high-quality, cost-effective patient care. With the intention to implement vitamin B12 testing utilization management, we found various algorithm recommendations. These algorithms differ in the threshold value of vitamin B12 to initiate reflex testing and more importantly, in whether to include homocysteine as a follow-up test. When homocysteine is included, these algorithms also differ in defining vitamin B12 deficiency as having elevations in both methylmalonic acid (MMA) and homocysteine, versus in either MMA or homocysteine.

Methods: We evaluated the testing algorithms performance for our inpatient population and to determine the best testing strategy for our population. From January 2016 – December 2016, we analyzed 914 vitamin B12 tests (reference range: 180-914 pg/mL) that were ordered concurrently with MMA (reference range: 0-378 nmol/L) and homocysteine (reference range: 6.6-17.5 µmol/L) at our medical center.

Results: The mean vitamin B12 concentration was 468.6 ± 280.6 pg/mL and the median age of the patients was 68 (male: 92%; female: 8%). Stratified serum vitamin B12 concentrations were assessed for percentage of elevated MMA (>400 nmol/L) and/or homocysteine concentrations (>18 µmol/L). We found that 4% of the patients with a serum vitamin B12 above 914 pg/mL have elevated MMA concentrations. This percentage remains the same in patient with a serum vitamin B12 above 400 pg/mL, but increases as vitamin B12 concentration decreases. This supports the concept that a serum concentration above 400 pg/mL represents vitamin B12 replete status in this population. Importantly, we also found that in the equivocal range of vitamin B12 concentrations from 150-400 pg/mL, 28% of patients have elevated homocysteine concentrations, 13% have elevated MMA concentrations, but only 2% have both elevated homocysteine and MMA concentrations. Because the percentage of patients with elevated homocysteine is twice as high as those with elevated MMA concentrations, we further investigated whether the patients with elevated homocysteine but normal MMA concentrations represent true vitamin B12 deficiency by reviewing hematologic indices (red blood cell count, hemoglobin and MCV), neurologic symptoms including cognitive and mood changes, peripheral neuropathies, and whether abnormalities can be reversed by vitamin B12 supplement. Interestingly, only 10% of the these patients may represent true vitamin B12 deficiency, while over

50% of these patients have conditions including folate deficiency, renal deficiency, alcohol abuse or use of medications such as fibrates and hydrochlorothiazide that can elevate homocysteine concentrations.

Conclusion: Our data suggest that for evaluating vitamin B12 deficiency, homocysteine should be used in patients with equivocal vitamin B12 results, but normal MMA concentrations, and when all other compounding factors that can increase homocysteine levels can be excluded. Furthermore, each institution needs to analyze data from their own patient population before implementing testing algorithms.