Wednesday, August 1, 2018

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

Nutrition/Trace Metals/Vitamins

B-295

Serum Level of Some Micronutrients as a Biomarker of Immunity in Antiretroviral-naïve HIV-infected Individuals

T. A. Adedeji[1], A. A. Akande[2], N. O. Adedeji[3], M. B. Fawale[1], B. A. Okesina[2], S. A. Adebisi[4], O. A. Jeje[3], A. K. Alaje[1], A. O. Ajose[1]. *[1]Obafemi Awolowo University, Ile-Ife, Nigeria, [2]University of Ilorin, Ilorin, Nigeria, [3]Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria, [4]Benue State University, Makurdi, Nigeria*

Background: HIV infection may lead to micronutrient deficiencies. These micronutrient deficiencies may affect the risk of having HIV/AIDS and low $CD4^+$ count level in the patients which is a measure of their level of immunity. This study was to correlate the $CD4^+$ count in antiretroviral-naïve patients with the serum levels of some micronutrients as measures of relationship between immunity and nutrition/malnutrition. **Method:** Ninety consecutive newly diagnosed HIV/AIDS patients (age 18-59 years) attending the clinics were recruited for this descriptive cross sectional study. Ninety apparently healthy, screened blood donors were enrolled for comparison. Blood samples were collected from the control subjects and the patients before HAART treatment and were assayed for serum zinc, selenium, copper, manganese and magnesium using atomic absorption spectrophotometer (AAS). Vitamin B_{12} level was measured using high performance liquid chromatography (HPLC). **Results:** Mean serum vitamin B_{12} was significantly higher in the study participants than the controls (304.59±86.31 pmol/l and 279.77±81.58 pmol/l respectively, p=0.036); while the participants had significantly lower mean serum zinc (14.25±2.93 µmol/l and 14.58±3.69 µmol/l respectively, p= 0.493), significantly lower selenium (0.38±0.08 µmol/l versus 0.78±0.22 µmol/l, p <0.001), manganese (7.06±0.87 µmol/l versus 11.23±3.27 µmol/l, p <0.00), and magnesium (1.02±0.21 mmol/l versus 1.21±0.28 mmol/l, p <0.001) values when compared with the controls. Mean concentration of copper is similar in both participants and controls (18.88±3.1 µmol/l and 18.82±5.12 µmol/l, p= 0.921). There was no correlation between the micronutrients levels and $CD4^+$ count, however, there are strong positive correlations between the serum levels of zinc and copper; zinc, selenium and magnesium; zinc and selenium; copper and magnesium (p values, <0.001 respectively). Multivariate regression analyses among variables with significant correlations showed that all micronutrients were independent predictors of one another (p values of <0.001). Significant positive correlation exists between duration of HIV infection and vitamin B_{12}. There were significant differences in the mean values of vitamin B_{12} among the WHO categories of three degrees of immunosuppression (based on $CD4^+$ count; Anova, p=0.047). The highest concentration was in those with mild immunosuppression, CDC category 1($CD4^+$ ≥500 cells/µl; vitamin B_{12}: 324.1±89.9 pmol/l); declining in those with moderate immunosuppressive state, category 2 ($CD4^+$ 200 – 500 cells/µl; vitamin B_{12}: 317.9±74.5 pmol/l, with multiple comparison (post hoc) analysis p=0.047 between category 1 and 2), and lowest in severe immunosuppression, category 3 ($CD4^+$ < 200 cells/µl; vitamin B_{12}: 274.1±88.2 pmol/l, with multiple comparison (post hoc) analysis p=0.029 between category 2 and 3). The mean difference in vitamin B_{12} between moderate- and severe immunosuppressive state was greater than the mean difference between mild- and moderate immunosuppression. **Conclusion:** HIV/AIDS results in depletion of serum micronutrients with strong positive correlations between their serum levels. Although serum levels of micronutrients may not be qualified as direct markers or surrogates for $CD4^+$ count in antiretroviral-naïve HIV-infected patients, serum vitamin B_{12} could differentiate between mild- to moderate and severe immunosuppressive states; inverse relationship exists between serum levels of vitamin B_{12} and HIV/AIDS disease severity and progression.

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Impact of new values for vitamin D in Brazil assessed in sampling of a reference laboratory

L. G. S. ASSUNÇÃO, B. S. Moura, M. D. Farace, L. Moutinho. *LAB REDE, BELO HORIZONTE, Brazil*

Background: In Brazil, hypovitaminosis D has been documented in several regions of the country, which justified a critical analysis of diagnostic criteria by a committee of specialists from the Department of Bone Metabolism of the Brazilian Society of Endocrinology and Metabolism (SBEM) and the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML) for the development of recommendations based on scientific evidence available from current literature on vitamin D. The main change was the adoption of the vitamin D sufficiency criterion to values above 20ng/mL for healthy individuals aged 19 to 60 years versus the previous 30ng/mL. Current criteria consider sufficiency between 30ng/mL to 60ng/mL for risk groups and aged above 60. The study aims to analyze the difference between the adopted criteria, the behavior and the impact in this sampling. **Methods:** Data from 231,830 vitamin D analyses were obtained in adults aged 18-90 years from the southeast region of the country, performed at a reference laboratory, on Abbott-Architect platform, for a period of one year (September 2016 to 2017). Sampling was categorized according to gender, age and ranges of values used for vitamin D in clinical evaluation. The results were subject to a descriptive statistical evaluation and the Mann-Whitney and Kappa tests were applied to estimate the agreement between the previously adopted and current ranges of vitamin D suitability. A significance level of 5% (p <0.05) was adopted in the statistical software SPSS®, version 19.0 and STATA®. **Results**: Sampling is very homogeneous, but non-Gaussian. The mean age of this population was 52,93 years old; the median and the mean ± SD of the vitamin D concentration was 29.65ng/mL and 28.60 ± 10.81 ng/mL, respectively. It was observed that the majority were female (76.91%) and the predominant age group was between 19 and 59 years old (62.36%). The parameters of adequacy for the current levels of vitamin D were evaluated in frequency and percentage, obtaining the following: Insufficient or deficient (<20ng/mL) 10.2% [n=23647]; Sufficient (20 to 29ng/mL) 43.2% [n=100150]; Sufficient and recommended for adults above 60 years (> or = 30ng/mL) 46.6% [n=108033]. The concentration of vitamin D in females vs. male was lower, 28.20 ng/mL vs. 28.40 ng/mL (p = 0.003). Similarly, among individuals aged 19-59 years old the dosage was also lower, 28.10 ng/mL, when compared to the elderly, 28.60 ng/mL (p <0.001). It was observed that there is a low agreement (0.210) between the two classifications, with statistically significant values (p <0.001). **Conclusions:** It is well established that the parameters diverge as for the current and the previously established ranges. We did not analyze clinical criteria or use of replacement medication, which may have an effect, especially in the elderly population data. However, there is a significant difference, in the age group above 60 years old, higher levels as predicted by the current sufficiency criterion. The study provides the impact of this transition of criteria regarding the clinical decision, notably in the southeast region of Brazil, since previously 53.4% of the dosages would be insufficient, contrasting with the current 10.2%.

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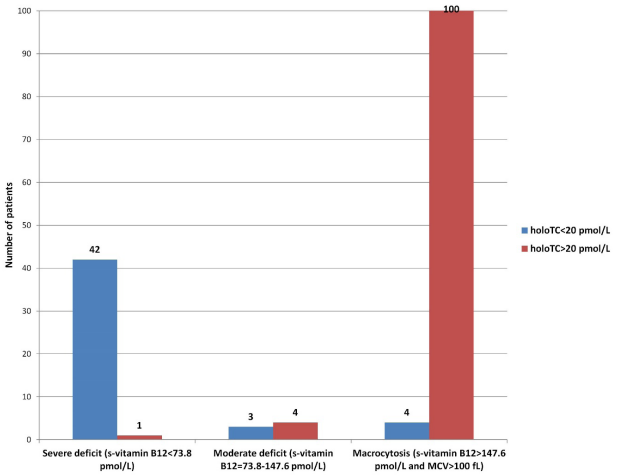
Holotranscobalamin still in debate.

M. Salinas[1], C. Hernando de Larramendi[2], E. Flores[1], M. Lopez-Garrigos[1], C. Leiva-Salinas[3]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]Fundación Ignacio Larramendi, Madrid, Spain, [3]University of Missouri Health, Columbia, MO*

Background: The diagnosis of vitamin B12 deficit is still a challenge for the clinical laboratory since serum vitamin B12 assay (s-vitamin B12) is not standardized. Methylmalonic acid (MMA) and total homocysteine (HCt), the ultimate tests to confirm deficiency, are expensive and not available in daily practice. Holotranscobalamin (HoloTC) might be a more reliable indicator of intracellular vitamin B12 status than s-vitamin B12. The aim was to study holoTC in three groups of primary care patients classified according to s-vitamin B12 and MCV values. **Methods:** The Laboratory located in a Community University Hospital covers a Health Department of 216210 inhabitants. Participants were primary care patients classified into two groups depending on s-vitamin B12<73.8 pmol/L, between 73.8 and 147.6 pmol/L, named "severe" and "moderate vitamin B12 deficit", and a third group with MCV>100 fL and s-vitamin >147.6 pmol/L, named "macrocytosis group". S-vitamin B12 was measured through immunoassay on Modular E170 (Roche Diagnostics, Switzerland), and holoTC by the Abbott Architect Assay (Abbott, USA), with a deficiency cut off of 20 pmol/L. Each patient sample was tested for s-vitamin B12 and holoTC and results were compared.

Results: There were 43 and 7 patients with severe and moderate deficit, respectively; and 104 in the macrocytosis cohort. Among the 43 patients with severe vitamin B12 deficit, 42 had holoTC deficiency, showing 97.7% of coincidence. Only 3 had low holoTC values in the moderate group, with 42.8% coincidence. Among the 104 patients with macrocytosis, only 4 (3.8%) had low holoTC (Figure). **Conclusion:** The high concordance between serum vitamin B12 and holoTC in severe vitamin B12 deficiency and macrocytosis groups shows that any of those markers might be appropriate when dealing with severe deficiency and macrocytosis with non-pathological s-vitamin B12 values. However in moderate deficiency, the use of additional MMA or HCt might be still necessary.



B-298

Method comparison of two different Vitamin D immunoassays by assessing Chinese serum samples

J. Chen, S. Song, C. Yun, L. Yang. *National Institute of Nutrition and Health, Chinese Center for Disease Control and Prevention, Beijing, China*

Background: Total 25-hydroxyvitamin D [25(OH)D] is the most reliable indicator of vitamin D status. Radioimmunoassay kit was used in many large-scale surveys, like China National Nutrition and Health Survey (CNNHS) 2010-2012. On the other hand, enzyme immunoassay kit was widely applied in many researches as well. The application of different analyzing methods brought difficulty for cross comparison of researches. To evaluate the difference between radioimmunoassay and enzyme immunoassay, we compared the most widely used enzyme immunoassay kit and radioimmunoassay kit through assessment of human serum samples from the Chinese population in this study. **Methods:** In total, 653 Chinese serum samples were tested using the IDS 25-Hydroxy Vitamin D EIA kits and DiaSorin 25-Hydroxyvitamin D RIA kits. **Results:** Spearman's rank correlation coefficient (rho) between DiaSorin-RIA and IDS-EIA was 0.7509. The Cusum test showed no significant deviation from linearity for DiaSorin-RIA vs IDS-EIA (P = NS). The Bland-Altman plots were constructed for all evaluated samples to determine the between-assay bias. The 25(OH)D concentrations obtained by IDS-EIA were lower on average (mean bias, -3.2 ng/ml, -6.4%) than the results of DiaSorin-RIA. The weighted kappa values was 0.527 in the assessment of vitamin D deficiency (<20 ng/mL). **Conclusion:** The results indicated acceptable correlation between DiaSorin-RIA and IDS-EIA. They can be recommended for routine use in the Chinese population.

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Levels of Trace Elements and Their Binding Proteins in the Blood of Rheumatoid Arthritis Patients in Saudi Arabia

S. H. Sobki. *PSMMC, Riyadh, Saudi Arabia*

Background: Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory joint disorder affecting about 1% of the world population. The severity of this disease may vary from mild arthralgias to destructive erosive disease involving all joint of upper and lower extremities. The pathogenesis of RA is not clearly understood. Several studies have shown altered levels of trace elements in the blood of RA patients as well as their deposition in synovial membranes. **Aim**: This study was aimed to determine the levels of serum iron, copper and zinc; and their binding proteins, hemoglobin, ferritin and albumin of RA patients in a tertiary care referral hospital in Saudi Arabia. **Method**: The RA patients were graded into 3 categories (mild, moderate and severe) using the standard procedures. The study included 34 patients and 17 matched controls. Clinical data and medical history were recorded for all subjects. Blood samples were collected from all subjects and serum concentration of trace metals, zinc, copper were analyzed by Atomic Absorption Spectrophotometry. Iron, ferritin, and albumin were analyzed on Roche Cobas system. Hemoglobin was measured on Sysmex XN system. **Results**: Our results showed a significant decrease (P<0.01) in serum iron (5.11±1.25 vs 11.35±1.25 umol/l) and zinc (10.62±0.95 vs 13.50+0.56 umol/l) levels in RA patients of severe category versus normal subjects. On the other hand serum copper was found to be significantly higher in RA patients (21.59±0.88 umol/l) as compared to controls (17.13±0.87 umol/l). Among the proteins, the level of serum ferritin was significantly higher (P<0.001) whereas the levels of hemoglobin and albumin were significantly lower (P<0.05 and P<0.01 respectively) in RA patients. The regression analysis about the levels of trace elements and proteins with the severity index of RA showed the following correlation coefficients: serum iron (0.35), copper (0.33), zinc (-0.42), ferritin (0.53) , hemoglobin (-0.37) and albumin (-0.66). **Conclusion**: These findings clearly indicated significant alterations in the levels of trace elements and proteins in the blood of RA patients. Low levels of iron and zinc and elevated levels of copper appeared to be associated with the risk of RA. The role of trace elements can be investigated further in the pathogenesis of RA and to test the efficacy of chelation/supplementation therapy for the treatment of RA.

B-300

Development of a New Biochip Based Immunoassay for the Measurement of Total 25-hydroxyvitamin D in Serum and the Accurate Classification of Vitamin D Status

T. McConnell, S. Marshall, D. Vance, R. I. McConnell, S. P. FitzGerald. *Randox Laboratories Ltd, Crumlin, United Kingdom*

Background: 25hydroxyvitamin D [25(OH)D] concentration is directly related to the storage of vitamin D in the body and is the most widely used indicator of vitamin D status. Accurate measurement of 25(OH)D has well documented clinical implications for the diagnosis of vitamin D deficiency associated with musculoskeletal health and severe liver and kidney disease. More recent studies have shown that Vitamin D deficiency also has important implications in chronic illnesses including cancer and cardiovascular diseases. As a result of the emerging agreement on the implication of severe vitamin D deficiency on people 's health, there is an increasing need to assess vitamin D status in individual patients. The aim of this study was to evaluate a new biochip based immunoassay for the measurement of total 25(OH)D in serum on the fully automated, Evidence Evolution analyser. The immunoassay results were compared with a LC-MS/MS method, traceable to NIST Standard Reference Material STM 972 **Methods:** A direct competitive chemiluminescent immunoassay on a biochip platform and applied to the fully automated Evidence Evolution was utilized. Assay sensitivity was determined as functional sensitivity in accordance with Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A2. Repeatability was determined following CLSI protocol EP05-A3: 2 runs per day in duplication for 20 days (n=80). A method comparison study was conducted by analysing 20 serum samples from healthy individuals using the biochip based immunoassay, a LCMS/MS method and another commercially available immunoassay platform, the results were expressed as a % agreement, after being classified against established guidelines for each system. **Results:** The functional sensitivity evaluation showed sensitivity of 13.00 nmol/L. Repeatability was expressed as CV (%) for samples at the following concentrations; 45.5, 52.1, 76.7 nmol/L and was 12.8%, 10.3% and 12.9% respectively. In terms of the clinical classification of patient samples, the Evidence Evolution platform and LCMS/MS agreed on the classification of 20 samples out of 20 (100% agreement). The other commercially available immunoassay platform and LCMS/MS had an agreement on the classification of just 1 sample (5% agreement). **Conclusion:** The results show that this new biochip based immunoassay for the determination of 25(OH)D in serum and applied to the fully automated Evidence Evolution, presents optimal analytical performance and compares very favourably with LCMS/MS (100% agreement) in the classification of vitamin D status of serum samples. The Evidence Evolution is a high throughput analyser with random access and STAT capabilities. This platform is therefore a valuable and reliable analytical tool for the measurement of 25(OH)D.

B-301

Assessing diagnostic accuracy of serum Holotranscobalamine (Active-B12) in comparison with other markers of vitaminB12 deficiency.

J. D. Bondu. *Christian Medical Collge, vellore, India*

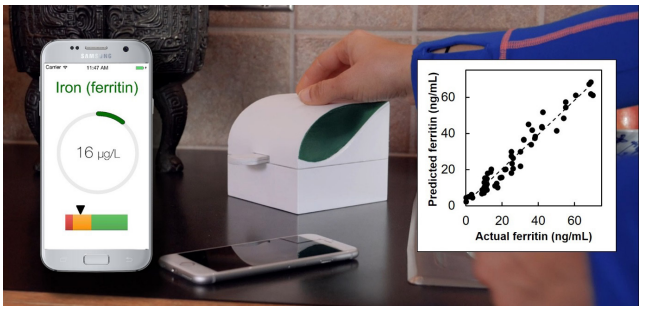
Background: Vitamin B12 deficiency has a prevalence of 15 - 40% in India and serum total vitaminB12 is the most commonly used test in its diagnosis. Total vitamin B12 exists in two bound forms: (i) bound to haptocorrin to form holohaptocorrin (70%-80%), (ii) bound to transcobalamin to form Holotranscobalamine (holoTC) (20%-30%). Body cells can only take up vitamin B12 in the form of holoTC. Therefore, measuring holoTC is more reflective of vitamin B12 status than measuring total vitamin B12 only. **Aim:** To assess the diagnostic accuracy of serum holoTC in comparison with total Vitamin B12 and total Homocystine as indicator of serum Vitamin B12 status. **Materials and methods:** 217 human subjects were assessed of which 99 were males and 118 were females ranging from 17 to 83 years of age. They were divided into 3 groups: (i) Vitamin B12 deficient (n= 70) with total vitamin B12 levels <200 pg/ml, (ii) borderline deficient (n=100) with levels 200 -350 pg/ml and (iii) sufficient group (n=47) with normal levels (>350 pg/ml). Markers of Vitamin B12 deficiency assessed were serum active vitamin B12, total Homocystine, Mean Corpuscular Volume (MCV), Folate, heamoglobin and creatinine for renal function. The samples were analysed using Siemens Advia Centaur Xpi. **Results**: Among the deficient group (n=70) most cases (14.3%) had low total vitamin B12 levels, low active Vitamin B12 but normal homocystine levels. 12.8% had low total B12 but normal active vitamin B12 levels and normal homocystine. 4 cases (5.7%) had low total vitamin B12, normal active vitamin B12 and elevated homocystine. However, among borderline deficient group (n=100) 14% had borderline total vitamin B12, low active vitamin B12 and elevated homocystine. While most cases (17%) had borderline total vitamin B12, low active vitamin B12 and normal homocystine. 15% had borderline total vitamin B12, normal active vitamin B12 and elevated homocystine . Also among the normal group (n= 47) only one case had low active vitamin B12 levels. Additionally, elevated MCV levels were found in 15.7% of the deficient group and 8.3% in the borderline group. **Conclusion:** Our study findings suggest that using combination tests of total B12, active B12 and plasma homocystine is required for improving diagnostic accuracy of Vitamin B12 deficiency.

B-302

VitaScan: Fast, portable, and low cost nutrition deficiency test

L. Jiang, D. O'Dell. *VitaScan, Ithaca, NY*

Background: Malnutrition is one of the most significant challenges in global health. For example, 2 billion people in the world are anemic, which is largely caused by iron deficiency and contributes to 20% of all maternal deaths. Existing bottlenecks include the limited access to tests and the inability to monitor people's health and provide feedback. Mobile diagnostic technologies largely have not reached technical requirements in speed, precision, and cost to viably address this problem. We present the solution through our rapid, low cost, and portable platform for quantifying nutrition biomarkers. Our initial focus is iron deficiency, and here we demonstrate strong analytical agreement between laboratory results and our ferritin assay, which is the strongest indicator of a person's iron status. **Methods:** Our platform technology incorporates simple sample processing, unique lateral flow design, and novel test imaging and analysis to enable quantitative determination of nutrition biomarkers at picomolar concentrations from a finger stick of blood. We demonstrate this with our disposable ferritin assay. A 40µL drop of finger stick blood is dispensed onto the assay, followed by running buffer. Optical contrast agents in the assay produce test and control lines at signal intensities relative to the native ferritin concentration in the blood. The assay cartridge is inserted into our dedicated reader platform, which analyzes the test and control lines to produce a quantitative result in under 10 minutes. **Results:** Our ferritin assay was tested against the Siemens Immulite ferritin lab test on 55 human samples. Samples showed linearity across the entire range of tested concentrations from 0 to 70 µg/L, producing an R^2 value of 0.95 and CV of 0.15. **Conclusion:** Our ferritin assay has demonstrated accuracy compared to laboratory instrumentation while showing superiority in portability, cost, and ease of use.



B-303

Measurements of blood trace elements in patients undergoing online hemodiafiltration

M. Ji[1], E. Bae[1], H. Kim[1], W. Lee[2], S. Chun[2], Y. Choi[1], W. Min[2]. *[1]VHS Medical Center, Seoul, Korea, Republic of, [2]University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea, Republic of*

Background: Essential trace elements play key roles in multiple biological systems, and hemodialysis patients are at risk for deficiency of essential trace elements. Blood levels of trace elements were previously measured in patients undergoing conventional hemodialysis, but there has been no data about those with online hemodiafiltration (online HDF). The aim of the study was to measure the serum concentration of copper (Cu), zinc (Zn), selenium (Se), and manganese (Mn) in patients with end-stage renal disease undergoing online HDF in outpatient dialysis clinic. Methods: A total of 28 patients (mean age 70.2 years, male 89%) were included. All were Korean. Blood samples were collected before and after one hemodialysis session, and serum concentrations of 4 trace elements were simultaneously measured by inductively coupled plasma mass spectrometry (PerkinElmer NexION 350D ICP-MS). Patients' clinical and recent laboratory data were also collected from medical records. Results: After the hemodialysis session, concentrations of all trace elements were significantly increased than the pre-hemodialysis levels. Mean post-hemodialysis levels were increased by 13.4% for Zn, 10.1% for Cu, and 8.1% for Se than pre-hemodialysis levels. Mn remarkably increased after session in 7 patients, and the remaining patients showed mean increase of 0.46 µg/L. Se and Zn deficiency were observed in 71% and 36% of the study participants with median 83.6 µg/L (reference range, 93-150 µg/L) and 70.8 µg/dL (66-110 µg/dL). Conclusion: The preliminary data suggest that the patients undergoing online HDF are also at increased risk of trace elements deficiency, especially for Se.

B-304

A quantitative method for magnesium measurement in red blood cells by ICP-MS

Y. Zheng, W. Cieslak, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Magnesium is a mineral that is important for bone development, muscle contraction, nerve function and energy production. It is the fourth most abundant cation in the body with total content of about 25 g. The majority of magnesium are stored in the bone and inside cells with only ~ 1 % is present extracellular within the body; therefore, the measurement of plasma/serum magnesium concentrations correlates poorly with total body magnesium level. Hence, we developed an ICP-MS assay to accurately measure the intracellular magnesium level in the red blood cells. **Methods:** This method was developed on a Thermo Fisher X Series 2 ICP-MS with a collision/reaction cell controlled by PlasmaLab (v. 2.6.2.337). Scandium was used as the internal standard, and the total assay time is 1 min. The calibration standards were prepared at 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 mg/dL in 0.5 % nitric acid. Whole blood was first spin down for 5 min at 4000 rpm. The collected red blood cells were then diluted 1:100 with 0.5 % nitric acid and directly injected to MS for analysis. **Results:** The use of 0.5 % nitric acid as the artificial matrix for building calibration curve was verified in 10 individual patient samples through a mixing study. No interference was observed from exogenous compounds such as carbon (20,000 µg/dL), iron (25,000 µg/dL), sodium (65,000 µg/dL), calcium (30,000 µg/dL), chloride (200,000 µg/dL) and MRI contract mix (10,000 µg/dL of iodide, gadolinium and barium). Analytical measuring range was determined to be between 0.7 mg/dL to 29.3 mg/dL with analytical recovery ranging from 99.5 % to 118.8 %. No significant carryover was observed from a sample at 40.0 mg/dL. CV for total precision and intra-assay preci-

sion was between 3.1 % to 4.2 % and 1.5 % to 3.5 %, respectively at three concentration levels of 3.2 mg/dL, 4.6 mg/dL and 6.0 mg/dL (n = 30 each). This method was compared to another ICP-MS method offered at an independent lab using 44 patient samples with a concentration range of 3.0-6.6 mg/dL; and the Deming regression showed R of 0.8837, slope of 0.937, intercept of 0.35 and overall bias of 1.55 %. RBC magnesium was stable for 1 week at ambient, 1 month at refrigerated and unstable at frozen. Whole blood samples should be separated from cells within 4 h. **Conclusion:** This new ICP-MS method for quantifying magnesium in red blood cells is highly sensitive and accurate. It has been validated for clinical use.

B-305

Iodine- and Gadolinium-based Magnetic Resonance Imaging Contrast Agents Interfere with Inductively Coupled Plasma Tandem Mass Spectrometry-based Trace Element Testing

H. Boswell[1], K. Bernardin[1], H. Li[2], D. Bailey[1], P. Catomeris[2], A. S. Ptolemy[1]. *[1]Dynacare, London, ON, Canada, [2]Dynacare, Brampton, ON, Canada*

Background: Gadolinium (Gd)- and iodine (I)-based magnetic resonance imaging (MRI) contrasting agents have been reported to interfere with the inductively coupled plasma mass spectrometry-based (ICP-MS) quantitation of trace elements in human matrices. However, limited comprehensive literature describing the effect of these agents on the inductively coupled plasma tandem mass spectrometry-based (ICP-MS/MS) quantitative testing of plasma and urine trace elements is available. ***Objective:*** Evaluate potential interference from the MRI contrast agents: gadobutrol (Gadovist™); gadodiamide (Omniscan™); iohexol (Omnipaque™); ioversol (Optiray™); and iodixanol (Visipaque™) on plasma and urine trace element quantitation by ICP-MS/MS. ***Methods:*** Plasma and urine specimen pools were respectively created from *N*=10 specimens. *N*=5 unique aliquots of each matrix pool were then created with subsequent aliquots respectively spiked with 0, 0.05, 0.1, 0.5, 1, 5, 10, 15 and 20 µg·mL^{-1} of an individual MRI contrast agent. Prior to their introduction to an Agilent 8800 ICP-MS/MS, all plasma and urine samples were diluted in a solution containing nitric acid and ethanol. Aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iodine (I), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), strontium (Sr), thallium (Tl), tin (Sn), uranium (Ur), vanadium (V) and zinc (Zn) were quantified in both plasma and urine. All specimens were analyzed in duplicate. Interference from the MRI contract agent was considered significant if the percentage difference between the experimental and expected trace element concentration exceeded the tests defined total allowable error at any of the tested MRI contrast agent concentrations. ***Results:*** Iodine contamination of the ICP-MS/MS instrument occurred following the analysis of any specimen with an iohexol, ioversol or iodixanol concentration ≥5 µg·mL^{-1}. Maintenance was required to remove this contaminant and eliminate the high iodine background ion count within this ICP-MS/MS system. MRI contrast agents causing significant interference with plasma trace elements: gadobutrol (Al, I and Hg); gadodiamide (Al, Be, B, Co, Cu, Hg, Mo and Zn); iohexol (Cr, I and Sn); ioversol (B, Co, Cu, I, Mo, Sn, V and Zn); and iodixanol (As, Co, Cu, I, Sn and V). MRI contrast agents causing significant interference with urine trace elements: gadobutrol (Al, As, Co, Cu, Fe, Pb, Mn, Hg, Ni, Se, Tl and Zn); gadodiamide (Co, Cu, Fe, Pb, Mn, Hg, Ni, Se, Tl and Zn); iohexol (Al, I and Tl); ioversol (Al, I and Tl); and iodixanol (Al, As and I). All other plasma and urine trace element tests were not interfered with by the MRI contrast agents at the concentrations tested. ***Conclusions:*** Plasma and urine specimens containing iohexol, ioversol or iodixanol can introduce significant iodine contamination to an ICP-MS/MS system. If I- and Gd-based MRI contrast agents are administered to a patient, plasma and urine specimen collection for trace element testing should be delayed due to the potential for significant analytical interference with multiple analytes.

B-306

Urine cadmium analysis - Is molybdenum oxide a concern?

C. Tan, W. Ng, C. Yeo. *Singapore General Hospital, Singapore, Singapore*

Introduction:
Cadmium is a toxic and carcinogenic metal associated with increased risk of cancer and cardiovascular diseases in prolonged extreme exposure. Urine Cadmium is commonly measured in individual either with clinical presentations suggesting acute toxicity or as compliance to regulatory requirement of safe biological exposure indices. SGH Clinical Biochemistry offers the Urine Cadmium test as routine clinical services using Inductively Coupled Plasma Mass Spectrometry. It is widely recognized that the presence of Molybdenum Oxide may introduce false positive to Cadmium analysis. This paper describes the use and compares the mathematical correction against Dynamic Reaction Cell in removing of Molybdenum polyatomic interference in Cadmium Urine analysis.
Methods:
2 solutions containing; i) Molybdenum standard (100ppb) and ii) blank using acid diluent were prepared and measured for Cadmium amu111 and Molybdenum amu95 signal intensity counts. Fresh gravimetric-preparation of Cadmium standards (6 points; 20xdilution of Multi Calibration Standards) were prepared and analyzed for calibration of Cadmium test on Perkin Elmer Elan DRC II, using 2 different applications of standard mode and dynamic reaction cell (oxygen gas at 2.0ml/min). Recovery studies were performed on samples spiked with 100ppb of Molybdenum and 0.4ppb of Cadmium.
Results:
The first method uses the mathematical equation to correct the interfering Molybdenum from the Cadmium. The factor (F) is calculated by [(111 Mo - 111 blank)/(95 Mo - 95 blank)] to be 0.04 and applied to all analyzed sample. The second method uses reaction gas oxygen to eliminate the bulkier polyatomic interference of molybdenum oxide in a dynamic reaction cell. The recovery of Cadmium with mathematical calculation and DRC mode is calculated to be 94 to 104 % and 112 to 114%, respectively.
Conclusion:
This study demonstrated that mathematical equation is more effective in correcting the interference of Molybdenum in urine Cadmium measurement using PerkinElmer Elan DRCII. Molybdenum oxide is a known polyatomic interference of cadmium analysis and must be corrected to minimize false positive result.

B-307

Significant Loss of Blood Amino Acids and Free Carnitine in Newborns Receiving Continuous Renal Replacement Therapy (CRRT)

U. Garg, M. Thompson, B. Warady, V. Chadha. *Children's Mercy Hospitals and Clinics, Kansas City, MO*

Background: Newborns with acute kidney injury (AKI) or end-stage renal disease (ESRD) often receive prolonged CRRT when the early initiation of peritoneal dialysis is either contraindicated or unable to be performed. These patients often receive total parenteral nutrition (TPN) to meet their nutritional goals. A little to no information exists on the loss of blood amino acids (AA) and free carnitine during CRRT in these patients. The objective of this study was to determine the amino acids and free carnitine losses in newborns receiving prolonged CRRT and TPN.
Methods: Three newborns who received prolonged (> 2 weeks) CRRT and TPN were included in the study. Blood and CRRT effluent were simultaneously collected from these patients. The effluent specimens were collected over 8-12 hours and the results were extrapolated to 24 hrs. Plasma was separated from blood for the analysis of 30 amino acids and free carnitine. Amino acids in plasma and CRRT effluent were analyzed using amino acid analyzer which uses ion-exchange chromatography and post-column ninhydrin derivatization (Biochrom System). Free carnitine was determined by HPLC-tandem mass spectrometry (HPLC-MS/MS) using flow injection, electrospray ionization and precursor ion scan. The total amount of amino acids and carnitine received by each patient was calculated from the amino acids concentrate and carnitine added to TPN solution. The sieving coefficients (SQ) for each measured amino acid and carnitine was determined, while the losses of amino acids and carnitine losses were calculated as mg/day, and as percentage of the intake.
Results: The amino acid profile of 30 amino acids was determined in 3 patients and free carnitine was performed in 2 of these patients. The CRRT clearance ranged from 68-115 mL/kg/hr (1.4 -3.2 L/1.73m2/hr). Interestingly there was significantly different SQs for different categories of amino acids. The SQ for all essential amino acids was >80% with loses of 10-20% of their intake. SQ for acidic AAs (glutamic acid and aspartic acid) was <40% with <5% of their intake. SQ for cystine exceeded 100% and all patients had a low plasma cystine level. SQ for carnitine was >84%, and carnitine losses were 80% of its intake. This led to decreased free plasma carnitine concentrations.
Conclusions: CRRT leads to significant loss of many amino acids and free carnitine. The amino acids losses during CRRT are not uniform and can result in significantly low concentrations of certain blood amino acids. Additional studies are needed to determine if patients receiving CRRT require special amino acids formulations as part of TPN to account for these amino acids losses. As carnitine is rapidly and freely filtered during CRRT, appropriate increase in carnitine dose is necessary to avoid its deficiency.

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Vitamin D Status and Glycemic Control: Implications for Pre- and Postmenopausal Women

L. A. FONDJO. *KNUST/KATH, KUMASI, Ghana*

Background
Emerging evidence indicates that vitamin D deficiency is associated with several chronic diseases. Vitamin D levels has been implicated with abnormal glycemic control and estrogen levels. Aging and the subsequent decline in oestrogen during menopausal stages promotes hypovitaminosis D. Nonetheless, the interaction between vitamin D, menopause, lifestyle and T2DM requires extensive study. This study provides the first evidence of vitamin D status among pre- and postmenopausal T2DM in the Ghanaian population, determined the association between vitamin D status and glycemic control and also assessed the influence of lifestyle habits on hypovitaminosis D.
Methods
In a cross-sectional study conducted at the Komfo Anokye Teaching Hospital, Kumasi, Ghana. Structured questionnaires were administered to 192 consenting pre- and postmenopausal T2DM women with more than 6months disease duration. Their blood samples were collected for estimation of (25OH) D and Insulin using ELISA. Fasting blood glucose (FBG), Lipid profile, Glycated haemoglobin (HbA1c) and calcium were measured enzymatically. Statistical analyses were performed using Graphpad Prism 6.
Results
The prevalence of vitamin D inadequacy was 92.2%. Hypovitaminosis D was more prevalent among the postmenopausal T2DM (63.8% vs 58.2%). Hypovitaminosis D significantly associated with Insulin [R^2=0.01760,p=0.0008], HbA1c [R^2=0.3709,p=<0.0001], FBG [R^2=0.3465,p=0.0001] in only the postmenopausal women. A higher risk of developing vitamin D deficiency was associated with unemployment (aOR=1.612, 95% CI (0.828-3.138), p = 0.160), being both uneducated (1.095 95% CI (0.094-12.800), p = 0.943) and educated 2.236 95% CI (0.222-22.529), p= 0.495) having diabetes mellitus for > 5 years (aOR = 1.842, 95% CI (0.926-3.664), p = 0.082), higher WHR (aOR = 1.419, 95% CI (0.594-3.392), p = 0.431) and WHtR (aOR = 1.336, 95% CI (0.723-2.468), p = 0.355)
Conclusion
Vitamin D deficiency is prevalent in pre- and postmenopausal T2DM but higher among postmenopausal women. Adequate vitamin D levels in both groups was associated with improved glucose control while hypovitaminosis D in the postmenopausal women was related to poorer glucose control. Vitamin D screening should be incorporated into the management plan for T2DM to serve as an early tool for prevention of Vitamin D deficiency.
Keywords: Vitamin D, diabetes, postmenopausal women, insulin resistance, glycemic control.

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Analysis of biomarkers of calcium metabolism in bariatric surgery patients

L. M. Johnson, S. Ikramuddin, D. Leslie, B. Slusarek, A. A. Killeen. *University of Minnesota, Minneapolis, MN*

Background: Bariatric surgery patients have an increase in bone metabolism after surgery. One hypothesis is that their rapid weight loss results in skeletal unloading, reducing the bone mass density (BMD). However, studies have found that bariatric patients are at high risk for vitamin D deficiency and secondary hyperparathyroidism which lead to pathological changes in BMD. Therefore, it is important to monitor biomarkers of calcium metabolism, such as PTH, vitamin D, and calcium. We examined how these three markers were related in bariatric surgery patients over time with multilevel mixed-effects models.
Methods: A retrospective chart review of 358 sleeve gastrectomy (SG) and 110 Roux-en-Y gastric bypass (RYGB) patients who had recorded vitamin levels and a bariatric procedure from April of 2012 to April of 2016 was performed. Data were collected for vitamin D (Abbott Architect), PTH (Siemens Centaur), albumin, calcium (corrected for albumin, Siemens Vista), estimated GFR, and the presence of diabetes and hypertension. Mean levels, deficiencies in vitamin D, and elevations in PTH were analyzed and grouped according to pre-operative, ≤1 year post-operative, and >1 year post-operative period. The cut-off for vitamin D deficiency was <20 ng/mL, and the reference range for PTH was 12-72 pg/mL. Statistical analysis was performed using Stata. Multilevel mixed-effects quadratic models were used to determine if the change in analyte level was significant over time (months) and related to changes in the other biomarkers. We investigated what factors contributed to elevated PTH in our bariatric patients by examining the effects of vitamin D deficiency, surgery type, and chronic kidney disease. The study was approved by our IRB.
Results: Vitamin D deficiencies decreased the first year after surgery, from approximately 27% pre-operative deficiencies to 5.2% in SG and 11.5% in RYGB patients. In the ≥1 year post-operative period, SG participants had 13.4% and RYGB had 20.3% vitamin D deficiencies. Elevated PTH remained constant at approximately 20% of SG patients; however, 45% of RYGB patients had at least one elevated PTH level in the ≥1 year post-operative period. Mean PTH levels for RYGB patients were 52 ± 27 pg/mL at baseline (pre-op) and 73 ± 29 pg/mL at greater than 1 year post-surgery. The multilevel models showed some predictable trends in the data, such as increasing levels of vitamin D and corrected calcium were inversely associated with levels of PTH. Vitamin D deficiency was significantly associated with an average increase of 16 pg/mL of PTH (p<0.005). For the effect of surgery type, RYGB patients had an increase of 11 pg/mL for PTH and decrease of 4 ng/mL for vitamin D when compared to SG patients (p<0.05). Additionally, CKD stages had an effect on PTH levels: baseline was no CKD or stage 1, 5 pg/mL increase for stage 2 (not significant), 19 pg/mL increase for stage 3 (p<0.005), and 42 pg/mL increase for stage 4 (p<0.05).
Conclusions: Risk factors associated with increased PTH in our bariatric patients were vitamin D deficiency, RYGB surgery type, and CKD status. Patients with these risk factors may need more aggressive management to prevent decreased BMD.