Tuesday, July 29, 2014

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

Endocrinology/Hormones

A-152

Assessing macroprolactin interference in prolactin assays after polyethylene glycol precipitation in two automatized platforms

R. R. Rodrigues, R. C. Basso, R. C. A. Sansão, P. Osorio, C. F. Pereira. *Diagnostico da America (DASA), Barueri, Brazil*

Background: Prolactin serum levels over 30 ng/ml in the absence of pregnancy and postpartum breastfeeding are indicators of hyperprolactinaemia which could impact in more complexes and expensive diagnostic protocols. Seric Prolactin are classified in three main forms: monomeric which is the predominant form; dimer, also known as big prolactin; and the high molecular weight form which is usually known as macroprolactin or big-big prolactin (bbPRL). It is known that macroprolactinaemia may correspond to approximately 20 - 25% of cases of hyperprolactinemia and it is a common disorder in a healthy population. Thus, the investigation of macroprolactinaemia as the main cause of hyperprolactinaemia would avoid clinical investigation of prolactinoma and other diseases. The reference test for detecting macroprolactin is gel filtration chromatography, but the test based on polyethylene glycol (PEG) is simpler and cheaper and has been validated in 1999 by Olokoga and Kane. All commercially available prolactin immunoassays have a cross-reactivity level with macroprolactin. The purpose of this study was to validate the PEG precipitation test using Siemens Healthcare Diagnostics ADVIA Centaur System when compared to Abbott Architect.

Methods: 46 patient samples presenting levels over 30 ng/mL previously dosed were re-tested in ADVIA Centaur and Abbott Architect after PEG treatment. Analysis of mean, standard deviation and correlation were calculated. Moreover, a cutoff of 60% was established to determine the presence of bbPRL.

Results: The samples were measured before extraction and the results were: 69.29 ng/mL average in ADVIA Centaur and 94.42 ng/mL in Architect with a standard deviation of 30.12 and 42.10 respectively and R2 of 0.88. 60% was regarded as cutoff for bbPRL screening. When comparing the ADVIA Centaur with Architect, a relative sensitivity of 100% and a Relative Specificity of 83.33% with positive predictive value of 97.56% and negative predictive value of 100% were obtained.

Conclusion: The Prolactin assay varies according to the elected methodology for Macroprolactin detection. Correlation results between compared instruments were satisfactory. Moreover, ADVIA Centaur is capable of dosing the three main forms of seric prolactin. Among 46 samples only one sample showed doubtful result for Architect and positive for ADVIA Centaur. As we can observe macroprolactin is a major interference source and may lead to diagnostic errors and processing errors involving patients with hyperprolactinemia. Samples present greater dispersion measurements prior to PEG precipitation for Architect results and the difference between the values of pure samples can be related to low interference that ADVIA Centaur system presents, being considered as a positive point once risk in releasing high results decreases. This provides a smaller number of high results which can generate inadequate diagnostic or request a further test to make the diagnosis. In conclusion, we can confirm that the evaluation of bbPRL methods, such as PEG precipitation is still necessary, even in trials that have low reactivity for macroprolactin as the ADVIA Centaur.

A-153

Vitamin D status in healthy and rheumatoid arthritis groups.

S. Kang, M. Lee, J. Yang, M. Kim, W. Lee. *KyungHee University Hospital at Gangdong, Seoul, Korea, Republic of*

Introduction: Vitamin D is important for maintenance of calcium homeostasis and bone metabolism. Its association with chronic and inflammatory diseases including rheumatoid arthritis (RA) has been suggested while consensus on the optimal level of vitamin D is yet to be made. The aims of this study are 1) to assess vitamin D status among healthy and RA groups from Korean population; 2) to evaluate biochemical markers of their relationship with vitamin D level; and 3) to determine an alternative cutoff level and assess the relationship with RA.

Materials and methods: The study includes 346 healthy individuals and 222 diagnosed RA patients during winter (January-February) and summer (July-August). C-reactive protein (CRP), adjusted calcium (aCa), erythrocyte sedimentation rate (ESR), parathyroid hormone (PTH), ß-isomerized C-terminal telopeptides (ß-CTx) and serum 25(OH)D levels were measured. Each groups were classified by vitamin D status and related markers into subgroups for comparison. Statistical analyses were made using t-test, ANOVA, chi-squared test and logistic regression analysis. Segmented linear regression analysis was used to determine an alternative cutoff. Statistical significance was determined at $P<0.05$.

Results: 25(OH)D level was lower in female, younger age group, high PTH subgroup and during winter. Vitamin D insufficiency/deficiency was highly prevalent among healthy group (95.7%) and RA group (98.2%) but the distribution of vitamin D status was not different. 25(OH)D andshowed a negative correlation with PTH ($P<0.01$, r=-0.29), however the increase of PTH level was mostly within reference range and increase of PTH beyond reference range level in vitamin D deficient subgroup was rare (2.3%). Despite vitamin D deficiency, ß-CTx and aCacalcium levels were not different among vitamin D status subgroups and no correlation with 25(OH)D or PTH was found. Comparison between healthy and RA group also revealed similar seasonal differences, RA group had lower 25(OH)D level during summer, although the distribution of vitamin D status was not different. The alternative cutoff 22.08 ng/mL, classified healthy and RA group as vitamin D insufficiency/deficiency in 85.54% and 90.54% respectively. The prevalence of vitamin D insufficiency/deficiecy was low but the difference between healthy and RA group was not found. Logistic regression analyses showed that 25(OH)D level was not associated with RA, and ESR and CRP as markers of disease activity.

Discussion: Most healthy individuals are categorized as vitamin D insufficiency/deficiency under the currently used conventional criteria for vitamin D status. The level of vitamin D deficiency from this study did not result in increment of PTH, ß-CTx or aCa greater than reference range. This finding is suggestive that the status of vitamin D deficiency using the current criteria may not be clinically practical. In contrast to previous reports on the relationship of vitamin D and chronic inflammatory diseases, application of lower cutoff in this study also did not exhibit association with RA and correlationor with markers of disease activity. Future investigation of vitamin D should be conducted through a large, randomized controlled trial and focus on deciding the optimal vitamin D level in correlation with clinically meaningful results, regarding calcium homeostasis and bone metabolism.

A-154

Evaluation of Hb A1c bias and precision across eight platforms in the presence of Hb AS and Hb AC

J. M. Rhea[1], M. Richter-Roche[1], A. Woodworth[2], N. Korpi-Steiner[3], J. Miller[4], D. Koch[5], R. Molinaro[1]. *[1]Emory University, Atlanta, GA, [2]Vanderbilt University, Nashville, TN, [3]University of North Carolina, Chapel Hill, NC, [4]University of Louisville, Louisville, KY, [5]Grady Memorial Hospital, Atlanta, GA*

Introduction: Changes in serial Hb A1c results are due to changes in the clinical condition or to inherent sources of biologic and analytic variation. Results of the 2012 CAP GH2-B proficiency survey which contained an Hb AS sample, suggest that samples containing an Hb variant may impact assay precision for some methods, and potentially reference change values (RCVs). Objective: To calculate the effect of Hb variants on RCV for Hb A1c by determining the precision of each method. Methods: Seven different NGSP-certified Hb A1c platforms were used to measure imprecision and bias using patient samples containing Hb AA, Hb AS, or Hb AC. Precision was determined following CLSI EP05-A2, and bias by calculating the percent difference between each test method and the comparative method (NGSP Secondary Reference Lab). RCVs were calculated using the standard formula. Results: Differences in imprecision and bias were observed, and were typically greater in samples containing an Hb variant. RCVs for all methods except two at were ≤0.5% Hb A1c; overall, RCVs were slightly increased in the presence of Hb AS and Hb AC at ≤0.8%. Conclusions: The total analytical error for the majority of assays was significantly greater in samples containing Hb AS or Hb AC, and may indicate a need for including proficiency samples containing the most common Hb variants, especially when assays are used to measure Hb A1c in populations with a high prevalence of Hb variants. In addition, while the clinical relevance of increased was beyond the scope of this study, the change in RCV suggests a difference in how serial Hb A1c results may be interpreted.

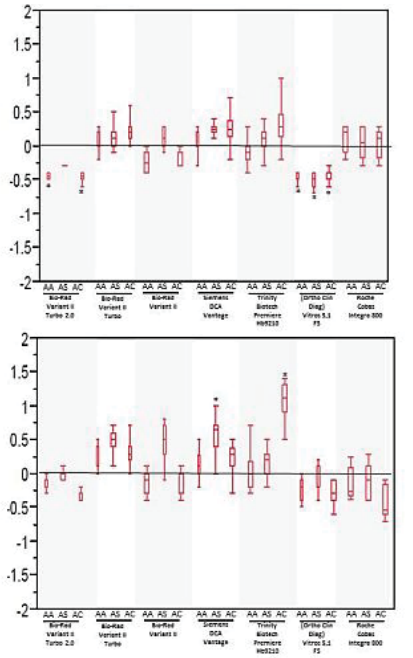


Figure 1. Box plots showing the absolute differences between each assay and the comparative method for each hemoglobin type at a low (A) and high (B) Hb A_{1c} concentration. The upper and lower limits of each box correspond to the 25th and 75th percentiles of the differences, respectively. The upper and lower bars represent the maximum and minimum differences between the comparative method. Clinically significant differences (±6%) are indicated with a (*).

A-155

Is it possible to make insulin tolerance test (ITT) better?

M. P. Campagnoli[1], A. Menna Barreto[1], M. Freire[2], M. Scharf[1]. *[1]DASA, Curitiba, Brazil., CURITIBA, Brazil, [2]DASA, Curitiba, Brazil., Rio de Janeiro, Brazil*

Background The diagnosis of growth hormone (GH) deficiency in children with growth retardation is complex. Because of the pattern of pulsatile secretion of GH, isolated determinations have no value. So, the functional stimulus tests for evaluation of GH secretory reserve are fundamental for the diagnosis. The Insulin Tolerance Test (ITT) is considered to be the gold standard test for diagnosis in children over 2 years of age. This test consists on the administration of intravenous insulin and sampling a multi amostral sequence of glucose and GH, and sometimes, cortisol.

The given insulin should be able to reduce 50% of fasting glucose or reaching below the normal range, promoting the stimulus needed to boost the production and release of GH and cortisol .

Bedside blood glucose control is done, during the lab test, through the measurement of plasma glucose with blood glucose strips. Decisions like, correction of blood glucose as well as administering another dose of insulin, are based on these values .

We observed that in many patients glucose measured at the bedside shows superior values to subsequently assayed by enzymatic method (hexokinase).

This study aims to determine the difference between plasma glucose measured by blood glucose strips (bedside) and enzymatic method and propose new targets for bedside blood glucose to increase the security of ITT.

Methods From March 2013 to January 2014 we conducted 423 ITT. In all these, measurements of plasma glucose were performed using blood glucose strips (bedside) and enzymatic method. The blood sampling was done at the same time for both methods. The results were matched and compared statistically.

Results: There is statistically significant difference between the plasma glucose levels measured by blood glucose strips and enzymatic method . The value assayed by the enzymatic method is smaller than the other (bedside). The median difference between all values is -9.624mg/dl (-10.11 to -9.137)

Regarding extreme glycaemia (under 60 mg / dL and greater than 99mg/dl) , the difference between the two methodologies is constant , statistically significant and even higher. The enzymatic method is -10.87 mg / dL (-11.0 to 10.64) lower than the equivalent sample at the bedside method (strips). At standard deviation (SD) we found a deviation of + / - 21.75mg/ dL.

Conclusion There are statistically significant and consistent difference between the two methods compared. In extreme glycaemia (outside normal range) this difference is even greater. Our data show that if the glucose levels at the bedside (strips) were held on average 10 mg / dl of hypoglycemia target to be achieved in the plasma assay by enzymatic method, we can increase exam security without compromising its reliability and possibly reduce clinical patient discomfort.

A-159

Laboratory process improvement through adjustments in calibrations of immunoassays

A. L. Camilo, D. Waltrick, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: Workflow optimization identifying the specific processes and improvements not only improves operational efficiency, it also reduces error and contains costs. Because system capabilities are not the only considerations when selecting the optimal system for the DASA laboratory, created customized workflow scenarios to help maximize your productivity.

Objective: The objective of this study is to show that continuous monitoring and standardization of quality system improved the flow in large laboratory routines, increase productivity and yield of reagents reducing the turnaround time on the pre-analytical (TAT), impacting on operational costs, reducing interfering in quality control.

Methods: Based on an extensive statistical analysis on the performance of systems ADVIA Centaur XP (Siemens Healthcare Diagnostics), were established changes in calibration routine for Progesterone test, setting 6 equipments in which the calibration for this analyte would be performed every seven days and for other six systems calibration would be performed every twenty-eight days, number of replicates of the calibrator was changed from 3 to 5 according to the instructions of reagents after a period of four months. The performance analysis of the test and the variation in assay constitute evaluated.

Results: The number of disparate control represented 15.4% with a confidence interval from 14.6 to 16.2%. In 8,862 processed control results, 1,363 were outside 2SD range. After implementation and improvement processes in the system, in between January and April 2012, the disparate controls number decreased to 7.8% with confidence interval of 7.2% to 8.4%. In 7,931 only 615 control results were outside 2SD range. The number of test calibrations decreased from 600 to 420 in the respective time periods and processed control number decrease 10%.

Conclusion: The preanalytical phase may cause the inaccuracy of the results when there is a long time for the release of the operating systems in laboratories of large routines. The results suggest that variations in the time interval between calibrations can impact the quality of the result, interfere with the performance of quality control and increase the release time of the equipment for routine, compromising the productivity of clinical laboratory routine.

A-160

Performance evaluation of novel C-peptide immunoassay reagent using a fully-automated immunoassay analyzer

Y. Watanabe, I. Sato, N. Hayashi, J. Saegusa, S. Kawano. *Department of Clinical Laboratory, Kobe University Hospital, Kobe, Japan*

Background: C-peptide is co-secreted with insulin in equimolar amounts from pancreatic β cells. Assessment of endogenous insulin production with C-peptide immunoassay requires sufficient sensitivity and high specificity. In this study, we evaluated the analytical performance of newly developed C-peptide reagent.

Methods: The ST AIA-PACK C-Peptide II reagent* on Tosoh AIA-2000 fully-automated immunoassay analyzer is an enzyme immunoassay which is performed entirely in a single cup. C-peptide in the sample is bound with monoclonal antibody immobilized on magnetic beads and alkaline phosphatase-labelled monoclonal antibody. After 10 minutes incubation at 37 ℃, the beads are washed to remove unbound materials and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate. The amount of enzyme-labelled monoclonal antibody that binds to the beads is directly proportional to the C-peptide concentration in the sample. A standard curve is constructed using the Calibrator Set and unknown concentration of C-peptide is automatically calculated using this curve. In this study we evaluated the precision, functional sensitivity, interference, recovery and cross-reactivity of this new reagent toward human proinsulin. Method comparison, against ARCHITECT C-peptide immunoassay based on chemiluminescent immunoassay, was evaluated with clinical specimens from patients. Correlation of the C-peptide

concentrations between serum and EDTA plasma samples was also studied. Results: The standard curve extended from 0.02-30ng/mL for serum. Within-run and between-run coefficients of variation ranged from 2.5% to 3.5% and from 2.5% to 3.6%, respectively. Based on the imprecision profile, functional sensitivity (at 10% CV) of this reagent was 0.017ng/mL. Assay correlation with ARCHITECT C-peptide immunoassay was determined: y=0.97x+0.02, r=0.997, (n=50 x; ARCHITECT, y; AIA-2000). There was a good correlation between serum and EDTA plasma concentrations of C-peptide: y=0.96x-0.07, r=0.996, (n=220 x; EDTA plasma, y; serum). The cross-reactivity against purified human proinsulin was below 0.4%. Conclusion: ST AIA-PACK C-Peptide II reagent using a fully-automated analyzer, a novel enzyme immunoassay for detecting C-peptide, exhibited extremely low cross-reactivity with proinsulin, and showed high sensitivity for C-peptide. Our results demonstrated that this reagent is a reliable method for the rapid and accurate quantification of C-peptide in clinical laboratories, and turns out a useful tool for both the screening and the management of diabetic patients. *) This product has not been approved by the FDA yet.

A-161

Low testosterone concentrations: only mass spectrometry?

A. Fortunato[1], M. Caputo[2], P. Garofalo[3], C. Marchetti[1], R. Castello[4]. *[1]Laboratorio di Chimica Clinica ed Ematologia Ospedale "S. Bortolo", Vicenza, Italy, [2]Laboratorio Chimica Clinica e Microbiologia, Ospedale "Orlandi", Bussolengo (VR), Italy, [3]UOC Endocrinologia AOOR "Villa Sofia-Cervello", Palermo, Italy, [4]UOC Medicina generale indirizzo endocrinologico, AOUI, Verona, Italy*

Background: total testosterone level measurement is the most requested one among steroid hormones assays. Unfortunately, the diagnostic accuracy at low concentrations of the most common immunoassays proved to be insufficient. In 2007 the Endocrine Society recommended the determinations of testosterone in children and in women has to be done only with one reference method (extraction, chromatography and determination by mass spectrometry). Due the method related difficulties in most of the laboratories the testosterone determinations are still done by immunoassays.

Samples and methods: we measured testosterone with three different fully automated immunoassays present in most of the clinical labs and repeated the determinations both with a commercial RIA and LC-MS/MS method. The latter one, considered the reference method, has been done in the Perkin Elmer labs (Turku, Finland), with updated equipment, by skilled personnel and determinations carried out in replicated. The serum samples were collected from 70 patients, male and female in pediatric age. The obtained concentrations by LC-MS/MS, considered as reference, ranged from 11 to 110 ng/dL.

Results: the distribution of the concentrations obtained with the methods used should be noted that, although the averages and medians of the concentrations obtained with the LC-MS/MS method are less, the differences are not such as to distort the clinical information can be obtained: the 3 automated methods show values ranging from 10 to 134 ng/dL with correlations coefficients respectively to LC-MS/MS ranging from 0,829 to 0,934; whereas the RIA method shows a higher concentration's dispersal, values ranging from 20 to 149 ng/dL and a worse correlation to the reference method. (r=0,705).

Conclusions: the position of the scientific community on total blood testosterone measurement at low concentrations is critical to the use of direct immunoassays because without the necessary diagnostic accuracy, and recommends the use of methods that are not within the reach of general laboratories. Our results, although preliminary, open an interesting perspective on the possibility of arriving at a reasonable future to employ even the immunoassays, certainly more feasible, as an aid to diagnosis of common and important endocrine syndromes of the woman and the child.

A-162

Classification of Children with New-Onset Diabetes Mellitus Using an Auto-Antibody Algorithm

S. Prakash[1], V. Leung-Pineda[2], S. Suzuki[1], J. Radack[2], J. Dallas[2], P. S. Thornton[2], D. P. Wilson[2]. *[1]University of North Texas Health Science Center, Fort Worth, TX, [2]Cook Children's Medical Center, Fort Worth, TX*

Background: Historically the diagnosis of Type 2 diabetes mellitus (T2D) in children has relied on a typical clinical phenotype. The ability of experienced clinicians to correctly classify the type of DM based upon the clinical phenotype has, however, recently been challenged. Since the appropriate classification has important implications with regard to treatment options, expected outcomes and genetic counseling, a systematic, cost-effective algorithm to assist in the initial classification of DM is needed.

Objective: To evaluate the use of an auto-antibody algorithm to classify new onset diabetes patients and its use in curtailing testing costs

Methodology: Data from children (<18 yrs of age) hospitalized at CCMC from Jan 2010 - Oct 2012 with new-onset DM was analyzed. In contrast to T2D, T1D is an autoimmune disease (AD) characterized by the presence of ≥1 diabetes-related antibodies (DR-Ab). Historically the initial evaluation, including DR-Ab testing, has been left to the discretion of individual Pediatric Endocrinologists. Other Abs are often measured to assess concurrent autoimmune diseases that commonly occur in individuals with DM, such as Hashimoto's thyroiditis and celiac disease. Inclusion Criteria: 1) Age < 18 yrs at diagnosis; 2) New-onset DM; 3) Onset Jan 2010-Oct 2012.

Results & Conclusions: The American Diabetes Association classifies DM into T1D, T2D, gestational diabetes, and diabetes due to other causes. While the majority of those <18 yr of age have T1D, the number with T2D is increasing. Individuals with T2D are often obese. With the exponential increase in the number of children who have become overweight/obese, classifying DM based on a child's phenotype has become problematic. In children with overt signs/symptoms of DM the presence of ≥ 1 DR Ab is generally considered sufficient evidence of auto-immunity (i.e. T1D). In our study, subjects were routinely tested for 2 DR-Abs (GAD_{65} 97.9%; ICA 95.9%). Since 73.3% of subjects were positive for GAD_{65}, additional testing for ICA increased cost w/out additional benefit. While not included in the present study, additional screening tests are sometimes also requested for celiac and thyroid disease. For those whose initial screening was positive (celiac 12.5%; thyroid 16.1%), eliminating further testing would have helped reduce cost.

Ab testing to help classify children with new-onset DM may be enhanced with use of an algorithm, especially if it includes reflex testing. Reflex tests are tests automatically performed by the laboratory if the initial test requested fails to meet preset criteria. Subsequent tests can generally be performed w/out need for additional samples and may consist of ≥ 1 sequential tests. Although there is a charge for additional tests, if the likelihood of the criteria being met with the initial sample is high, reflex testing has the potential to reduce medical cost.

A-163

Quality control management improving immunoassays systems in the clinical laboratorial routine

D. Waltrick, A. L. N. Camilo, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: Automated analyzers provide several advantages on processing immunoassay methods. The literature demonstrates that laboratory errors can be associated with pre-analytical (30.6%), analytical (31.6%) and post-analytical (30.8%) processes or even due to combined processes (6%). Errors in the analytical phase are commonly related to lack of preventive maintenance, inappropriate Quality Control (QC) management, and improper handling of samples or reagents. Errors due to analytical problems have been significantly reduced over time, but there is evidence that this interference may have a serious impact on patient results, especially for immunoassays.

Objective: The aim of the study was to identify the potential causes for quality control variability in immunoassays to improve the laboratory routine productivity when adopting best quality control practices.

Methods: 32,760 quality control points of Immunoassay Plus QC Lot 40240 (BioRad) were collected during eight months using 14 ADVIA Centaur Systems (Siemens Helathcare Diagnostics), in the clinical laboratories. The statistics evaluated were: Coefficient of variation (CV), Standard deviation (SD), observed mean and outliers. Data was compared to those reported on Biorad International Quality Control Program. All assays that presented better or equal statistic results were considered acceptable. Assays with higher CV than the reference, Biorad worldwide report, were submitted to further technical investigation and corrective actions. After critical analysis of the first four months data, some improvements were implemented, such as: a new plan of the preventive maintenance, increasing periodicity from once per quarter to once each three months matching laboratory number of tests; a new definition of mean and standard deviation for each QC target level for each assay. Once improvements were implemented, quality control data was collected during the following four months.

Results: In the first four months period, before implementation of proposed improvements, the CV mean was 18.8%, 5,402 tests were spent in calibrations, 16,841 tests were used in QC material analysis and reagents profitability was 96.4%.

Thereafter, data of a new four months period were collected, showing CV mean decreased to 10.7%; 4,708 tests were spent in calibrations; 15,919 tests were used in QC material analysis and reagents profitability raised to 98.0%.

Conclusion: Through statistic analysis, it was possible to identify the importance on studying clinical lab routine in order to implement a best plan for preventive maintenance and appropriate rules of the quality control management. The new definition of a single target value for all ADVIA Centaur Systems was useful for reducing time to evaluate daily internal quality control and also to ensure the commutativity between the systems. This project brought financial and nonfinancial gains, such as lower consumption of reagents, reduced downtime and reported results confidence.

A-164

A Comparison of CVD risk in newly diagnosed hypothyroid and type 2 diabetes mellitus subjects using Framingham risk score sheet

P. Purohit, P. Sharma. *All India Institute of Medical Sciences, Jodhpur, India*

Background Thyroid disorders and type 2 diabetes are known for their association with CVDs owing to their effect on derangement of the lipid metabolism. However there are no studies to document a comparative CVD risk in these two disorders.

Aim We aimed to compare the various CVD risk parameters in thyroid disorder and type 2 DM subjects at the time of diagnosis.

Material and methods The study participants were 150 hypothyroid and 180 type 2 DM subjects reporting for the first time to our endocrinal clinics. The patients were selected on the basis of symptomatology, a TSH >5µIU/ml and a FBS > 126 mg/dl. All participants were evaluated for BMI, Blood Pressure, serum Insulin, HOMA - IR, Lipid profile, apo -B and A1. CVD risk was assessed using the Framingham risk score. Statistical analysis was done using the students -'t' test to assess significance.

Results At diagnosis the hypothyroid and T2DM subjects presented with raised BMI ($p>0.001$), hypertension (SBP 132.98 ±17.40 v/s 132.60± 12.18 [NS]; DBP 86.52± 9.82 v/s 88.79± 8.02 [NS]), insulin resistance (30.63±16.18 v/s 17.29 ± 15.61, $p<0.0001$), gross dyslipidemia, with the T2 diabetic subjects showing significantly raised total cholesterol (231.15± 22.19 v/s 213.50 ± 38.95, $p<0.0001$), triglycerides (197.35± 35.31 v/s 187.91 ± 39.12, $p<0.0001$), reduced HDLc (33.56± 2.67 v/s 42.99 ± 4.70, $p<0.0001$) and significantly reduced apo B (154.47± 12.87 v/s 175.58 ± 34.56, $p<0.0001$) and apo A1 (96.94± 8.55 v/s 139.76 ± 17.40, $p<0.0001$). The CVD risk ratios T.chol/HDLc 6.93 v/s 5.03 and apoB/apoA1 were 1.60 v/s 1.29. The ten year risk of CVD in the T2DM subjects was 25% and in the hypothyroid subjects was 13%.

Conclusion: The present study concludes a significantly raised CVD risk in T2DM as compared to hypothyroid subjects at diagnosis.

A-165

Serum ghrelin, obestatin and nesfatin1 levels in pregnant women with hyperemesis gravidarum

G. Ozturk[1], S. Erdinc[2], F. Ucar[1], Z. Ginis[1], G. Erden[1], N. Danisman[2]. *[1]Department of Clinical Biochemistry, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey, [2]Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey*

Backgrounds: Hyperemesis gravidarum, which affects 0.3-2.3% of pregnancies, is defined as excessive vomiting during pregnancy and usually starting at 4 to 5 weeks' gestation , which may lead to severe outcomes including weight loss, dehydration, ketonemia, ketonuria, fasting acidosis, alkalosis due to hydrochloric acid loss and hypokalemia. Although, their exact cause is unknown, various metabolic and neuromuscular factors have been implicated in the pathogenesis of Hyperemesis gravidarum. The aim of this study was to investigate the levels of prealbumin, total ghrelin , nesfatin 1, obestatin in Hyperemesis gravidarum.

Methods: A total of 40 pregnant women with Hyperemesis gravidarum and 38 pregnant women who were perfectly healthy and whose pregnancy had a normal course were included in this study. After an 8-12 hour overnight fast, blood samples were collected into plain tubes for obtaining serum. Blood samples were centrifuged at 2.500 g for 15 min at 4 oC within 30 min of collection, and serum samples were stored at -80oC until analysis. Measurements of ghrelin (Phoneix, USA) obestatin (Biovendor,Czech Republic) and nesfatin1 (Phoneix, USA) were per¬formed in an EPOCH system (BioTek Instruments, Inc, USA) using the com¬mercially available enzyme-linked immunosorbent assay kit in accordance with the manufactures' instructions. Prealbumin levels were measured by spectrophometric method. All statistical analyses were performed using the software SPSS 15.0 (SPSS inc., Chicago, Il, USA) program. For all statistical tests, two-tailed p value <0.05 indicated the statistical significance of the results.

Results: There were no differences in age, gestational week and BMIs between the patients with Hyperemesis gravidarum and control subjects. Serum ghrelin and prealbumin levels were significantly lower in patients with Hyperemesis gravidarum than the control group ($p<0.05$). Serum obestatin and nesfatin1 levels were not statistically different between the two groups.

Conclusions: Hyperemesis gravidarum is a disease of severe nausea, vomiting, and anorexia in early pregnancy resulting in dehydration and weight loss. Prealbumin is an indicator to assess nutritional status, so our data also suggests that prealbumin levels are decreased in patients with Hyperemesis gravidarum. Ghrelin is involved in stimulation of appetite, control of energy balance, and gastric motility. Ghrelin administration increases food intake through central mechanisms. One possible explanation might be that the decreased levels of ghrelin in Hyperemesis gravidarum may be a mechanism to lose of appetite and the energy balance of the Hyperemesis gravidarum pregnant women.

A-166

Evaluation of the Impact on IGF-I Control of Pharmacological Treatment with Octreotide LAR isolated compared to Association with Cabergoline in Patients with Acromegaly

L. F. A. Nery, C. M. Araújo, R. H. Jácomo, S. S. S. Costa, L. A. Naves. *Laboratório Sabin de Análises Clínicas, Brasília, Brazil*

Background: Discrepancies concerning GH and IGF-1 levels in acromegaly patients can occur in patients submitted to pharmacological treatment. Objectives: The aim of this study was to compare the efficacy in decrease and normalization of IGF-I values of Octreotide LAR treatment isolated and associated with cabergoline, based on IGF-1 determinations.

Methods: This is a case series study that enrolled 34 patients with confirmed diagnosis of acromegaly recruited from outpatient clinics of the Neuroendocrine Unit of the University Hospital of Brasilia. All of them received the diagnosis of acromegaly confirmed by clinical findings suggestive of the disease, elevated GH and age-matched IGF-I levels, GH not suppressible by the oral glucose load and evidence of pituitary adenoma on CT or MRI. The statistical analysis was performed using SPSS 17.0 software. Values are expressed as the mean ± standard deviation. The values of IGF-I are presented both as absolute values as percentage values of the upper normal limit normal range of IGF-I (% ULNV-IGF-I).

Results: The cohort was composed by 15 men and 19 women; mean age 54 (27-74) years old, divided in two groups, group 1, treated by Octreotide LAR (OC-LAR) 30 mg/month, and group 2 treated by Octreotide LAR (OC-LAR) 30 mg/month associated with cabergoline 2,0 mg/week (OC-LAR + CBG). Mean serum IGF-I and % ULNV-IGF-I pretreatment were significantly higher in the group OC-LAR + CBG. Those variables decreased 6 and 12 months after treatment in both groups, and the values of OC-LAR+CBG group became inferior to OC-LAR group. However, no significant difference was found between the OC-LAR and OC-LAR + CBG group neither 6 nor 12 months after treatment.

Conclusion: OC-LAR + CBG association resulted in a significantly higher decrease of IFG-I, both 6 and 12 months after treatment, compared to those treated with OC-LAR

A-167

ADVIA Centaur® Vitamin D Total Assay*: Expected Vitamin D Values in a Healthy Pediatric Population

H. Mindicino[1], J. Freeman[1], R. Christenson[2], B. Plouffe[1], A. Woods[3], M. Zgela[1]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]University of Maryland School of Medicine, Baltimore, MD, [3]Siemens Healthcare Diagnostics, Berkeley, CA*

Background: Vitamin D is a hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. It is a key regulator of bone metabolism. Vitamin D_3 is derived from skin exposure to sunlight while food supplements contain either Vitamins D_2 or D_3. Vitamin D deficiency is caused by a lack of exposure to sunlight and by insufficient dietary intake. Measurements of serum or plasma levels of the metabolite 25-hydroxyvitamin D (25[OH]D) are the best indicators of nutritional Vitamin D status.

Objective: The goal of the study was to test healthy pediatric donor specimens in order to establish pediatric observed values for the ADVIA Centaur Vitamin D Total assay*.

Methods: Serum samples were obtained from donors with ages ranging from 1 year up to 21 years. Donors resided in 8 regions, geographically distributed across the continental U.S. Donations occurred throughout one calendar year. All donors were free of chronic or active diseases, and were not receiving any prescription medications within 7 days of donation. All samples were assayed for iPTH and TSH on the Siemens IMMULITE® 2000 Immunoassay System. Samples with abnormal iPTH or TSH levels were excluded. The remaining samples were assayed for total vitamin D Total on the Siemens ADVIA Centaur. The ADVIA Centaur Vitamin D Assay used was aligned to the ID-LC/MS/MS 25(OH)vitamin D Reference Measurement Procedure (RMP), the reference procedure for the Vitamin D Standardization Program (VDSP).

Results: After all inclusion criteria were met, 227 samples were assayed for Vitamin D Total. Values were calculated for each age group, by season, and by geographic location. The lower and upper reference limits were estimated as the 2.5th and the 97.5th percentiles of the distribution of test results for each age group. The n, mean, median, 2.5th and the 97.5th percentiles for each population sub-group were:

Northern: n=119, mean=23.55, median=22.17, 25th% = 12.36, 75th = 38.92

Southern: n=108, mean=26.33, median=25.47, 25th%= 9.70, 75th% = 49.16

Summer: n=136, mean=26.64, median=24.86, 25th% = 12.46, 75th% = 46.58

Winter: n=91 , mean=22.23 , median=22.10 , 25th% =9.70 , 75th% = 32.38

1yr-3yr: n=22 , mean=23.34 , median=24.41 , 25th% = 13.98, 75th% =32.45

3yr-12yr: n=114 , mean=24.99 , median=23.10 , 25th% =12.46 , 75th% =45.96

12yr-21 yr: n=91 , mean=25.09 , median=23.95 , 25th% =8.16 , 75th% =45.83

All Donors: n=227 , mean=24.87 , median=23.37 , 25th% =11.36 , 75th% =45.83

Conclusion: Vitamin D levels were consistent across the 3 age groups with no apparent changes with age. There was no statistical difference between those receiving and not receiving vitamin supplements. Vitamin D levels were statistically higher for the southern region versus northern region, and for summer (maximum sunlight) versus winter (minimum sunlight). There was extensive overlap in ranges for all sub-populations and the expected range for the entire population can applied to any of the sub-populations.

* This version of the ADVIA Centaur Vitamin D Total assay is not available for sale in the U.S.

Product availability varies from country to country and is subject to local regulatory requirements.

A-168

Enigma behind Thyroid Function Tests: Harmonization Efforts

B. Das. *Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai, Maharashtra, India*

Background: Thyroid function tests (TFTs) form a very important set of tests in a pathology laboratory; a tool that clinicians and patients alike depend on to pin down the symptoms for treatment and relief However, it is this very set of tests that have come in question. First, what is the normal and acceptable range (upper & lower limits) of TSH in a particular population has been debated in different scientific fora A second problem which the doctors and technicians have to grapple with is the variability of test-results in itself; even a broadly similar set of instruments and methods can give up to 40% more or less values in TSH levels. In our laboratory, we validated thyroid function test and established reference range of TSH for Indian population.

Methods: In our laboratory, validation of thyroid function test were done [with particular reference to Thyroid Stimulating Hormone (TSH)] by verifying analytical accuracy and precision, and Analytical measurement range (AMR) as well as sigma metrics. We have also verified the reference range for Indian Population. We have screened 800 subjects. 630 healthy subjects were chosen in the study group for reference interval verification.

Results: In our laboratory, we have seen, high degree of analytical accuracy between two instruments (r2 = 0.985). Within Run (Repeatability) Precision and Within Laboratory Precision were comparable with the manufacturer's claim. Our obtained reference range (0.62 - 4.22 micro IU/ml) was within that of the manufacturer's (0.35 - 4.94 micro IU/ml). AMR was also verified with C.V. 1.70%, 1.89% and 2.51%, for control sera. Our obtained reference range (0.33 - 4.90 micro IU/ml) was correlating with that of the manufacturer's (0.35 - 4.94 micro IU/ml).

Conclusion: In our laboratory, we have verified thyroid function tests in our hospital set up. However, standardization of TSH and other thyroid function test is still a formidable challenge, due to the lack of proper reference intervals and standardized measurement procedures.

Our laboratory validation protocol will help any laboratory personnel from any part of the world to validate & establish reference interval based on their own population demographic variation.

A-169

Glutamate Decarboxylase Antibody Positivity in Diabetics

L. Ong, M. Tan, S. Saw, S. Sethi. *National University Hospital, Singapore, Singapore*

Background: Glutamate decarboxylase antibody(GAD) testing is useful in identifying patients with latent autoimmune diabetes in adults (LADA), and some studies showed that higher antibody titres were associated with specific phenotypes. We looked at patients with positive GAD and reviewed their laboratory and clinical features.

Methods: All GAD tests performed in 2013 were included, and positive results were analyzed with respect to demographic parameters, indication for testing (evaluation of diabetes mellitus or neurological signs or symptoms), presence of other autoantibodies or autoimmune diseases. GAD was performed using radioimmunoassay using the CentAK® kit and a positive result was defined as ≥0.9U/L. Statistical analysis was done using SPSS Version 17.0.

Results: There were 454 GAD requests in 2013, with 75(16.5%) positive cases. The median age was 39.3 years old, with female: male ratio of 0.55.

In patients with positive GAD, the median age was 43.4 years old, with female: male ratio of 1.06. There were 45 Chinese, 10 Indians, 9 Malays and 10 from other ethnic groups. 94.6% were requests to exclude LADA, and 21.3% had concurrent requests for anti-islet cell antibody, with 50% positivity. 4 patients had co-existing autoimmune diseases (myasthenia gravis, thyroid disease with positive thyroid peroxidase antibody, vitiligo and pernicious anemia).

GAD titres showed a Gaussian distribution with a left skew and peak at 0-25U/L. There was no association between GAD titres and age or C peptide levels.

Conclusion: GAD levels were predominantly ordered to evaluate diabetes mellitus in younger patients and there was an association with anti-islet cell antibody and other autoimmune diseases. Further studies may be performed to determine the clinical significance of high GAD titres in diabetics.

A-170

New therapeutic effect of metformin due to increased levels of FGF21 ?

D. Stejskal[1], R. Ochmanova[2], M. Uvirova[3]. *[1]Agel Research and Training Institute, branch Novy Jicin, Laboratories Agel, Novy Jicin, Czech Republic, [2]Agel Research and Training Institute, branch Prostejov, Central Moravian Hospital Trust, Department of laboratory medicine, Prostejov, Czech Republic, [3]Agel Research and Training Institute, branch Ostrava, CGB Laboratory, Ostrava, Czech Republic*

Background:: Fibroblast growth factor 21 (FGF21) is an endocrine hormone that exhibits anti-obesity and anti-diabetes effects. Recently was presented that metformin in patients with type 2 diabetes modulates FGF21 expression and blood concentration. Results indicate that metformin induced expression of FGF21 through an ATF4-dependent mechanism by inhibiting mitochondrial respiration independently of AMPK and it´s concentration in blood. AIM: Studying the effect of metformin on the concentration of FGF21 in serum in type 2 diabetes patients.

Methods: The study was approved by the Ethics Commission of the Hospital Šternberk. Study was monocentric, prospective and randomized. A total of 108 individuals were recruited for our study (18 healthy controls (HC), 18 T2D individuals without anti-diabetes therapy (W), 18 T2D individuals with diabetes monotherapy with derivate of sullfonylurea (SU),18 T2D individuals with diabetes monotherapy 500 mg metformin/day (M5), 18 T2D individuals with diabetes therapy 1000 mg metformin/day (M10), 18 T2D individuals with diabetes therapy 1500 mg metformin/day (M15). Anthropometric (height, weight, BMI, waist circumference (WC)), clinical (systolic and diastolic pressures) and laboratory fasting analyses were performed. Serum samples were separated in a cooled centrifuge and immediatelly analyzed for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, high sensitivity CRP, creatinine, uric acid, AST, ALT (all Siemens, Advia 1800). FGF21 serum level was determined by a commercially available ELISA kit (Biovendor, DS2, Dynex) in serum samples stored at -80o C.

Results: The study analysed 108 subjects, of which 18 were in good health while 100 probands suffered from T2D. In-defined subgroups, we found no significant differences by gender in FGF21 concentration. Healthy individuals had the lowest

values of FGF21, in other subjects are increased by the value of the diagnosis, the type of therapy and dose (HC 84.2 ng/l vs W 111.6 vs. SU 158.6 vs. M5 269.7 vs. M10 404.1 vs. M15 558.7, P <0.01). Changes remained significant after adjustment for age, sex and BMI. Serum glucose levels fluctuated in subgroups below 8 mmol/l.

Conclusion:: in a randomized prospective study, we for the first time confirmed the recently presented hypothesis that metformin leads to the rise of FGF21. The new finding was the fact that this happens regardless of gender, weight and age of probands. FGF21 induction by metformin might explain a portion of the beneficial metabolic effects of metformin

A-171

Pre-analytical assessment of AMH stability in human serum using a well-characterized midpro-mature immunoassay

A. Kumar[1], B. Kalra[1], A. S. Patel[1], G. Savjani[1], E. E. Eklund[2], G. Lambert-Messerlian[2]. *[1]Ansh Labs, Webster, TX, [2]Women and Infants Hospital, Providence, RI*

Background: The aim of the study was to assess the stability of AMH in human serum using a well-characterized midpro-mature immunoassay.

Relevance: AMH is a homodimeric glycoprotein composed of two 55 kDa N-terminal and two 12.5 kDa C-terminal homodimers, non-covalently linked by disulfide bridges. Recently, there have been concerns related to AMH stability in serum/plasma and complement interferences affecting the end result. This has generated numerous debates and publications related to reproducibility of AMH measurements and impact of pre-analytical sample handling. To date, no publication has clearly stated if the AMH variability is related to process (pre-analytical) or the assay. The AMH in female serum is mostly pro-mature associated form. The kinetics of association of pro and mature is rapid. Assay design that includes stable epitope antibodies and is not impacted by molecule association or complements will generate reproducible results.

Methods: A prospective study (n=16) was designed in which serum samples were tested within 3 hrs of draw, aliquoted and stored at room temperature (RT), -20°C, 2-8°C and re-assayed at 7, 10, 24, 48 and 168 hours. Multiple samples were thawed up to 4 cycles and measured at two independent sites. A well-characterized two-step, ELISA (Ansh Labs, US AMH, AL-105) was used to measure AMH levels in 25 µL of sample in < 3 hours. The assay is specific for human and measures pro-mature AMH complex. The assay is calibrated (0.09-19.4 ng/mL) against standardized recombinant human AMH.

Results: No significant changes were observed when samples were stored at RT, 2-8°C and -20°C. The median AMH concentration (16 serum samples, range 0.34-20 ng/mL) measured at 7, 10, 24, 48 and 168 hrs were 5.2, 4.9, 5.1, 4.9, 5.0 ng/mL at RT, 5.0, 5.0, 4.7, 4.3, 4.4 ng/mL at -20°C and 4.8, 4.8, 5.0, 4.7, 4.9 ng/mL at 2-8°C. The average CV on multiple runs at RT, 2-8°C, -20°C was 8.7%, 6.9%, 9.3%, respectively. Total imprecision (all data points) on stored samples and two controls were 9.3%, 4.8% and 3.1%, respectively. Freeze thaw analysis on two independent sample sets showed that AMH levels were stable over 4 thaw cycles, with median levels of 4.5, 4.7, 4.8, 4.7 ng/mL obtained at site 1 (n=4) and 1.2, 1.2, 1.3, 1.3 ng/mL, respectively at site 2 (n=5).

Conclusion: AMH as a biomolecule is very stable. This finding helps to resolve the uncertainty related to AMH sample stability and reliability of AMH measurements that have been debated. This study demonstrates that well-characterized assays and good pre-analytical methods will produce reliable and reproducible results.

A-172

Performance of Thyroid Hormone Assays on Mindray CL-2000i Chemiluminescence Immunoassay System

Y. Qiu[1], J. Hao[2], C. Kang[1], P. Feng[1], C. Yang[1], S. Yuan[1], Y. Zhang[1], F. Xia[3]. *[1]Nanfang Hospital, Southern Medical Univeristy, Guangzhou, China, [2]Nanshan Hospital, Huazhong University of Science and Technology, Shenzhen, China, [3]Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China*

Background: Thyroid hormones are among the first-line tests subject to international collaborative investigations, aiming at assessing the key performances and comparability of results between the available tests. Mindray CL-2000i Chemiluminescence Immunoassay System (CL-2000i) is a recently launched automatic immunoassay system. We have evaluated the performance of the system with serum thyroid-stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), total thyroxine (TT4), and total triiodothyronine (TT3).

Methods: Imprecision studies were performed according to the CLSI EP5-A2 guideline, and have been evaluated using two samples with low and high concentration. The within run imprecision was performed by measuring each sample for 20 times. The total imprecision was evaluated by measuring each sample continuously for 20 days with the same lot of reagent. The imprecision was expressed as coefficient of variation (CV%). Patient samples from healthy subjects, hypo and hyperthyroidism were freshly collected from the clinical laboratory of our hospital. The comparison studies were performed using CL 2000i and the reference methods in our laboratory, Siemens ADVIA Centaur and Beckman Coulter DxI 800.

Results: The CVs of TSH, FT4, FT3, TT4, and TT3 are in a range from 1.09 - 6.16% for within run, and from 3.22 - 9.16% for total imprecision. TT4 shows relative higher CVs for both within run and total imprecision comparing to other parameters, but within the manufacturer's claim (≤ 10%). The comparison studies indicated slopes for the five thyroid hormone parameters ranged from 0.748 to 1.051 and the intercepts from -0.48 to 0.6. All of the five assays displayed high interassay correlation (r^2 > 0.92). TSH displayed the highest correlation between CL 2000i system and Centaur XP system (slope = 0.993; r^2 = 0.971), while TT4 showed the lowest agreement between CL 2000i system and the Centaur XP (slope = 1.051; r^2 = 0.921).

Conclusion: The imprecision was highly acceptable for all the five parameters of thyroid hormones tested. The method comparison between CL-2000i system and the reference methods evidenced high concordance. Therefore, the parameters of CL-2000i system are well suited for the detection of thyroid hormones in clinical laboratories.

A-173

Linearity study of fertility assays assuring the quality control requirements

D. Waltrick, A. L. Camilo, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background:In the fertility trials, each step in the reproduction process is evaluated to monitor gynecological health. There are basic exams requested for each phase of the process. To certify released results accuracy, it is important to ensure the linearity of the tests.A Brazilian laboratory implemented an Easy Linearity Curve (ELC) tool to monitor the immune-hormone analytical systems, establishing a self-inspection program to verify the efficiency and accuracy of procedures and results. The use of a tool that checks assay linearity provides additional safety and reliability of the results. This study aims to use the statistic tool to monitor the AMR of prolactin, progesterone, LH, FSH, testosterone and estradiol.

Materials and methods: We selected samples from the laboratory routine, with concentrations within assay linearity range for each test. Samples were tested in Advia Centaur® XP (Siemens Healthcare Diagnostics) using a chemiluminescent method and analyzed with the ELC tool.

Results: AMR studies were carried out according to CLSI Guideline EP6-A. Results are demonstrated in the table below.

Discussion: The tests showed satisfactory linearity results with different samples. In this study, the sample pool was not used, because all the results from this dilution test presented high percentage of recovery, above the manufacturer's reference, due to a matrix effect. Because of that, we established the utilization of different samples, respecting the expected concentrations of the sample pool, if diluted samples were prepared. The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level.

Conclusion: The fertility trials tested in this study presented Assay Linearity Range as established by the manufacturer, within CLSI Guideline EP6-A and Total Error Laboratory's target. Thus tests were approved by the Quality Control Management in the laboratory.

Table 1 -

Assay	Assay Linearity Range	Obtained CV %	CV Target %	Obtained TE %	TE max Target %	Linear Regression	R2
Prolactin	0.3 - 200 ng/ml	4.9	5.75	12.65	21.1	1.013x + (-4.058)	0.99505
Progesterone	0.21 - 60 ng/ml	3.1	8.71	15.47	25	1.038x + (-0.67)	0.99821
LH	0.07 - 200 mUI/ml	3.2	4.78	10.48	19.8	0.988x + (-0.947)	0.99699
FSH	0.3 - 200 mUI/ml	4.5	5.5	8.9	17.1	1x + (-0.835)	0.99934
Estradiol	11.8 - 3000 pg/ml	3.5	4.53	12.6	21.6	1.029x + (15.194)	0.99797
Testosterone	10 - 1500 ng/dl	3.2	8.3	16.54	25	1.086x + (-30.634)	0.99356

A-174

Calcitriol and its free and bioavailable fractions are better markers than 25 hydroxy vitamin D for monitoring vitamin D status during Pregnancy

R. Pandian[1], J. Pandian[1], Z. Seres[2], A. Elias[1]. *[1]Pan Laboratories, IRVINE, CA, [2]Immunodiagnostic system LTD, Bolden, United Kingdom*

Objective To compare bioavailable 25-hydroxy vitamin D(25OH-D)concentrations with those of bioavailable calcitriol

(1, 25 di Hydroxy vitamin D) in sera of pregnant women.

Relevance Pregnancy is associated with major changes in calcium homeostasis with critical roles played by vitamin D and its metabolites.Current practice favors monitoring of vitamin D status in pregnancy using 25OH-D concentrations in serum. Calcitriol is the active form of vitamin D.The level of 25OH-D and calcitriol vary due to changes in maternal serum vitamin D binding protein(DBP).Serum concentration of bioavailable and free vitamin D are not influenced by DBP. Therefore, we measured total, bioavailable and free fractions of 25OH-D or calcitriol in serum samples of pregnant and non-pregnant women. Correlation between total 25OH-D and its fractions (bioavailable and free) and calcitriol and its fractions with PTH and CTX(C-terminal collagen degradation product) was explored in order to determine which compound or compounds were optimal markers of vitamin D status in pregnancy.

Methods Bioavailable 25OH-D or bioavailable calcitriol are fractions, not bound to DBP.They are the combined fractions of albumin bound and free fractions of 25OH-D or calcitriol.To obtain the bioavailable fraction, total vitamin D

(25OH-D or calcitriol)was quantitated using immunoassays(IDS, Phoenix,AZ). DBP was quantitated by an ELISA using reagents from R&D systems. Albumin was quantitated by a calorimetric method.Using the affinity constants of 25OH-D and calcitriol for DBP, and the affinity constants of 25OH-D or calcitriol for serum albumin, bioavailable 25OH-D,bioavailable calcitriol,free 25OH-D and free calcitriol were calculated.PTH and CTX assays were performed in pregnancy serum samples using IDS kits.Pregnant serum samples (n=54) were collected between 27 to 38 weeks of pregnancy.

Results Total 25OH-D was significantly lower in pregnant women despite a significant increase in DBP (276 ± 15 Vs 410 ± 30ug/ml).Bioavailable and free 25OH-D levels were lower than normal(n=55),and were in the deficiency or insufficiency range although the levels of PTH and CTX were in the normal range.The correlation between PTH with total 25OH-D was poor(r2= 0.3).There was also poor correlation between PTH and bioavailable or free 25OH-D (r2= <0.5). Calcitriol was high in the pregnancy samples (127.5 ± 15.5 pg/ml)compared to non-pregnant samples (36.2 ± 5.6 pg/ml) and the DBP-corrected bioavailable and free calcitriol was twofold higher than non-pregnant controls.Calcitriol and its fractions (bioavailable and free of calcitriol) correlated well with serum PTH and CTX (r2 = >0.9).

Conclusion Current assessment of vitamin D status in pregnant women by measurement of 25OH-D does not adequately reflect calcium homeostasis in pregnancy.25OH-D or its fractions do not correlate with PTH or CTX. On the other hand, calcitriol correlates well with PTH and CTX in pregnancy when determined either as total, bioavailable or free calcitriol.The data indicate that bioavailable or free calcitriol are the best markers for determining vitamin D status in pregnant women.

A-175

Analytical Measurement Range (AMR) Monitoring for Immune-hormone System

A. L. Camilo, D. Waltrick, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: Linearity tests verify if a method is able to provide linear results, proportionally to the analyte's concentration. It is extremely important to ensure the accuracy of the results released. A Brazilian laboratory established a self-inspection program to verify the efficiency and accuracy of procedures and results through the implementation of the Easy Linearity Curve (ELC) statistic tool to monitor an immune-hormone system. ELC checks assay linearity and provides additional safety and reliability to their results. This study aims to use the statistic tool to monitor the AMR of ten immune-hormone parameters.

Materials and methods: Samples were selected from the laboratory routine, electing concentrations within assay linearity range for each test. Then, samples were tested for Insulin-like Growth Factor 1 (IGF-1), Insulin-like Growth Factor Binding Protein 3 (IGFBP-3), Growth Hormone (GRH), Prolactin (PRL), Sex Hormone Binding Globulin (SHBG), C-Peptide (Pep-C), Homocysteine (HCY), Adrenocorticotropic Hormone (ACTH) and Intact Parathyroid Hormone (iPTH) on IMMULITE 2000® System (Siemens Diagnostics Healthcare) and results analyzed with the ELC tool.

Results and Discussion: AMR studies were carried out according to CLSI Guideline EP6-A. Results are shown in the following table bellow.Tested Samples showed satisfactory linearity results for each parameter. The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level. All results analyzed by ELC, presented Assay Linearity Range as established by the manufacturer, within CLSI Guideline EP6-A and Total Error Laboratory's target.

Conclusion: We conclude that the implementation of a self-verification program as ELC can increase efficiency and accuracy of procedures and results; aiding laboratory on accomplishing Quality Control Program requirements and guaranteeing safety and reliability of their released results.

Table 1 -

Assay	Assay Linearity Range	Obtained CV %	CV Lab max Target %	Obtained TE %	TE max Target %	Linear Regression	R2
IGFBP3	0.1 - 16 ug/mL	1.40	6.3	14.6	17.50	1.09x + (-0.236)	0.99377
IGF1	20 - 1600 ug/dL	2.80	4.7	8.8	14.90	0.985x + (2.037)	0.99995
GRH	0.05 - 40 ng/mL	3.20	4.6	12.6	20.00	0.982x + (-0.246)	0.99634
PRL	0.5 150 ng/mL	2.50	5.9	14.7	21.10	0.93x + (3.362)	0.99509
SHBG	2 - 180 nmol/L	3.20	6.2	13.2	21.10	0.946x + (1.952)	0.99837
Pep C	0.1 - 20 ng/mL	2.40	8.3	11.2	20.80	1.03x + (-0.044)	0.99956
HCY	2 - 50 umol/L	7.70	6.4	15.3	17.70	1.053x + (-0.145)	0.99986
ACTH	5 - 1250 pg/mL	3.94	4.6	9.1	11.82	0.986x + (3.936)	0.99844
IPTH	3 - 2500 pg/mL	6.73	13.0	20.4	30.20	1.042x + (51.56)	0.98744

A-176

Clinical Applications Of Adiponectin Measurements In Type 2 Diabetes Mellitus - Screening, Diagnosis and Marker of Diabetes Control

O. A. Mojiminiyi, N. Abdella. *Faculty of Medicine, Kuwait University, Kuwait, Kuwait*

Background: Adipose tissue-derived adiponectin has pleiotropic protective effects with suppression of inflammatory and metabolic derangements that may result in insulin resistance, metabolic syndrome, Type-2 diabetes (T2DM) and cardiovascular disease. No study has evaluated the potential clinical significance of adiponectin measurements that may be useful in routine practice. The aim of this study was to evaluate adiponectin as a screening tool and diagnostic marker of T2DM and diabetic control.

Methods: Fasting adiponectin, insulin and glucose and HbA1c were determined in 575 subjects with undiagnosed diabetes but with family history of T2DM. To evaluate adiponectin as a marker of DM control, we studied 376 patients with known T2DM duration of 12.4 ± 8.1 years. Clinical and anthropometric data were recorded and subjects were classified on the basis of the degree of adiposity, insulin resistance (IR) using the homeostasis model assessment, and achievement of target HbA1c levels < 53mmol/mol. Using standard cut off values for glucose and HbA1c, receiver operating characteristic curve (ROC) analysis was used to examine the diagnostic performance characteristics for undiagnosed DM.

Results: In undiagnosed subjects, adiponectin was significantly lower in subjects with IR (7.0 vs 8.5 µg/mL) and diabetic subjects (7.4 vs 8.6 µg/mL) compared with those without. 73 of 575 subjects were found to have T2DM. Binary logistic regression showed that the odds ratio of T2DM as predicted by adiponectin was 0.88 [95% confidence interval 0.80-0.96; p = 0.007]. At cut-off points of 7.5 µg/mL, the diagnostic sensitivity and specificity of adiponectin for T2DM were 88% and 51% respectively. Using the ADA glucose and HbA1c diagnostic criteria as reference, the area under the adiponectin ROC curve for diagnosis of DM was 0.740 (95% CI 0.570 - 0.910). In known T2DM subjects, those with good control (HbA1c < 53mmol/mol) had significantly higher adiponectin (8.5 vs 7.1 µg/mL) compared to subjects with poor control.

Conclusions: Adiponectin levels are associated with better glycemic control and could be useful adjunct for screening for IR and T2DM. Therapeutic measures that increase adiponectin levels might be valuable targets for improving diabetes control and decreasing complications.

A-177

Performance of GH stimulation tests at a private laboratory in Brazil: Is insulin tolerance test still the best?

P. B. M. C. Araujo, K. Marca, L. Santos, J. Duarte, A. C. Bispo, M. Freire. *Diagnósticos da America S.A., Rio de janeiro, Brazil*

Background: Growth hormone deficiency (GHD) is the most important endocrine cause of short stature in childhood. Once growth hormone (GH) secretion is pulsatile, diagnosis of GHD is made with a combination of clinical assessment, IGF1 and

IGFBP3 levels, and GH stimulation tests. In Brazil, the most common GH stimulation tests are insulin tolerance test (ITT), clonidine and glucagon. The aim of this study was to determine the performance of those GH provocative tests and to make comparisons among them and with IGF1 levels.

Methods: Retrospective assessment of GH stimulation tests, performed during the year of 2013 in children between 4 to 18 years old, including 141 ITT (mean age 11,72 2,58 yrs, 72,3% male), 285 clonidine tests (mean age 10,41 2,91 yrs, 76,5% male) and 42 glucagon tests (mean age 7,00 3,4 yrs, 66,7% male). The mean dose of each medication administered was respectively 0,074UI/kg, 0,138 mg and 0,568 mg and the mean glucose nadir at ITT was 27,95 8,02. Comparison among tests showed statistical difference regarding peak stimulated GH (GH > 5,0 ng/mL) between ITT and glucagon (p<0,01) and ITT and clonidine (p<0,01), but not between glucagon and clonidine tests (p=0,1014). Peak stimulated GH happened at 26,24% (mean GH 10,62 5,78), 82,10% (mean GH 11,88 5,79) and 71,43% (mean GH 11,63 7,38) from ITT, clonidine and glucagon tests, respectively. GH peaks concentration was at time of hypoglycemia at ITT (70,6%), at 60 minutes after stimulation with clonidine (60,3%) and at 2h after stimulation with glucagon (63,3%). Levels of IGF1 did not correlate with GH answer to the stimulation at insulin (p=0,6165), clonidine (p=0,4914) and glucagon tests (p=0,5551).

Conclusion: Our finding that clonidine and glucagon tests presented a better rate of response compared with ITT represents a new scenario for GH provocative tests, once ITT has been considered the gold standard test for GHD investigation. Maybe it could be explained by the fact that in a private laboratory environment, we can not let the patient recover spontaneously, which could increase side effects. Instead, the recovery from hypoglycemia is done with oral glucose, which may impair the GH peak, finding reported by Yeste and cols. Hence, clonidine and glucagon tests emerge as a reliable and safer alternative to ITT. Surprisingly, IGF1 levels did not correlate with rates of GH response to the tests, which highlights the importance of a clinical/laboratory combined evaluation.

A-180

A Fully-Automated 1,25-Dihydroxy Vitamin DXp Assay on the IDS-iSYS Automated System

J. Tran[1], D. Bautista[2], Z. Seres[3], L. Cornaut[4], T. Gundlach[3], A. Rousseau[4], H. Griesser[3]. *[1]Immunodiagnostic Systems Ltd, Westminster, CA, [2]Immunodiagnostic Systems Ltd, San Clemente, CA, [3]Immunodiagnostic Systems Ltd, Boldon, United Kingdom, [4]Immunodiagnostic Systems Ltd, Pouilly, France*

1,25-Dihydroxyvitamin D (1,25D) is one of the major regulators of calcium metabolism. Due to its lipophilic nature and low circulating concentration, the measurement of 1,25D concentration levels has been labour intensive and technique dependent in addition to multiple equipments required for the sample purification procedure. We reported the results of fully automated IDS-iSYS 1,25 VitD^{Xp} assay.

IDS-iSYS 1,25 VitD^{Xp} assay purifies 1,25D in human sera utilising the anti-1,25D antibody coated magnetic particles in cuvette 1. After incubation, the magnetic particles are washed and 1,25D is eluted. The eluate is transferred to cuvette 2 where the immunoassay procedure is performed utilising the IDS-iSYS 1,25-Dihydroxy Vitamin D test. The purified 1,25D competes with 1,25D-Acridinium (1,25D-ACR) for a limited amount of biotinylated anti-1,25D antibody sites. Bound complexes are captured via streptavidin-coated magnetic particles. Following washing, the bound 1,25D-ACR is measured where signal generated is inversely proportional to the 1,25D concentration in the sample. Below are the preliminary analytical performance of the IDS-iSYS 1,25 VitD^{Xp} (iSYS XP125) assay:

Performances	Results
Functional sensitivity	<12.0pg/mL
Inter-assay precision	16.0% (23.5pg/mL)
	8.6% (59.4pg/mL)
	9.6% (79.3pg/ml)
	6.9% (141pg/mL)
Linearity	92-109%
Method comparison against IDS-iSYS 125D (n = 81)	iSYS XP125 = 0.87 x (iSYS 125D) +6.4pg/mL r = 0.95

Combining the innovative on-board sample purification procedure with the already proven IDS-iSYS 1,25 Dihydroxy Vitamin D test, the IDS-iSYS 1,25 VitD^{Xp} delivers accurate results for patient care while enhancing the clinical laboratory 1,25D testing efficiency.

A-181

POST-FIRE STRESS REACTIVITY IN VOLUNTEER FIREFIGHTERS

M. A. LEGUIZAMON[1], S. UCA-Villarrica[2], A. Lird[3], C. Mora[2], M. Cabral[4]. *[1]Laboratorio Central del Hospital de Clínicas. Facultad de Ciencias Médicas. Universidad Nacional de Asunción. San Lorenzo-Paraguay, ASUNCION, Paraguay, [2]Universidad Católica "Nuestra Señora de la Asunción", Campus Guaira. Facultad de Medicina. Cátedra de Bioquímica. Guairá-Paraguay, VILLARRICA, Paraguay, [3]Laboratorio Central del Hospital de Clínicas. Facultad de Ciencias Médicas. Universidad Nacional de Asunción. San Lorenzo-Paraguay, SAN LORENZO, Paraguay, [4]Instituto de Investigaciones en Ciencias de la Salud., ASUNCION, Paraguay*

Firefighters belong to a population especially sensitive to stress factors. The responsibility for "people" sharpen the stress feeling due to the need of pushing oneself, sometimes more that one can. The physical and emotional stress, just like diseases, could increase the levels of cortisol because during the normal response to stress, the hypophysis secretes more corticotropin. **Objective:** To determine the stress level, the analysis of the level of cortisol in serum in Volunteer Firefighters of Caaguazú, before and after a 12-hour duty is presented. **Material and methods**: observational and longitudinal design. Subjects: Male and female volunteer firefighters of any rank and in activity from Caaguazú-Paraguay that accepted blood drawing and do not present disease of the suprarenal glands like Cushing Disease. The determinations performed were levels of cortisol before and after a 12-hour duty. **Results**: Thirty two firefighters participated, 81% were men and 34% were aspirants. Age range was 18 to 33 years. In the first simple, there was a mean of 10.58±5.2 ug/dl and in the second 12.42±5.3 ug/dl. (p=0.0001). There was an increase of 15.43% in the aspirants, 22.29% in the firefighters and 4.34% in the instructors. In the others positions, such as driver and members of the board of directors there was an increase in the level of cortisol of 18.39%. **Conclusions:** the rank of the firefighters as well as the years of experience in the community service influence considerably in the stress level as the aspirants presented more variation in the level of cortisol in serum after the stress situation.

Keywords: cortisol, stress, firefighters.

A-182

Evaluation of a direct HbA1c Assay on a fully automated Chemistry Analyzer versus two common HPLC methods.

D. Rampersaud[1], H. Lee[1], W. Walters[2]. *[1]Clinitox Diagnostix Inc, Mississauga, ON, Canada, [2]Pointe Scientific, Canton, MI*

Pointe Scientific, Inc. (now part of MedTest) has developed a latex-enhanced immunoturbidimetric assay that directly measures the % HbA1c in whole blood. This new method was adapted to a Roche Hitachi 917 clinical chemistry analyzer in a fashion that offline sample pretreatment was not required. The objective of this study was to evaluate the performance of this direct HbA1c assay on the Hitachi 917 chemistry analyzer versus a Tosoh G8 and a Bio Rad Variant II (HPLC methods) at a large commercial lab.

Samples with normal hemoglobin that were run on the Tosoh G8 versus the Hitachi 917 showed a correlation of R=0.987, with a regression equation of y = 1.18x – 0.80. (n=172). Additional samples containing various hemoglobin variants were compared to the results from the Biorad and Tosoh systems. Utilizing all samples (normal and variants) resulted in a correlation of R=0.958, with a regression equation of y = 1.15x – 0.77. (n=256). High and low Biorad QC materials were run for Day-to-Day and Within Run precision. Results are shown below:

Day to Day Precision				Within Run Precision		
Average	4.83	9.21		Average	4.75	9.27
Std Dev	0.144	0.166		Std Dev	0.165	0.082
%CV	2.97	1.80		%CV	3.47	0.89

The data shows that for normal samples, performance of the Pointe Scientific HbA1c assay is comparable to the Tosoh HPLC system. When Biorad and Tosoh variant sample results were included, the correlation was minimally affected. These results demonstrate that this assay can be a viable solution for large volume testing environments where HPLC systems may not be capable of handling a very high volume of samples.

A-183

Analytical evaluation of a glycated protein method on the Siemens ADVIA 1800

T. Higgins, K. Rodriguez Capote, K. Tovell, D. Holmes, J. Dayton. *DynaLIFEDx,, Edmonton, AB, Canada*

Background HbA1c has become the gold standard for assessment of glycemic control in individuals with diabetes mellitus. However the test cannot be used if the individual has a homozygous hemoglobin variant or a condition in which there is a rapid turnover of red blood cells. In these cases glycated protein has been proposed as an alternative assay. We wished to evaluate the analytical performance of the Diazyme Glycated Protein assay, establish a reference interval and to compare the glycated protein results against HbA1c results.

Methods The Diazyme Glycated Protein assay was programmed onto a Siemens ADVIA 1800 analyzer using a program supplied by the reagent manufacturer. Glycated protein was analyzed on samples that had serum albumin, total protein and HbA1c results available. Samples with a bisalbumin were also analyzed for glycated protein by 2 methods.

Results The within run imprecision was calculated at 1.2% and 0.4% respectively at glycated protein concentrations of 181.2umol/L and 684.2 umol/L. At the same concentrations the between run imprecision was 2.3% and 1.2% respectively. Correlation of glycated protein against HbA1c gave a regression equation of y (glycated protein) = 56.41(HbA1c)-35.72, (n=155, r^2=0.91) The reference range was calculated on 42 samples that had both glucose and HbA1c within the respective reference intervals and was 165 (90% CI 145 to 186) to 367 (90%CI 340 to 386) umol/L. This compares with the manufacturer's suggested reference interval of 100 to 295 umol/L. Using the ratio glycated albumin /albumin the reference interval was 10.5 (90%CI 9.8 to 11.4) to 18.5(90%CI 17.5 to 19.4). Linearity was established from 10 to 1150 umol/L. Comparison of Diazyme Glycated Protein results on bisalbumin samples with a glycated albumin method, Lucica GA-L, gave a correlation equation of y(Diazyme)=-0.5 (Lucica GA-L) +6.2 (n=25,) r^2=1, two tailed T test p=0.14).

Conclusion The analytical performance of the Diazyme Glycated Protein was satisfactory although the reference interval obtained was higher than that suggested by the manufacturer. Comparison against a glycated albumin method for bisalbunemia samples was good although the absolute values are different. The effect of albumin variants needs to be further evaluated.

A-184

Hypercalcemia with Normal PTH: A Diagnostic Puzzle?

O. Krasnozhen-Ratush, L. Bilello, S. Weinerman, L. K. Bjornson. *Hofstra-NSLIJ Scool of Medicine/North Shore-LIJ Health System, Manhasset, NY*

Introduction: The two major causes of hypercalcemia are primary hyperparathyroidism and malignancy, where patients generally have elevated PTH results in the former and suppressed PTH in the latter condition. However, there is a smaller group of patients who present with mild hypercalcemia and normal PTH that can be a diagnostic puzzle if clinical history and condition are unremarkable.

Objective: To determine the prevalence of hypercalcemia with normal PTH in a large endocrinology practice in an integrated health system and to determine what effect correcting (adjusting) the calcium concentration for albumin would have on the classification of hypercalcemia.

Results/Discussion: 3,958 calcium results were retrieved by a computer search of the data base from the endocrinology faculty practice at North Shore-LIJ Health System in Long Island, NY from January 2013 through January 2014, where 155 (3.9%) of these results have been classified as hypercalcemic, i.e., above 10.5 mg/dL. This study focused on calcium, PTH and albumin results without any patient history or other clinical information. Within this original hypercalcemic group a subgroup of 42 (27.1%) patients were identified with normal PTH results. For this subgroup albumin results were also retrieved and a corrected calcium concentration was calculated according to a standard textbook equation; Ca (corrected) = Ca (measured) + 0.8 x (4.0 – Albumin), where the calcium and albumin units of measure are mg/dL and g/dL, respectively. From the original subgroup of 42 patients, 18 (42.9%) were reclassified to normocalcemia based on corrected calcium results whereas 24 (57.1%) remained in the hypercalcemia classification. While the correction of calcium for albumin is not routinely performed or reported in most laboratories, its' primary application has been for patients with hypoalbuminemia and not for patients with normal or high normal albumin as in this subgroup. However, the fact that a corrected calcium concentration reclassifies nearly half of these patients as normocalcemic gives rise to questions regarding the original classification of hypercalcemia based on total calcium concentrations. While measurement of free (ionized) calcium is considered a more accurate assessment of calcium status than total calcium, most laboratories primarily perform total calcium measurements for outpatients since this assay is easily automated and provides rapid, cost effective results. Outreach physicians do not often order free calcium because it is more expensive, has special requirements for blood collection and is usually not required for diagnosing calcium abnormalities. However, corrected calcium results are an estimation and free calcium measurements should be performed if the total calcium measurement is in question.

Conclusions: We have investigated a subgroup of hypercalcemic patients that have normal PTH results and have found that nearly half (42.9%) are reclassified as normocalcemic when the total calcium concentration is corrected for albumin. Since corrected calcium concentrations are an estimation it is suggested that measurement of free calcium may provide a more accurate assessment of calcium status for some patients in this subgroup.

A-185

Validation of a productive platform for HbA1c testing

N. Z. Maluf, A. L. N. Camilo, C. Rosin, C. F. d. Pereira, O. V. P. Denardin. *DASA, Barueri, Brazil*

Background: The current guidelines for diabetes have recommended the use of HbA1c testing for diagnosis as well for monitoring of diabetes mellitus type 2. In HbA1c testing has the important role of diagnosis, there is the need for accurate and precise methods for quantification as recommended by the National Glycated Hemoglobin Standardization Program (NGSP). The purpose of exceeding the expectations of customers makes the labs have to consolidate their growth strategies and strengthen its activities in order to deliver results quickly, with quality and updated methodologies. Large routines demand faster methods which are efficient and have unquestionable quality results. DASA's routine faces an average of 917,000 tests per month where we need to ensure that the time spent for tests processing do not generate a negative impact on laboratory routine, while guaranteeing that the integrity of the samples and the results of high quality promote the positive impacts within established time to deliver results. This study aims to validate a platform which improves productivity, reduces the time for analysis of samples and the number of installed equipments, enables the allocation of resources and maintains acceptable correlation with the methodology currently used for HbA1c testing.

Methods: HbA1c for Siemens ADVIA 2400 Chemistry ® is a turbidimetric assay with range from 2.9% to 15.4%. To assess the assay's correlation, we analyzed 100 whole blood samples collected in EDTA K2 tubes. The samples were divided according to the three following ranges: 4 to 6%, 6 to 14% and >15%. The results were compared with results obtained from Tosoh G7 ® platform. To compare the productivity, time to analyze the samples and TAT (Turn Around Time) were used 5 days of LIS data for routine performed at Tosoh G7 ® and a second period held on ADVIA 2400 Chemistry®.

Results: The comparative analysis of the results revealed a correlation coefficient (r) of 0.98 for HbA1c in ADVIA ® 2400 Chemistry and a linear regression equation y = 0.9808 x + 0.2838 and R2 = 0.9579. When we verified LIS data analysis, the total processing time was 10.6 hours for a routine of 7,422 samples on five platforms Tosoh G7 ® and 4 hours for a routine of 8,550 samples on one platform ADVIA 2400 Chemistry ®. The TAT for ADVIA Chemistry 2400 ® was 06 hours and 33 minutes and the Tosoh G7 ® was 20 hours and 32 minutes.

Conclusion: In the present study, Siemens HbA1c assay for the ADVIA Chemistry System has a proper correlation with the results of the Tosoh G7 ®. Thus, it ensures that the migration of this assay to tested platform causes no significant difference in results and clinical conduct. Furthermore, ADVIA Chemistry ® 2400 presented higher throughput and a 68% reduction of TAT compared to the Tosoh G7 ®.

A-186

The correlation of Fasting and Post Prandial Plasma Glucose with Estimated Average Glucose Levels

S. Ozdemir, N. Ozcan, M. Yavuz Taslipinar, F. Ucar, Z. Ginis, A. Guneyk, A. E. Arzuhal, E. Bulut, G. Erden. *Ankara Diskapi Yildirim Beyazit Research and Training Hospital, Department of Clinical Biochemistry Ankara, Turkey*

Background: Association of fasting plasma glucose (FPG) and post prandial plasma glucose (PPG) on hemoglobin glycation is still controversial. Estimated average glucose (eAG) is a value calculated by hemoglobin A1c (HbA1c) that represents the integrated values for glucose over the preceding 8-12 weeks. The 'A1C-Derived Average Glucose (ADAG)' study showed a linear relationship between HbA1c and

eAG in both diabetic and non-diabetic patient populations. The aim of the present study was to investigate the correlation of fasting and post prandial plasma glucose with estimated average glucose levels.

Methods: This retrospective study includes 7887 subjects with HbA1c data(within a 4 months period). Three subgroups were created: group 1 with FPG (n=4596; 2218 diabetic, 2478 non-diabetic), group 2 with PPG (n=2775, 1683 diabetic, 1092 non-diabetic), and group 3 with FPG and PPG (n=516; 280 diabetic, 236 non-diabetic). The equation published by the ADAG study was used to obtain eAG with the following formula: eAG mg/dL = 28.7x HbA1c(NGSP,%)-46.7 [eAGmmol/L=1.59x HbA1c(NGSP,%)-2.59]. HbA1c values were measured by boronate affinity chromatography methods (Trinity Biotech, Premier Hb9210, Ireland). Glucose was measured by glucose oxidase method (ADVIA® 2400 Chemistry System, Siemens Healthcare Diagnostics Inc.,Tarrytown USA). The correlation coefficients and their significance were calculated using the Pearson test.

Results: In all subjects for group 1 and 2, FPG and PPG showed a strong positive correlation with eAG (r=0.817, p<0.01 and r=0.853, p<0.01 respectively). There was a positive correlation between FPG and eAG in group 1(diabetic subgroup r=0.643; p< 0.01 and nondiabetic subgroup r=0.397; p<0.01). A positive correlation was observed between postprandial glucose and eAG in group 2 (diabetic subgroup r=0.762; p< 0.01 and nondiabetic subgroup r=0.357; p<0.01). It was also found out that eAG had positive correlations with FPG and postprandial glucose in group 3 (diabetic subgroup r=0.725; p< 0.01 and nondiabetic subgroup r=0.581; p<0.01; diabetic subgroup r=0.632; p< 0.01 and nondiabetic subgroup r= 0.255; p<0.01 respectively).

Conclusion: eAG values obtained from HbA1c were highly correlated with FPG and PPG values. Thus, we may suggest that blood glucose expressed as eAG improves the understanding of blood glucose monitoring.

A-187

Development of quantitative Estradiol assay for fully automated analyzer LUMIPULS™ G1200

Y. Kitamura, Y. Ishii, H. Murakami, S. Kojima, M. Tomita, S. Sato, K. Aoyagi. *Fujirebio.Inc, Tokyo, Japan*

Background: Estradiol (E2) is one of a female steroid hormone which is produced in ovarian tissue. Mainly it is used for monitoring of ovarian hypo-function or infertility treatment. In this time, we developed new reagent (Lumipulse G E2-III) which has excellent correlation with ID-GC/MS and reference materials (IRMM) and improved cross reactivity to some drugs or E2 derivative. Lumipulse G E2-III is one-step immunoassay, and E2 in specimen samples and ALP-labeled E2 competitively react with anti E2 monoclonal antibody coated on the micro particles. It is finally detected based on CLEIA technology. Here we show the excellent fundamental performance with fully automated chemiluminescence analyzer LUMIPULSE G1200.

Methods: The sample types used for this study were serum or Heparin-Li. Correlation with ID-GC/MS, commercial competitive kit, matched pair correlation between serum and plasma, cross-reactivity to drugs, within-run precision, limit of detection (LoD) and limit of quantification (LoQ) were evaluated following recommendation from CLSI documents EP-5, EP-7, EP-12, EP-14 and EP-17. All evaluations were executed with LUMIPULSE G1200 (FUJIREBIO INC.).

Results: Correlation with ID-GC/MS with 25 specimen samples was excellent (slope: 1.04, regression: 1.00) and the measurement value in Lumipulse G E2-III calibrators was traceable to BCR577 reference materials. The significant correlation with the commercial available kit with 79 specimen samples was observed (Cobas, slope: 0.93, regression: 1.00, Centaur, slope: 1.06, regression: 1.00). Correlation between serum and heparin-Li with 56 matched pair samples was excellent (slope: 1.03, regression: 1.00). Within-run precision % CVs for our assay ranged from 1 to 3% when 3 different conc. of samples were tested, the calculated LoD is at 15 pg/mL and the LoQ ranged from 13 pg/mL to 17 pg/mL. As a result of evaluation with total 43 kinds of drugs and E2 derivatives, cross reactivity with almost all cross reactants were < 0.1 %.

Conclusion: These results demonstrated that improved Lumipulse G E2-III is a precise and highly useful for measuring E2 in serum and heparin-Li. Also this assay is perfectly traceable to ID-GC/MS and reference materials.

A-188

Plasma total homocysteine, high - sensitivity C- reactive protein and thyroid function in metabolic syndrome patients in Port Harcourt, Nigeria.

C. G. Orluwene[1], M. O. Mommoh[2]. *[1]University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria, [2]NNPC Zonal Industrial Hospital, Port Harcourt, Nigeria*

Aims and Objectives: Metabolic syndrome is assuming epidemic proportions in most populations of the world and so are its co-morbidities. Metabolic syndrome, hypothyroidism, total plasma homocysteine and C-reactive protein are independent risk factors for cardiovascular disease. There could be an association between these risk factors. The aim of our study was to examine the levels of thyroid hormones, total plasma homocysteine and C-reactive protein in metabolic syndrome patients and assess the possibility of an association between these four risk factors for cardiovascular disease.

Patients and Methods: A total of 93 subjects were recruited for this study. (48 with metabolic syndrome and 45 as the control group). Basic demographic data, components of the metabolic syndrome, thyroid hormones, high-sensitivity C-reactive protein and total plasma homocysteine were estimated for all subjects using standards methods. Appropriate statistic was used for data analysis.

Results: Components of the metabolic syndrome, thyrotropin, total plasma homocysteine and high sensitivity C-reactive protein were significantly higher in the study group (P<0.05) while free thyroxine and high density lipoprotein cholesterol were significantly lower in the study group (P<0.05). The predominant type of thyroid dysfunction in the study group was sub-clinical hypothyroidism (87.5% in the study group compared to 18% in the control; P<0.05). Logistic regression showed significant association between sub-clinical hypothyroidism, total plasma homocysteine and high-sensitivity C-reactive protein in the study group.

Conclusion: There was a strong association between metabolic syndrome, sub-clinical hypopthyroidism, total plasma homocysteine and high-sensitivity C-reactive protein. Females are at an increased risk of this association.

Keywords: Metabolic syndrome, Thyrotropin, Sub-clinical hypothyroidism, total plasma homocysteine, high-sensitivity C-reactive protein.

A-189

An Evaluation of Three Novel Biomarkers: Total-sLHCGR, LH-sLHCGR and hCG-sLHCGR.

M. Jansen[1], C. F. Rosmalen[1], S. Banerjee[2], A. E. Chambers[2]. *[1]NBCL B.V., Nijmegen, Netherlands, [2]Origin Biomarkers, Lanarkshire, United Kingdom*

Background: Researchers in the field of human reproduction need better research tools for the care of pregnant woman (e.g., prenatal diagnosis) and women who wish to become pregnant (e.g., fertility treatment). The assays for the soluble receptor for both human chorionic gonadotropin (hCG) and human luteinizing hormone (hLH) and the same soluble receptor bound to either hCG or hLH could be used as such research tools. The receptor is called the soluble LH/hCG receptor or sLHCGR. Three different ELISA assays have been developed and based on the type of HRP-labeled detector antibody employed, the user can quantitate Total sLHCGR, a complex of LH with sLHCGR or a complex of hCG with sLHCGR in a standard microtiter plate format. **Methods:** The levels of Total-sLHCGR, LH-sLHCGR or hCG-sLHCGR were measured using sandwich ELISA. The three ELISA formats are the same; samples were diluted 5-fold, incubated for 10 minutes in a microtiter plate coated with a monoclonal capture antibody directed against the receptor. Next the HRP-labeled conjugate was added and incubated for 1.5 hour to generate the sandwich. After a wash step the substrate was added and after 20 minutes the reaction was stopped and read at 450 nm using a microplate reader. The optical densities from the reader are directly proportional to the amount of Total-sLHCGR, hCG-sLHCGR or LH-sLHCGR present in the sample. **Results:** The analytical sensitivity for the assays are 0.01, 0.004, and 0.02 Pmol/mL for the Total-sLHCGR, hCG-sLHCGR and LH-sLHCGR, respectively. The within assay precision was done at three different levels for the three assays (n=16). For hCG-sLHCGR the CV% are 4.4, 3.5, and 3.5% at 2.1, 11.7 and 18.0 Pmol/mL, respectively. For LH-sLHCGR the CV% are 3.2, 3.7, and 3.5% at 2.1, 11.2 and 28.9 Pmol/mL, respectively. For Total-sLHCGR the CV% are 7.4, 6.5, and 9.0 % at 3.5, 4.3 and 11.5 Pmol/mL, respectively. Inter-assay precision was determined for the three assays (n=4) and were for the hCG-sLHCGR 2.7, 7.7 and 4.2% at 2.2, 12.7 and 19.1 Pmol/mL, respectively. For LH-sLHCGR the CV% were 3.5, 3.6, and 2.9% at 2.1, 11.6 and 30.3 Pmol/mL, respectively. For Total-sLHCGR the CV% were 8.9, 8.6, and 11.0% at 3.7, 5.2, and 11.8 Pmol/mL, respectively. Sample recoveries for the three assays are between 80.6 and 101.7% **Conclusion:** Three

simple, fast ELISA assays for the detection of Total-sLHCGR, hCG-sLHCGR and LH-sLHCGR in serum or plasma were evaluated and were shown to be precise and sensitive for the detection of these novel biomarkers.

A-190

Iatrogenic Vitamin D Toxicity in an Infant: Clinical Relevance of Vitamin D Metabolic Profiling

H. Ketha, H. Wadams, M. Fahse, A. Lteif, S. Grebe, R. J. Singh. *Mayo Clinic, Rochester, MN*

Background Public concern over vitamin D (vitD) deficiency has led to widespread use of over the counter (OTC) vitD supplements, containing up to 10,000 IU (400IU=10µg). Overzealous use of such supplements can result in vitD toxicity. Infants are particularly vulnerable to toxicity associated with vitD overdose. Mutations in the CYP24A1 gene have been shown to cause reduced serum 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) to 25-hydroxyvitamin D 25(OH)D ratio (<0.02), elevated serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), hypercalcemia, hypercalciuria and nephrolithiasis. Additionally, studies in infants have shown that C3 epimer of 25(OH) D can contribute upto 9-61.1% of the total 25-OH)D. Therefore, measurements of parathyroid hormone (PTH) and vitD metabolites 25(OH)D, $1,25(OH)_2D$, 3-Epi-25-hydroxyvitamin D (3EPI-25(OH)D) and $24,25(OH)_2D$ are useful to diagnose and manage hypercalcemia due to vitD overdose or due to CYP24A1 mutations.

Relevance to Clinical Laboratories Measurement of vitD metabolites 25(OH)D, $1,25(OH)_2D$, 3EPI-25(OH)D and $24,25(OH)_2D$ is of clinical value for differentiating between genetic vs iatrogenic causes of hypercalcemia.

Case A significantly underweight four month old female presented with a three day history of emesis, diarrhea, lethargy and dehydration. The medical work-up revealed hypercalcemia, hypercalciuria and nephrocalcinosis. She had been exclusively breast-fed and had been given OTC vitD supplementation at a higher dose than recommended on the supplement's label. 25(OH)D, $1,25(OH)_2D$, 3EPI-25(OH)D, $24,25(OH)_2D$ and the vitD content of the OTC preparation were measured by LC-MS/MS.

Results Nephrocalcinosis was confirmed by ultrasound studies. Serum cacium (SCa) was 18.7 mg/dL (ref range: 9-11 mg/dL) and PTH was < 6pg/mL (ref range: 15-65 pg/mL) at presentation. Urine calcium was 157 mg/dL with a calcium to creatinine (Cr) ratio of 2618 mg/g of Cr (ref range <2100 mg/g). The vitD content of the supplement was threefold higher (6000 IU of D/drop) than listed on the label (2000IU). Combined with the gross overdosing, this was estimated to have resulted in a daily vitD dose of 50,000 IU for two months. The SCa gradually decreased upon calcitonin injection on day 3, but trended upward again (15.5, 13.3, 12.1, 10.4, 9.8, 10.7, 10.9, 10.3 and 11.6 mg/dL on days 1, 3, 5, 7, 11, 14, 20, 25 and 40 respectively). The 25(OH)D decreased slowly from 294 ng/mL on day 1 to 257, 227, 197, 189, 138, 124 and 84 ng/mL on days 3, 5, 7, 11, 14, 20, 25, and 40 respectively. Serum 3EPI-25(OH)D levels were 44 % to 29 % of the 25(OH)D levels(126-36 ng/mL). Serum $1,25(OH)_2D$ levels were elevated. The ratio of $24,25(OH)_2D$ to 25(OH)D was 0.11-0.14 (ref range: 0.07-0.18).

Discussion Genetic cause of hypercalcemia, hypercalciuria and nephrocalcinosis could be ruled out on the basis of normal $24,25(OH)_2D$ to 25(OH)D ratio. Clear warning labels regarding maximum allowable doses of OTC vitD supplements are of value from a public health perspective.

Conclusion Infants receiving high dose OTC vitD supplementation are vulnerable to vitD toxicity. Vitamin D metabolic profiling is of value for evaluating such cases and to exclude certain genetic causes. Our study also emphasizes the need for stricter regulation of vitD content in OTC supplements and prominent warning labels regarding maximum allowable daily doses.

A-192

Insulin resistance and secretory functions in Pre-diabetics and newly diagnosed diabetics of north-west India: role of adipocyte mediators

P. Sharma, P. Purohit. *All India Institute of Medical Sciences, Jodhpur, India*

Background: Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. There has been a dearth of studies evaluating the natural course of insulin secretion in pre-diabetics progressing to diabetes in north western India.

Aim: To examine the natural history of insulin secretory dysfunction and insulin resistance during the development of diabetes and to examine the role of adipocyte mediators non-esterified fatty acids (NEFA) in development of type 2 DM.

Material and Method: The study was conducted on newly diagnosed, untreated hyperglycaemic patients attending our diabetic clinic. The study subjects were grouped as-Group I: Healthy control (n = 56), Group II: Pre-diabetics (n=39), Group III: Diabetics (n= 124). All subjects were evaluated for waist to hip ratio (W: H), body mass index (BMI), fasting blood glucose, insulin, HOMA-IR, HOMA-beta, NEFA and lipid profile. Statistical analysis was done using ANOVA and Multiple logistic regression analysis.

Results: The diabetic subjects had significantly raised W: H and BMI as compared to the pre-diabetics (0.88±0.06; 27.44±4.62) and controls (0.87±0.06; 24.2±4.34) with the F value 14.64 ($p<0.001$) and 5.98 ($p=0.003$) respectively for W: H and BMI. Age adjusted base line characteristics according to BMI and W: H quintiles for predicting risk of type 2 Diabetes showed significant trends across quintiles for total cholesterol (TCH), triglycerides (TG), HOMA-IR and HOMA-beta. Similarly Age adjusted base line characteristics according to NEFA quintiles for predicting risk of type 2 Diabetes showed significant trends across quintiles for BMI, W: H, lipid profile, insulin, HOMA-IR and HOMA-beta. Finally Multiple logistic regression analysis in newly diagnosed type 2 diabetic subjects with family history (Negative v/s Positive) as a dependent variable showed the strongest risk due to raised NEFA (OR 3.83), followed by HOMA-IR (OR 1.38), TCH (OR 1.35), WC (OR 1.7) and TG (OR 1.13).

Conclusion: We conclude that the insulin secretory rates and IR in pre-diabetics and newly diagnosed type 2 diabetics are associated with BMI, W: H and NEFA. W: H and NEFA can prove to be a strong predictor of type 2 DM even with a negative family history.

A-193

Comparison of Aldosterone and Renin Determination by Conventional Radioimmunoassay and by Automated Chemiluminescente Immunoassay

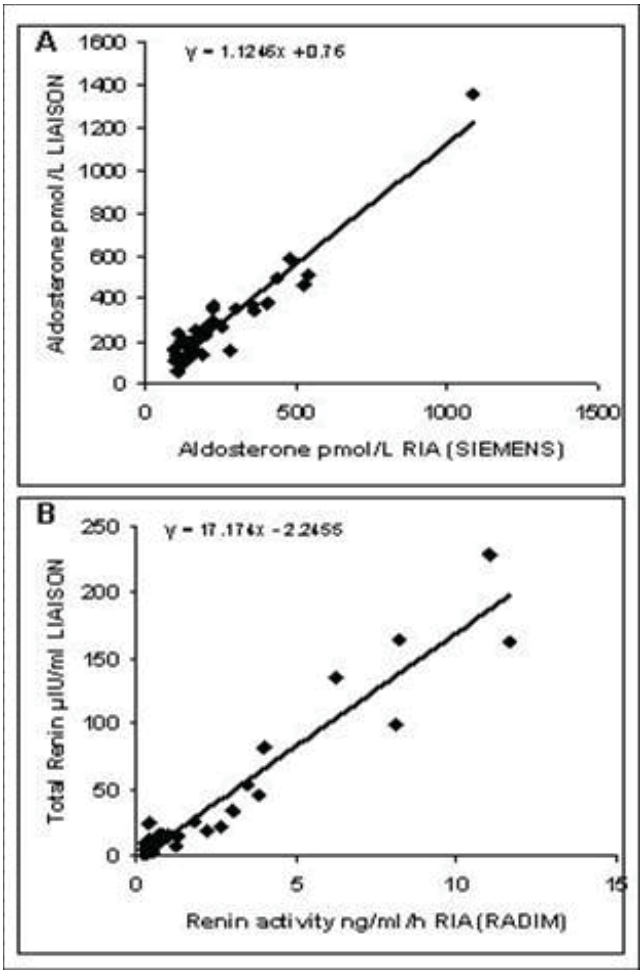
E. Zeruya, Y. Sharabi, H. Kanety, R. Hemi. *Sheba Medical Center, RAMAT-GAN, Israel*

Background: Renin-angiotensin-aldosterone system plays a paramount role in water homeostasis and electrolyte balance and in the regulation of arterial pressure; Aldosterone, major mineralcorticoid secreted by adrenal cortex, keeps water and salt balance helping in maintaining blood pressure. Renin, a proteolytic enzyme synthesized primarily by the juxtaglomerular cells of the kidneys converts angiotensinogen to angiotensin I, which is then cleaved by angiotensin converting enzyme to form angiotensin II. Angiotensin II increases blood pressure directly through vasoconstriction and indirectly by stimulating secretion of aldosterone. Measurement of plasma renin and aldosterone provides a marker of renin-angiotensin-aldosterone system activity.

Objective: To evaluate analytical performances of commercially available automated immunoassays for Aldosterone and direct renin (DiaSorin LIAISON®) and to compare LIAISON results to currently used radioimmunoassay (RIA).

Results: 43 plasma samples were measured for Renin activity (PRA) and Renin concentration (PRC) by RIA (RADIM) and LIAISON, respectively. In addition, 57 serum samples were analyzed for Aldosterone levels by RIA (SIEMENS) and LIAISON. High correlation was found between the two systems both for Aldosterone and Renin (R=0.95 and R=0.96, respectively, Figure 1 A&B). Satisfying clinical concordance of 95% and 100% was found between both LIAISON Aldosterone and Renin assays vs. RIA, respectively. Assays performance (precision and linearity) was tested and found compatible with the manufacturer's declaration.

Conclusion: LIAISON Aldosterone and Direct Renin assays are precise, accurate and reliable and can be used as an alternative to the conventional RIA which is technically demanding and laborious. Our data suggests that the LIAISON Direct Renin assay can be a good diagnostic and practice replacement for the RIA PRA assay, although biochemically, they measure different parameters. Setting normal ranges for the Renin/Aldoseterone ratio on the LIAISON is required in order to diagnose and monitor hypertensive patients.



A-196

Linearity study to define the Analytical Measurement Range (AMR) for immunoassays

A. L. Camilo, D. Waltrick, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: Linearity studies are crucial to assure analytical range accuracy and to validate reagents performance in clinical laboratory. Due to difficulties to evaluate linearity results, a Brazilian laboratory implemented a new tool (Easy Linearity Curve, ELC).ELC established self-inspection program, immune-hormone analytical systems monitoring and quality control requirements accomplishment. We described linearity data for nine different immunoassays: VB12, Folato(FOL), Ferritin(FER), BNP, Cortisol (Cor), C-Peptide(Pep C) and Insulin(Ins).

Materials and methods: Samples were selected from laboratory routine, with concentrations within assay linearity range for each test, performed by chemiluminescence on Advia Centaur® XP (Siemens Healthcare Diagnostics). Linearity was evaluated by calculating the recovery of repetition performed. The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level. In addition, they were analyzed with ELC tool to validate immunoassay's AMR according to CLSI Guideline EP6-A.

Results and Discussion: Tests showed satisfactory linearity results for different samples. They are summarized in the table below.The sample pools were not used, because all the results from this dilution test presented high percentage of recovery, higher than manufacturer's references, due to a matrix effect (data not showed).The main difficulty in linearity studies is to obtain samples in the ideal analytical range. ELC uses an algorithm that suggests samples' dilutions to contemplate the whole AMR for each parameter. In this study, for each analyte, five different samples were selected to simulate ideal concentrations covering the linear range.

Conclusion: Based on our results, we do not recommend the use of sample pools in linearity studies of hormones.The presented protocol permits laboratory autonomy to AMR self-management, solving linearity studies issues, ensuring additional safety and reliability of released results, and Laboratory's Quality Control Improvement.

Table 1 -

Assay	Assay Linearity Range	Obtained CV %	CV Lab max Target %	Obtained TE %	TE max Target %	Linear Regression	R2
VB12	45 - 2000 ng/mL	4.13	7.50	14.35	30.00	0.958x + (50.874)	0.99759
FOL	0.35 - 24 ng/mL	2.43	12.00	23.43	39.00	0.854x + (1.557)	0.96698
FER	0.5 - 1650 ng/mL	5.92	7.10	5.38	16.90	1.109x + (-32.411)	0.99532
BNP	2 - 5000 pg/mL	2.63	2.67	9.59	12.43	1.038x + (-76.888)	0.99672
Cor	0.2 - 75 ug/dL	3.97	10.50	13.76	29.80	1.057x + (-1.134)	0.99941
Pep C	0.05 - 30 ng/dL	5.14	8.30	12.70	20.80	0.985x + (-0.173)	0.99360
Ins	0.5 - 300 mU/L	4.19	10.60	12.74	32.90	0.993x + (4.202)	0.98842

A-197

Analytical performance of the Insulin-like growth factor I (IGF-I) assayin two Immunoassays Systems that used different standardization procedures

M. L. Moreira, M. D. C. Freire. *Diagnósticos da América, Duque de Caxias, Brazil*

Background: Insulin-like growth factor I (IGF-I) is intended for use in the diagnosis and monitoring of children who have growth-related disorders and adults with acromegaly.Large variability exists among different IGF-I assays owing to differences in calibration, antibody specificity, isoform recognition. The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) established criteria for standardization and evaluation of IGF-1 assays(Recombinant IGF-1, coded 02/254) and for the content monitoring of therapeutic products.

Objective:This study intends to evaluate the analytical performance of the IGF-I assay on Siemens IMMULITE 2000 IGF-I (Recombinant IGF-1, coded 87/518) and Liaison Analyser IGF-I Diasorin (Recombinant IGF-1, coded 02/254).

Methods:We tested59 patient's serum samples from DASA, Rio de Janeiro, with concentrations within the range of 25 ng/mL to 1039 ng/mL, in both analytical platforms.The IMMULITE 2000 IGF-I is a 2-cycle, sequential immunometric assay withcalibration range of 20-1600 ng/mL.In the assay procedure, prediluted patient sample is needed to reduce interference from binding proteins before analysis. The photon output is proportional to theconcentration of the analyte.The Liaison Analyser IGF-I is a 1-cycle, immunometric assay with calibration range of IGF-I 10-1500 ng/mL.

Results:Comparison between IMMULITE 2000 and Liaison assaysyielded a correlation coefficient of 0.97, with linear regression of x(IMMULITE 2000)=0.792(Liaison) + 30.48ng/mL. Moreover, means were 313 and 278 ng/mLfor IMMULITE 2000 and Liaison Analyser, respectively. Test t-student was 1.049 (expected is 1.98) and estimated total error (TE) was 20.2% which is lower than the allowed TE (22.35%) in all levels of decision-making practice.

Conclusion:Preliminary assessment of these clinical evaluation results indicates that the IMMULITE 2000 IGF-I immunoassay is a precise method for measuring IGF-I in serum across a wide range of clinically relevant concentrations and shows good correlation to the Liaison IGF-I assaydespiteof using different standardization.

A-198

Diiodothyropropionic acid interferes with TT3 and FT3 measurements on common Immunoassay Platforms for Thyroid Function Panel.

X. Yi, S. Refetoff, E. K. Y. Leung, K. T. J. Yeo. *The University of Chicago, Chicago, IL*

Background: Monocarboxylate transporter 8 (MCT8) is a thyroid hormone-specific cell membrane transporter. MCT8 deficiency produces in young males an unusual pattern of thyroid hormone abnormality with elevated serum triiodothyronine (TT3) and causes severe neuropsychiatric defect. A thyroid hormone analogue, diiodothyropropionic acid (DITPA) was found to enter cells independently of MCT8. Therefore, this compound was tested in children with MCT8 deficiency and found to normalize their thyroid function tests and improve their nutritional status. A problem in the follow up of DITPA treatment is the interference of DITPA in the routine laboratory measurement of TT3. This necessitates the measurement of DITPA by LC-MS/MS to correct for its interference in TT3 determination by immunoassay. The objective of this study was to evaluate the possible interference of DITPA on commercial thyroid assays available from four in-vitro diagnostic companies. **Method**: Pooled human serum was collected and stock DITPA (1mg/ml) was added to create a serum set containing 3 -75 µg/dL DITPA. This sample set was then assayed for TT3, TT4, free T3, free T4, and TSH on the Roche Elecsys, Siemens IMMULITE, Siemens ADVIA Centaur, Siemens Dimension EXL, Siemens Dimension RXL,

Beckman Access, and Abbott Architect platforms, where available, respectively. All samples were analyzed in duplicate and the respective assay values in the DITPA-treated samples were expressed as the difference above the baseline sample. In addition, to investigate if the interference of DITPA could be overcome by excess TT3, serum samples at a constant concentration of 20 µg/dL DITPA were spiked with increasing amount of TT3 and measured on Roche Elecsys TT3 method. **Results**: At 75 µg/dL DITPA, the overestimation above the baseline endogenous values were: Elecsys: TT3, 193 ng/dL, FT3, >3000 pg/dL; Access: TT3, 745 ng/dL, FT3, 2405 pg/dL; IMMULITE: TT3, 146 ng/dL; Architect: TT3, 33 ng/dL, FT3, 96 pg/dL, respectively. Minimal interference was observed for ADVIA Centaur TT3 assay at all concentration of DITPA tested. TT4, FT4, and TSH tests were not affected by DITPA on all the immunoassay platforms tested. It is also interesting that with excess TT3, the DITPA interference on the Elecsys TT3 declined from 208% to 114% above baseline. **Conclusion:** DITPA significantly interferes with several commercial TT3 and FT3 assays with the exception of the Siemens ADVIA Centaur TT3 assay. This suggests that the TT3 antibody reagents used in the Elecsys, Access, Immulite and Architect cross-react significantly with DITPA. For patients undergoing treatment, the ADVIA Centaur TT3 assay is suitable for monitoring response to DITPA therapy.

A-199

Gender Differences in the interactions between Adipokines and the Insulin-Like Growth Factor-I System in a Metabolically High Risk Population

O. A. Mojiminiyi, N. Abdella. *Faculty of Medicine, Kuwait University, Kuwait, Kuwait*

Background: Accumulating evidence indicate important roles for the insulin-like growth factor (IGF)/IGF-binding protein (IGFBP) system in metabolic homeostasis. Despite potential molecular mechanisms that link obesity and insulin resistance with the IGF system, the pathophysiological metabolic interactions between adipose tissue derived adipokines and the IGF-I system remain unknown due to conflicting reports in the literature. In this study, we test the hypothesis that gender differences could be responsible in part for the conflicting reports on the associations of some adipokines with the IGF system.

Methods: Fasting adiponectin, resistin, leptin, leptin receptor (sOB-R), insulin, glucose, total IGF-I, IGFBP-3 and full lipid profile were determined in 590 (238M and 352F) first-degree relatives of patients with Type 2 Diabetes Mellitus. Sex hormone binding globulin (SHBG), oestradiol (E2), testosterone (T), were also measured. Free androgen index (FAI), Free leptin index (FLI), bioavailable IGF-1 (BIGF1), beta-cell function (%B), insulin sensitivity (%S) and insulin resistance (IR) (Homeostasis Model Assessment) were calculated. The data were analysed using simple and multivariate regression analyses.

Results: There are significant differences in mean (SEM) BIGF1 between males (87.6 (9.1)) and females (67.7 (4.6)). There were also significant gender differences in adiponectin, leptin, sOB-R, FLI, %S and IR. There were no gender differences in resistin and IGFBP3. Significant gender differences were found in the correlations of BIGF1. The following showed significant correlations with BIGF1 in females but not in males: adiponectin, sOB-R, FLI, SHBG, glucose, insulin, %S, IR, waist circumference, BMI, Apo B, total cholesterol, triglycerides and LDL-cholesterol. Males and females showed similar correlations of all other variables with BIGF1. Correlations with sex hormones (E2, T, SHBG, FAI) were not significant in males and females. Multivariate linear regression analysis showed that age, BMI, WC, adiponectin, FLI were significant determinants of BIGF1 in females but not in males. Age was the only significant determinant of BIGF1 in males.

Conclusions: There are significant gender differences in the metabolic interaction between adipokines and the IGF-1 system. Despite the putative links with obesity, sex steroids do not play a role in the gender differences.

A-200

LC-MS/MS detection of increased Androstenedione levels in patients receiving Danazol therapy

V. Gounden[1], D. El-Maouche[2], B. R. Stolze[1], R. Muniyappa[3], S. J. Soldin[4]. *[1]National Institutes of Health (Clinical Center), Bethesda, MD, [2]National Institutes of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, [3]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, [4]National Institutes of Health (Clinical Center) andDepartment of Medicine, Georgetown University, Washington, District of Columbia, Bethesda, MD*

Background : Danazol is a 17-ethinyl testosterone derivative. Danazol has long been used in the management of endometriosis, however its reported immunomodulatory effects such as reducing interleukin-1 and TNF-α have led to the use of danazol in management of immune related conditions such as aplastic anemia. The side effects associated with danazol are largely due to its androgenic effects. Danazol has been reported to act as an interference in the immunoassay measurement of various androgens (including androstenedione), resulting in falsely elevated values for these hormones. As a consequence measurement of these hormones in patients receiving danazol is best performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Here we report eight cases of significantly elevated androstendione (AND) levels following LC-MS/MS measurement in patients receiving danazaol for aplastic anemia.

Method: LC-MS/MS measurement: AND is measured as part of a steroid hormone panel. Samples were prepared as per previously published method (Guo T et al. Simultaneous determination of 12 steroids by isotope dilution liquid chromatography-photospray ionization tandem mass spectrometry. Clin Chim Acta 2006;372:76-82 and Mendu DR et al Clin Chem 2011 Abstract E-57, pA212). An Agilent 6460 triple quadropole mass spectrometer (Agilent, USA) equipped with an atmospheric pressure photoionization was used, employing isotope dilution with deuterium labeled internal standard for each analyte. Quantitation by multiple reaction monitoring (MRM) was performed in the positive ion mode. The quantifier MRM transition for androstenedione used was 287.2> 97.1 and the qualifier MRM of 287.2 >109 was used to confirm. The ratio of response of the two MRMs used, ranged from 56-58%. Retention times (RT) for AND were 6.540 -6.556 minutes for patient samples and 6.529- 6.554 minutes for the accompanying internal standard (IS). The ratio of androstenedione RT to internal standard RT for each specimen run was 1.00, thus confirming the identity of the peak.

Results: A total of 8 adult patients (female n=5; male n=3) were identified with increased AND values at either 6 or 12 month follow- up post danazol initiation. Baseline AND values for the female patients ranged from 38 - 176 ng/dL (reference interval 17- 175) and at 6 or 12 month follow up values increased markedly and ranged from 8128-33703 ng/dL. For the male patients baseline AND values ranged from 105- 240 ng/dL (reference interval 25-125) and at follow up values increased to range from 5609 to 17325 ng/dL. Similar increases were not observed for the other androgens measured. Of interest two of the three males had elevated LC-MS/MS estradiol levels above the reference interval on follow-up while all the female patients had estradiol concentrations that remained within the appropriate estradiol reference interval during therapy. Whilst on therapy patients responded well and side effects of therapy were reported to be minimal.

Conclusion: One of the important advantages of MS analysis is the greater specificity of over immunoassay based testing In the above described cases use of two MRM transitions (quantifier and qualifier) enabled the laboratory to confirm the presence of elevated AND and exclude the likelihood of an interference.

A-201

Evaluation of the effect of elevated Fetal Hemoglobin (HbF) on three HbA1c Assays Methods in Marshfield Clinic system.

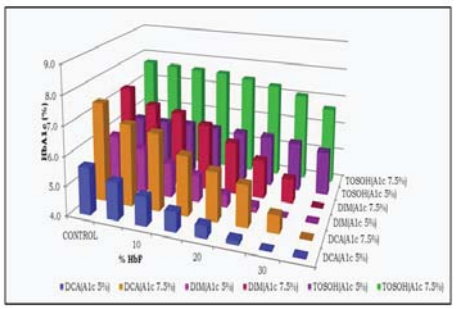
A. KHAJURIA, B. Scheibe. *Marshfield Clinic, Marshfield, WI*

Background: Accurate measurement of HbA1c is crucial for decision making in diabetic control and diagnosis. Elevated levels of HbF are reported to falsely decrease the HbA1c results. There are many clinical conditions presenting with elevated HbF and prevalence of elevated HbF can be as high as 7 to 8% in a diabetic population and Clinicians may be unaware of potential interference with HbA1c results. At a glycemic control target of 6.5% the critical difference between two results within a subject should not exceed ~0.4%. It is therefore, crucial that laboratories are aware, to what extent HbF interference affects HbA1c results.

Methodology: Following commercial assays were evaluated; TOSOHG8 (HPLC), Dimension EXL 200 & DCA 2000 (Immunoassay). Two whole blood EDTA patient pools as Normal (5 to 6%) and Abnormal (7 to 8%) were prepared and incubated with varying concentration of HbF (5 to 40%) by mixing umbilical cord blood with known HbF levels (estimated by G8). The effect of HbF interference was then evaluated relative to control pools. Percent decrease in HbA1c greater than 5% was considered a significant change.

Results: TOSOH G8 did not show any interference with up to 25% HbF concentration. Dimension & DCA 2000, however exhibited a dose dependent interference with HbF (>5%) at 5% HbF concentration.

Conclusions: Elevated HbF can be identified in Ion-exchange HPLC but not in Immunoassays. Laboratory professionals should make clinicians aware of potential interference from elevated HbF levels on a particular method that could adversely affect HbA1c results. Clinicians can then make informed decisions if HbA1c results appear discrepant related to patient history and glucose hemostasis.



A-202

VALIDITY OF A SEMI-QUANTITATIVE METHOD FOR MICROALBUMINURIA SCREENING IN DIABETES MELLITUS

J. D. Santotoribio, C. Cañavate-Solano, A. García de la Torre, F. Arce-Matute, S. Pérez-Ramos. *Puerto Real University Hospital, Cádiz, Spain*

INTRODUCTION Urine Albumin is an early marker of chronic kidney disease (CKD) in diabetic patients. The excretion of recent urine albumin greater than 20 mg/L (microalbuminuria) is considered as a predictor of diabetic CKD. The aim of this study is to analyse the validity of a semi-quantitative method for microalbuminuria screening in diabetes mellitus.

METHODS Recent urine microalbuminuria of diabetic patients were determined by two methods: 1. Semi-quantitative: Colorimetric method using the strip H13 in DIRUY H-800 PLUS (RAL®). The content of microalbuminuria is inversely proportional to the quantity of the color of the reagent pad. The instrument measures the color change of the reagent pad on a scale of 0 to 4000.

2. Quantitative: microalbuminuria was measured by immunoturbidity in COBAS C311 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the quantification of microalbuminuria: positive (microalbuminuria > 20 mg/L) and negative (microalbuminuria < 20 mg/L). Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC).

RESULTS We analyzed 469 diabetic patients between 27 and 85 y.o. (mean age = 56.3), 82 patients (17.5%) had a positive microalbuminuria and 387 patients (82.5%) were negative. The AUC was 0.985 ($p < 0.0001$). With a cut-off color scale less than 1305 determined by the test strip, we obtained a sensitivity of 100% and a specificity of 86.3%. With these results, it would only be necessary the quantification by immunoturbidimetry the samples with a value lower than 1305. In this case it would not have been necessary to measure microalbuminuria in 334 samples of the 469 studied, getting a saving of 71%.

CONCLUSIONS The semi-quantitative method by test strip, can be used as screening for microalbuminuria in diabetic patients with a sensitivity of 100%. Microalbuminuria would only be measured in samples with positive test strip.

A-203

Procollagen of type-1 N-terminal propeptide levels by Elecsys assay correlates with bone formation rate in Chronic Kidney Disease

S. Cremers, D. Dempster, H. Zhou, E. Dworakowski, M. Kamanda-Kosseh, T. Nickolas. *Columbia University Medical Center, New York City, NY*

Background: Renal osteodystrophy is a common metabolic bone disorder due to chronic kidney disease (CKD) that is associated with high risk of bone loss, fracture and death. It is characterized principally by a spectrum of abnormal bone turnover ranging from extremely low (adynamic bone disease) to high (osteitis fibrosa cystica). Bone turnover assessment is an absolute required for treatment. However, bone turnover is currently determined by tetracycline double-labeled transiliac crest bone biopsy with histomorphometry, an invasive, and not widely available procedure. Non-invasive turnover determination in CKD is controversial because most bone turnover markers (BTMs) are renally cleared; thus, in CKD they may be non-specifically elevated and not reflect accurately remodeling activity. Procollagen of type-1 N-terminal propeptide (P1NP) is a biochemical marker of bone formation, it circulates in mono- and multi-meric forms and multimeric P1NP is cleared non-renally. Our group reported that higher circulating total levels of P1NP predicted incident bone loss among pre-dialysis and dialysis-dependent CKD patients. We hypothesized that in CKD high levels of total serum P1NP would correlate with high bone formation rate (BFR) measured by biopsy.

Methods: In 22 patients (male=8; female=14; mean age±SD 68±11 years,) with CKD stages 2-5D, fasting morning blood was collected within 6-months of double tetracycline double-labeled transiliac bone biopsy. Estimated glomerular filtration rate (eGFR) was by MDRD formula. Total serum PINP (mono- and multi-meric) was measured by Electrochemiluminescence immunoassay (Elecsys 2010 analyzer, Roche Diagnostics, Indianapolis, IN). Intra- and inter-assay precision were 1.1% and 5.5% respectively (Reference range = 20-100 ug/L). BFR (µm3/ µm2/day) was determined histomorphometrically in trabecular, endocortical and intra-cortical bone from biopsy using American Society for Bone and Mineral Research (ASBMR) criteria. Data are presented as mean±SD. P1NP and BFR were log transformed prior to analyses and relationships were determined by Pearson correlations.

Results: Five patients were on hemodialysis and mean eGFR in pre-dialysis patients was 36±17 mL/min. Mean BFR at trabecular, endocortical and intracortical regions were 0.018±0.031, 0.019±0.035 and 0.030±0.038 respectively and there were no significant differences in BFR between pre-dialysis and hemodialysis patients. Mean PINP for the total, pre-dialysis and hemodialysis cohorts were 332±585ug/L, 98±51ug/L and 1125±878ug/L respectively. P1NP levels were significantly greater in hemodialysis compared to pre-dialysis ($p=0.004$) and there were no significant relationships between P1NP and eGFR among pre-dialysis patients. There were significant, moderate and direct associations between PINP and BFR in the three envelopes (R2 0.41, 0.34 and 0.34, all $p<0.05$ for trabecular, endocortical and intracortical bone, respectively).

Conclusion: These data suggest that measurement of total serum PINP by the Elecsys assay correlates well with BFR in CKD. Larger studies are needed in CKD populations to validate these data, and to determine whether P1NP predicts future fracture and can be used to guide treatment to protect against bone loss and fracture.

A-204

The contribution of angiotensin II-dependent oxidative stress to megalin expression in the renal cortex during the normoalbuminuric stage of diabetes mellitus in the rat.

Y. Kurosaki[1], Y. Yamada[2], P. K. Carmines[3], T. Tsukushi[4], A. Imoto[1], T. Ichikawa[2], M. Yokoba[1], S. Obata[4], H. Ikenaga[5], Y. Aoki[6], T. Takenaka[7], T. Ichikawa[1], M. Katagiri[1], N. Ishii[1]. *[1]Kitasato University School of Allied Health Sciences, Sagamihara, Japan, [2]Kitasato University Graduate School of Medical Sciences, Sagamihara, Japan, [3]University of Nebraska College of Medicine, Omaha, NE, [4]Kitasato University Hospital, Sagamihara, Japan, [5]Ikenaga Clinic, Ohtawara, Japan, [6]Kanagawa Health Service Association, Yokohama, Japan, [7]International University of Health and Welfare, Sanno Hospital, Minato-ku, Japan*

Background: Renal albuminuria can result from impaired albumin handling by the glomerulus or the renal tubules. Megalin plays a critical role in proximal tubular albumin reabsorption, and altered megalin expression or function can contribute to renal tubular albuminuria. Elevated glucose reabsorption during acute hyperglycemia results in impaired tubular handling of protein and proximal tubular damage (*Enzyme Protein* 8:243-50, 1994-1995). Moreover, renal albuminuria during diabetes mellitus (DM) is associated with decreased megalin expression in the proximal tubule (*Diabetes* 56:380-388, 2007). During the early stage of diabetes mellitus (DM) in rats, prior to development of albuminuria, the renal cortex exhibits oxidative stress that can be suppressed by renin-angiotensin system (RAS) inhibition (*Clin Sci* 24:543-52, 2013); however, it is not known whether these events arising during the normoalbumuric stage of DM influence megalin expression and protein excretion.

Objective: The goal of this study was to evaluate impact of oxidative stress suppression, achieved by angiotensin II receptor blocker (ARB) treatment, on proximal tubular megalin expression during the normoalbuminuric stage of DM in rats.

Methods: Four groups of rats were examined: 1) STZ group (n=5): rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg,*i.p.*), 2) Sham group (n=5): rats receiving the STZ vehicle, 3) STZ+TLM group (n=4): STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day in chow for 2 wks), and 4) Sham+TLM group (n=4): TLM-treated Sham rats. In each rat, blood glucose, blood pressure, glomerular filtration rate (GFR) were measured, as well as urinary albumin levels and activity of N-acetyl-β-D-glucosaminidase (NAG; a proximal tubule-derived enzyme) in urine. Further, we measured renal cortical 3-nitrotyrosine (3-NT) production (oxidative stress marker) by HPLC and megalin expression (Western blot analysis).

Results: Blood glucose levels were higher in STZ and STZ+TLM groups than in Sham and Sham+TLM groups ($P<0.05$), confirming development of DM; however, blood pressure and urine albumin level did not differ among groups. GFR and urinary NAG activity (an index of proximal tubule damage) were increased in the STZ group compared with Sham (each $P<0.05$), and these changes were prevented by TLM-treatment (each $P<0.05$ STZ vs. STZ+TLM). Renal cortical 3-NT production in the STZ group was 70% greater than in the Sham group (Sham, 34.0±3.0 pmol/mg protein; STZ, 58.4±2.1 pmol/mg protein; $P<0.05$ vs. Sham); however, this phenomenon was completely suppressed by TLM treatment (STZ+TLM, 35.5±3.5 pmol/mg protein; $P<0.05$ vs. STZ). Renal cortical megalin expression was elevated in the STZ group (303±89% of Sham; $P<0.05$); however, the enhanced expression of megalin in the STZ group was not evident in the STZ+TLM group (149±61% of Sham; $P<0.05$ STZ vs. STZ+TLM).

Conclusions: These observations demonstrate that increased renal cortical megalin expression accompanies oxidative stress during the early stage of DM, prior to development of albuminuria. The ability of ARB treatment to prevent the DM-induced elevation of megalin implicates the renin-angiotensin system in this phenomenon, perhaps through an oxidative stress-dependent mechanism.

A-205

Analytical validation of the new Roche Thyroglobulin II eletrochemiluminescent immunoassay.

M. C. Batista, P. R. S. Ferreira, C. E. S. Ferreira, A. C. L. Faulhaber, C. L. P. Mangueira. *Albert Einstein Hospital, São Paulo - SP, Brazil*

Background: Thyroglobulin (Tg) is a serum marker of thyroid cancer. It is thus critical to measure it accurately in the low range of 0.1 to 1.0 ng/mL level. Unfortunately, this is not possible with most commercial kits, including the traditional Roche eletrochemiluminescent Tg assay. One exception is the Beckman-Coulter Tg chemiluminescent assay (Brea, USA), which has a limit of quantitation (LOQ) of 0.1 ng/mL and, for this reason, is considered by many the reference method. In this study, we evaluated the analytical specifications of the new Roche Tg II eletrochemiluminescent immunoassay (Mannheim, Germany).

.Methods: All serum samples selected for this protocol were routine clinical specimens previously assayed for Tg in our lab using the Beckman-Coulter Access II® assay. They were all rerun within one week in Roche E170 Modular Analytics® using the new Tg II assay. This is an eletrochemiluminescent immunometric assay using 2 monoclonal antibodies to form a sandwich complex with Tg. Three samples with low, normal or high Tg levels were run in Modular E170, all in duplicate in the morning and afternoon for 5 days, and then used to calculate intra and interassay variation and LOQ (lowest concentration with CV ≤ 20%). Linearity and analytical measuring range (AMR) were evaluated in Modular E170 by mixing a low and high Tg sample in different proportions. Correlation studies were performed in 46 samples assayed in both Access II and Modular E170. Results were analyzed with EP Evaluator 11.0 software (Data Innovations, South Burlington, USA).

Results: Intra and interassay CV were 2.3% and 5.1% at 0.28 ng/mL; 1.5% and 2.7% at 2.2 ng/mL; and 0.7% and 2.3% at 45.9 ng/mL, respectively. LOD was set at ≤ 0.2 ng/mL, based on the low CV found at this level. The assay was linear at 0.21-493.3 ng/mL, which was defined as the AMR. Modular E170 results were compared with Access II using Deming regression over a range of 0.20 to 34.4 ng/mL. The correlation coefficient was 0.96, average error index 1.50 (range -3.71 to 6.41), slope 1.47, intercept -0.46 and standard error of estimate 3.17. The methods were not considered equivalent within allowable total error of 21.9% (biologic variation database, Ricos C 2012).

Conclusion: The new Roche Tg II assay exhibited an excellent precision and linearity down to 0.2 ng/mL. Although results correlated well with the Beckman-Coulter Tg assay, they were not considered equivalent to this reference method.

A-206

Prediabetic Importance of Serum Zinc Alpha Glycoprotein and Ghrelin Levels in Subjects Classified According to Oral Glucose Loading Test and Fasting Glucose Levels

E. Kurtuluş, D. Konukoglu. *Istanbul University, Cerrahpasa Medical Faculty,Biochemistry Department, Istanbul, Turkey*

Background: Increasing evidence suggests that the postprandial state and fasting hyperglycemia are a contributing factor to the development of Diabetes Mellitus. It has been recently suggested that an adipokine, zinc-α2-glycoprotein (ZAG), may also have a protective role in the prevention of obesity and its associated disorders. ZAG has been proposed to play a role in the pathogenesis of insulin resistance and suspected to be related with Type 2 Diabetes. Ghrelin a peptide hormone secreted mainly by the stomach, increases appetite and food intake. It has been suggested that Ghrelin hormone plays role in insulin secretion and glucose metabolism. In the present study we determined serum ZAG and Ghrelin levels, and evaluated whether the relationship between serum ZAG and ghrelin levels in prediabetic stages. **Methods**: Subjects were categorized according to WHO criteria as Controls (n:23, women:13, men:10, mean ages:55,6 ± 7,7 years) , Impaired Fasting Glucose (IFG; women:29, men:23, mean ages: 55,1 ± 7,0 years), Impaired Glucose Tolerance (IGT; women:26, men: 20,mean ages: 59,1 ± 8,4 years) and Diabetic Glucose Tolerance (DGT; women:15,men: 15, mean age 59,9 ± 11,1 years) in our study. There was no any difference in Body Mass Index and plasma lipids levels (total cholesterol, triglyceride, HDL and LDL -cholesterol) between groups. Subject's patients did not use any medication or vitamin pills. Baseline serum ZAG and Ghrelin levels were determined by ELISA. Serum insülin levels were determined by chemiluminesans assay. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Results: Serum ZAG levels in Control group were found to be significantly higher than in DGT, IGT and IFG groups (p<0,005, p<0,001 and p<0,001 respectively). IFG group have significantly lower serum ZAG levels than both DGT and IGT groups (p<0,005 and p<0,001). Serum Ghrelin levels in IGT was significantly higher than in IFG, DGT and Controls (p<0,001, p<0,001 and p<0,001 respectively). Subjects with IFG have significantly higher serum ghrelin levels than Controls (p<0, 05). There was a significant negative correlation between ZAG and HOMA-IR (r:-0,321, p<0,001), as well as Ghrelin levels.(r=-0,530,p< 0,001). A positive correlation were obtained from serum ZAG and 2-hours post challenged plasma glucose levels (r=0,187, p<0, 05). Conclusion: The results of our study suggest that ZAG and Ghrelin are involved prediabetic stages and their levels can be important in the regulation of glucose metabolism. ZAG and ghrelin was found to be effective in the opposite direction. Also, we thought that basal ZAG levels can be a predictive marker for the 2-hours post challenged glucose levels. The present workwas supported by the Research Fund of Istanbul University. Project No. 29822

A-207

Angiotensin II-dependent oxidative stress and increased hypoxia-inducible factor-1α expression in the renal cortex during the normoalbuminuric stage of diabetic mellitus in the rat.

Y. Yamada[1], Y. Kurosaki[2], P. K. Carmines[3], T. Tsukushi[4], A. Imoto[2], T. Ichikawa[1], M. Yokoba[2], H. Ikenaga[5], Y. Aoki[6], T. Takenaka[7], T. Ichikawa[2], M. Katagiri[2], N. Ishii[2]. *[1]Kitasato University Graduate School of Medical Sciences, Sagamihara, Japan, [2]Kitasato University School of Allied Health Sciences, Sagamihara, Japan, [3]University of Nebraska College of Medicine, Omaha, NE, [4]Kitasato University Hospital, Sagamihara, Japan, [5]Ikenaga Clinic, Otawara, Japan, [6]Kanagawa Health Service Association, Yokohama, Japan, [7]International University of Health and Welfare, Sanno Hospital, Minato-ku, Japan*

Background: Hypoxia-inducible factor-1 (HIF-1) is composed of α and β subunits, with HIF-1α considered to be a master regulator of the hypoxic response. HIF-1α and oxidative stress associate with the progression of diabetic nephropathy (*Clin Exp Pharmacol Physiol* 33:997-1001, 2006). We previously reported that renal cortical oxidant production is already increased during the early stage of diabetes mellitus (DM), prior to development of albuminuria, and that renin-angiotensin system (RAS) inhibition suppresses development of renal oxidative stress under these conditions (*Clin Sci* 124:543-52,2013). However, the relationship between oxidative stress and the hypoxic response in the kidney during normoalbuminuric stage of DM has not been established.

Objective: The goal of this study was to determine the effect of oxidative stress suppression by RAS inhibition (treatment with an angiotensin II receptor blocker; ARB) on HIF-1α expression in the renal cortex during the normoalbuminuric stage of DM.

Methods: Four groups of rats were examined: 1) STZ group (n=5): rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg, *i.p.*), 2) Sham group (n=5): rats receiving the STZ vehicle, 3) STZ+TLM group (n=5): STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day in chow for 2 wks), and 4) Sham+TLM group (n=4): TLM-treated Sham rats. In each rat, blood glucose, blood pressure and glomerular filtration rate (GFR) were measured. Production of 3-nitrotyrosine (3-NT; an oxidative stress marker) in the renal cortex was measured by HPLC, and HIF-1α expression was quantified by the Western blot analysis.

Results: Blood glucose levels were significantly higher in STZ rats than in Sham rats, and was unaffected by TLM (similar to other treatments that suppress the RAS). Blood pressure did not differ among groups. Compared with the Sham group, GFR

was increased in the STZ group (P<0.05), and this was prevented by TLM treatment (P<0.05 STZ vs. STZ+TLM). Renal cortical 3-NT production in the STZ group was 70% greater than in the Sham group (Sham, 35.2±3.4 pmol/mg protein; STZ, 59.6±1.4 pmol/mg protein; P<0.05 vs. Sham); however, this phenomenon was completely suppressed by TLM treatment (STZ+TLM, 37.3±3.5 pmol/mg protein; P<0.05 vs. STZ). The STZ group also displayed an increase in renal cortical HIF-1α expression (257±26% of Sham; P<0.05); however, the DM-induced increase in HIF-1α expression was not evident in the STZ+TLM group (157±11% of Sham; P<0.05 STZ vs. STZ+TLM).

Conclusions: An increase in renal HIF-1α expression accompanies oxidative stress during the normoalbuminuric stage of DM in the rat, and both of these phenomena are prevented by ARB. These observations indicate that the hypoxic response arises in the renal cortex early during the course of DM, and that this occurs either directly or indirectly under the influence of the RAS, possibly secondary to the presence of oxidative stress.

A-208

Evaluation of TSH Levels in Rio de Janeiro State/Brazil Neonatal Screening.

M. F. M. C. Pinheiro, C. M. M. Oliveira, S. V. L. Argolo, O. F. Silva Filho. *DASA, Rio de Janeiro, Brazil*

Background: Congenital Hypothyroidism (CH) is the most common congenital endocrine disorder and it is a leading preventable mental retardation. Its incidence ranges from 1:2000 to 1:4000 live births in iodine-sufficient countries. In Brazil, the screening for CH is mandatory by law and usually done by TSH determination on dried blood spot on filter paper samples collected by heel puncture. Diagnostic confirmation is required dosing TSH and free T4 in serum. The objective of this study was to evaluate the distribution of TSH levels in newborn´s blood samples from Rio de Janeiro state and the frequency of CH confirmed patients. We also compared our results to those described by the manufacturer.

Methods: We evaluated 18,609 dried blood spots on filter paper samples for TSH of newborns from Rio de Janeiro state over one year period (2013). The range of age was 3 to 30 days of life. We used an automatic immunofluorimetric system, GSP Neonatal hTSH kit (Wallac Oy, Turku, Finland). The cutoff value for TSH was 10.0 mUI/L, children with levels above these limits were recalled for confirmation with serum TSH and FT4.

Results: The most of TSH levels, 85.3%, were less than 2.0 mUI/L. The percentiles 95 and 99 were 3.0 and 4.7 mUI/L, respectively. Comparing to the percentiles described by the manufacturer, 7.9 and 10.7mUI/L, our results were much lower. We found 9 samples (0.05%) above the TSH cutoff. All these patients underwent measurement of serum TSH and FT4. Congenital Hypothyroidism was confirmed in 7 (1:2658) patients. Their initial TSH filter paper level ranged from 12.8 to 279.0 mUI/L, with mean 115.0 mUI/L and median 42.8 mUI/L.

Conclusion: Our data are indicative that, in this Brazilian population, the distribution of TSH levels in newborn's blood filter samples were lower to those presented by the manufacturer, based on European individuals studies. This reinforces the need for each laboratory to evaluate the TSH levels in its specific population. Using 10.0 mUI/L as the TSH cutoff we found, in this Brazilian population, a CH incidence (1:2658) similar to others iodine-sufficient regions.

A-209

Linearity study of thyroid assays assuring the quality control requirements

D. Waltrick, A. L. Camilo, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background:The ability to quantitate circulating levels of thyroid hormones is important in evaluating thyroid function. It is especially useful in the differential diagnosis of primary (thyroid) from secondary (pituitary) and tertiary (hypothalamus) hypothyroidism.To certify released results accuracy, it is important to ensure the linearity of the tests.A Brazilian laboratory implemented an Easy Linearity Curve(ELC) tool to monitor the immune-hormone analytical systems, establishing a self-inspection program to verify the efficiency and accuracy of procedures and results. The use of a tool that checks assay linearity provides additional safety and reliability of the results. This study aims to use the statistic tool to monitor the AMR of TSH,T4, FT4, T3 and FT3.

Materials and methods: We selected samples from the laboratory routine, with concentrations within assay linearity range for each test. Samples were tested in Advia Centaur® XP (Siemens Healthcare Diagnostics) using a chemiluminescent method and analyzed with the ELC tool.

Results:AMR studies were carried out according to CLSI Guideline EP6-A.Results are demonstrated in the table below.

Discussion: The tests showed satisfactory linearity results with different samples. In this study, the sample pool was not used, because all the results from this dilution test presented high percentage of recovery, above the manufacturer's reference, due to a matrix effect. Because of that, we established the utilization of different samples, respecting the expected concentrations of the sample pool,if diluted samples were prepared.The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level.

Conclusion: The thyroid trials tested in this study presented Assay Linearity Range as established by the manufacturer, within CLSI Guideline EP6-A and Total Error Laboratory's target.Thus, tests were approved by the Quality Control Management in the laboratory.

Table 1 -

Assay	Assay Linearity Range	Obtained CV %	CV Lab max Target %	Obtained TE %	TE max Target %	Linear Regression	R2
TSH3UL	0.008 - 150 uIU/mL	4.92	9.70	6.93	23.70	0.990x + (0.312)	0.99973
T4	0.3 - 30 ug/dL	2.38	5.10	8.98	10.55	1.003x + (-0.017)	0.99983
FT4	0.1 – 12 ug/dL	9.68	5.26	11.79	15.00	0.975x + (0.057)	0.99684
T3	10 – 800 ng/dL	6.15	5.95	13.10	20.00	1.028x + (-9.808)	0.99778
FT3	0.2 – 2 ug/dL	5.72	4.00	14.00	30.00	1.003x+ (0.031)	0.99074

A-210

A REVIEW OF 312 GROWTH HORMONE STIMULATION TESTS PERFORMED AT A REFERENCE LABORATORY (DASA-RJ) IN RIO DE JANEIRO - BRAZIL

Y. Schrank, M. C. Freire, R. Fontes, D. M. V. Gomes, O. F. Silva. *DASA - laboratory medicine, rio de janeiro, Brazil*

Background: There is still no consensus on the stimulation test considered the "gold standard" for the diagnosis of GH deficiency. The optimal criteria for a definitive test of growth hormone function are not met by any single stimulus. Lack of standardization of GH response to each type of stimulus, poor reproducibility and lack of correlation between the response to the test and "growth" are some of the various limitations of these tests. The aim of our study was to examine the main tests of GH stimulus applied in our environment so as the response to these tests.

Materials and Methods: We did a retrospective review of 312 patients submitted to GH stimulation test in a period of 12 months. A test was considered responsive when peak GH >5ng/mL.

Results: GH stimulus with Clonidine was the most requested test .The mean age of our patients was 10,2 years, and male:female ratio was 2,4:1. Most of patients were therefore male. Interestingly, however, among patients submitted do the Glucagon simulation test, the majority were female. The greatest GH peak was seen with Glucagon stimulus. No significant complications were observed with the applied tests.

STIMULUS	N (%)	Mean AGE (years)	GENDER (M/F ratio)	RESPON-SIVE (%)	mean GH peak (ng/mL)	GH peak (time)
CLONIDINE	202 (63,7%)	10,1	2,9	73	9,5	60' and 90'
INSULIN	85 (26,8%)	11,6	2,1	50	7,0	60'
GLUCAGON	28 (8,8%)	6,5	1,2	68	10,7	120'
PIRIDOSTIGMIN	2 (0,7%)	11,5	1	0	0,05	-

Conclusions: The high number of tested patients so as the high rate of GH response to Clonidine suggests that this is the preferred screening test by test prescribers. On the other hand, the Insulin stimulation seems to be preferably reserved to confirm the diagnosis of GH deficiency, since the number of patients submitted to these test was significantly smaller and the percentage of responders was only 50%. The use of Insulin as a second-line test in the investigation of GH deficiency is easily explained by the justified fear of potential complications associated with this test. Moreover, Glucagon stimulation is the preferred screening test in children under 6 years.

A-211

Strategy to Improve Diabetes diagnosis in Primary Care: Preliminary Results and Evaluation.

M. Salinas[1], M. Lopez-Garrigos[1], A. Asencio[2], M. Gutierrez[1], J. Lugo[1], C. Leiva-Salinas[3]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]CAP Mutxamel, Mutxamel, Spain, [3]University of Virginia, Charlottesville, VA*

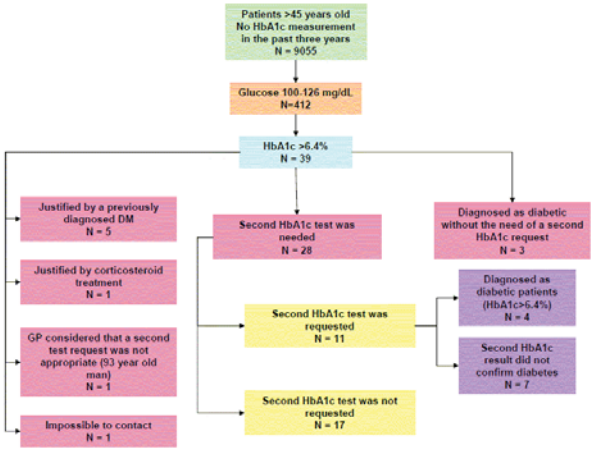
BACKGROUND: With the introduction of HbA1c as a tool to diagnose diabetes, a strategy was designed, established and evaluated in consensus with general

practitioners (GPs) to detect diabetic patients through an opportunistic study to improve HbA1c requesting, and to ascertain if previous HbA1c demand was appropriate to detect diabetes.

METHODS: The laboratory decided to approach the GPs to design a strategy that would improve the diabetes diagnosis efficiency: Laboratory Information System (LIS) automatically would add HbA1c to every sample from primary care patients older than 45 years, without an HbA1c request in the previous three years and glucose results between 100 and 126 mg/dl. If results were above 6.4% , LIS recommended a second request in 3-6 month period. In a last meeting the strategy was approved, established March 1st 2013 and evaluated after a 6 month period. HbA1c was measured using a Variant™ II Turbo Hemoglobin Testing System (Bio-Rad Laboratories, Madrid, Spain).

RESULTS: 412 HbA1c were added automatically, causing 39 HbA1c values above 6.4%. After medical record review, 6 HbA1c results above 6.4% were justified. In one case it was impossible to contact because a change of residency. 3 patients were diagnosed as diabetic without the need of a second request. To eleven patients a second HbA1c was requested, being 4 diagnosed as diabetic patients (HbA1c >6.4%) and in seven patients the second HbA1c result did not confirm diabetes. Despite their abnormal HbA1c results, until now, to 17 patients has not been requested a second HbA1c to confirm/discard the illness. Results are showed in figure.The cost of adding the 412 HbA1c was 535.6 US dollars. At this moment, each case of the seven diabetes diagnosed represented a cost of 76.5 US dollars.

CONCLUSION: Our proposed opportunistic screening to discover diabetes seems cost-effective. Hba1c was previously under requested.



A-212

Measurement of serum testosterone, androstenedione and dehydroepiandrosterone (DHEA) levels using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS)

R. Büttler[1], F. Martens[1], M. Ackermans[2], M. Blankenstein[1], A. Heijboer[1]. *[1]VU university medical center, Amsterdam, Netherlands, [2]Academic Medical Center, Amsterdam, Netherlands*

Background: The adrenal and gonadal androgens testosterone, androstenedione and dehydroepiandro-sterone (DHEA) play an important role in sexual development and fertility as well as in several other processes.

Methods: We developed a method to assess serum testosterone, androstenedione and DHEA levels in one run using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS). Sample preparation consisted of addition of internal standards ($^{13}C_3$-testosterone, $^{13}C_3$- androstenedione and $^{2}H_6$-DHEA) and a liquid-liquid extraction using hexane-ether. The samples were analyzed on an Acquity 2D UPLC system (Waters), equipped with a C4 column (Waters) and a Kinetex Fluorophenyl column (Phenomenex), and a Xevo TQ-S tandem mass spectrometer (Waters). The three analytes were baseline separated in a total run time of 9 minutes. The calibration curves ranged from 0.10 to 26 nmol/L for testosterone and androstenedione, and from 0.96 to 78 nmol/L for DHEA.

Results: The intra-assay CVs were <4.0%, <4.6% and <7.0% for testosterone, androstenedione and DHEA, respectively. The inter-assay CVs were <6% for testosterone and <8% for androstenedione and DHEA. At the lower concentrations inter-assay CVs were 15%, 7.0% and 9.3%, for testosterone (0.08 nM), androstenedione (0.47 nM) and DHEA (1.18 nM), respectively. Recoveries of spiked analytes were 101-107%, 99-106% and 92-104% for testosterone, androstenedione and DHEA, respectively. Linearity was shown in dilution series (mean R^2 was >0.999 for all analytes). This method tested negative for interference from DHEA-sulphate, estrone, 17β-estradiol, androsterone, 17-hydroxy progesterone, dihydrotestosterone, epi-testosterone, cortisone and cortisol and did not show ion suppression. The method was shown to be suitable for serum as well as EDTA and heparin plasma.

The present testosterone method compared well (y = 1.000 x + 0.035 nmol/L; r = 0,9982) to another ID-LC-MS/MS method for testosterone concordant with a published reference method (Bui et al. 2013). In the near future, the present method will also be compared to another LC-MS/MS method for androstenedione and DHEA.

Conclusion: We developed a sensitive and accurate method to measure serum testosterone, androstenedione and DHEA levels in one run.

A-213

Glucagon Quantification: Comparison of Radioimmunoassay and Sandwich ELISA methods

M. Raines, C. King, S. Christensen, P. Amin, D. Schranz. *Pacific Biomarkers, Seattle, WA*

Background: Accurate and robust measurement of glucagon is important in understanding glucagon's role in glucose metabolism and homeostasis as well as its role in the pathology of type 2 Diabetes and other metabolic diseases. It is also commonly used in clinical studies as a surrogate marker of drug efficacy. Competitive RIA methods, like the ALPCO Glucagon RIA (A), have been the gold standard for measuring glucagon but can be limiting due to the short shelf-life, long ordering lead times, long assay times, and large sample volume required (≥1 mL). The objective of this study is to evaluate the performance of the R&D Systems Glucagon Quantikine ELISA (R) and the Mercodia Glucagon ELISA (M) relative to the FDA cleared ALPCO Glucagon RIA.

Methods: All three assays were validated for precision, linearity, recovery, sensitivity, and normal glucagon ranges using fasting plasma samples collected in either K2EDTA or P800 tubes, frozen and stored at -70 ºC. A set of fasting and nonfasting P800 plasma samples were also used to directly compare glucagon results of all three assays.

Results: Precision for all three methods was acceptable with the intra-assay precision being less than 5% for all three assays and inter-assay precision being 3.8 - 11.9% (A), 4.9 - 9.2% (R), and 4.3-8.1% (M). Dilutional linearity was acceptable up to 40-fold (A), 16-fold (R), and 16,000-fold (M) dilutions for the three assays. The Mercodia ELISA was the most sensitive with a lower limit of quantitation (where the % CV is equal to 20%) of 1.5 pmol/L (M) versus 8.6 (A) and 9 pmol/L (R) for the two other kits. The most striking difference in the three assays was in the glucagon values observed in apparently healthy donor samples. The mean glucagon values for normal samples analyzed using the ALPCO RIA method were much higher (39.3 pmol/L) than the R&D systems (28.8 pmol/L) and Mercodia (9.1 pmol/L). Although the R&D systems ELISA had mean normal glucagon values that were more in line with those obtained for the ALPCO RIA, Deming Regression Analysis using the same set of P800 plasma samples yielded a correlation coefficient of 0.6445 and slope of 2.174, while the Mercodia ELISA had a correlation of 0.9093 and a slope of 0.606 when compared with the ALPCO RIA. The majority of the twenty samples analyzed with the R&D systems ELISA yielded glucagon values 30-50% lower than the ALPCO RIA method, but there were three samples that had glucagon values that were higher in the R&D Systems ELISA than the ALPCO RIA which resulted in poor correlation. The Mercodia ELISA glucagon values were consistently lower than the ALPCO RIA values with greater biases observed for samples less than 10.0 pmol/L. Potential cross-reactivity with other glucagon-related molecules is speculated and currently being investigated as it may account for some of the differences observed between the three assays.

Conclusion: The Mercodia Glucagon ELISA may be a suitable alternative to the ALPCO Glucagon RIA method especially when sample volumes are limiting and better sensitivity is required.

A-214

Associations of Leukocyte Telomere Length with Cardiometabolic Risk Factors and Circulating Biomarkers of Inflammation and Oxidative Stress

R. M. AlKhaldi, O. Mojiminiyi, F. Al Mulla, N. Abdella. *Kuwait University, Kuwait, Kuwait*

Background: Telomeres are TTAGGG sequences at the end of chromosomes necessary for and chromosomal integrity which upon reaching critical length,cell become senescent or otherwise dysfunctional. However, telomerase a reverse

transcriptase enzyme prevents telomere exhaustion and chromosomal instability. Telomeres and telomerase were linked to aging & associated diseases namely obesity, diabetes type 2 [T2DM] and cancer. We hypothesize that shortened telomere length would be associated with cardio-metabolic risk factors, and that this relationship might be mediated by obesity related metabolic changes.

Methods: Indices of obesity (Body Mass Index [BMI], Waist Circumference [WC], Waist to height Ratio [WHtR]), glycated Hemoglobin [HbA1c%], lipid profile, fasting glucose, serum human Telomerase Reverse Transcriptase [hTERT], total adiponectin, Insulin, Myeloperoxidase [MPO], Malondialdehyde [MDA], Total Oxidative stress status [TOS] and Leukocyte Telomere Length [LTL] were measured in 225 T2DM patients and 245 age and sex matched controls. Insulin resistance [IR] was estimated using Homeostasis Model Assessment [HOMA] calculator.

Results: T2DM patients had significantly ($p<0.0001$) lower LTL compared to controls [(Mean±SD:2.1±0.2) vs. (Mean±SD:4.1±0.1)] respectively. Levels of hTERT were higher in controls compared to T2DM patients [(Mean±SD: 32.9±8.9 ng/mL) vs. (Mean±SD: 21.4±4.7 ng/mL)]. Spearman's rank correlation coefficients showed that LTL correlated negatively with age [r = -0.2, p=0.009], BMI [r = -0.3, p=0.006], WC [r = -0.3, p<0.0001], and Insulin [r = -0.2, p=0.03]. The significance of these correlations disappeared after adjusting BMI but not age and/or sex. Additionally, LTL correlated negatively and strongly with WHtR [r = -0.5, p=0.004], and HbA1c% [r = -0.6, p=0.003]. These significant correlations were not affected by BMI, age or sex. Multivariate regression analysis showed that LTL negatively associated with BMI [β=-0.7, p=0.005], WC [β=-5.7, p=0.004], HOMA-IR [β=-1.1, p=0.003], MPO [β=-0.6, p<0.0001], MDA [β=-0.1, p=0.04], TOS= [β=-2.2, p<0.0001]. hTERT showed similar trends in relation to BMI [β=-0.2, p=0.004], WC [β=-1.4, p=0.006], HOMA-IR [β=-1.3, p=0.007], MPO [β=-0.6, p<0.0001], MDA [β=-0.42, p=0.002], TOS[β=-0.3, p=0.007]. On the other hand, LTL and hTERT were associated significantly and positively associated with adiponectin [β=3.1, p=0.02; β=1.5, p=0.003] respectively. Using binary logistic regression analysis, higher BMI was associated with higher risk of telomeres' shortening [OR=2.4, p=0.008]. Higher WC and WHtR were associated with higher risk of telomere shortening [(OR=2.4, p=0.001); (OR=1.9, p=0.002)] respectively. Other obesity related factors such as IR, hyper-insulinemia and hyper-triglyridemia [(OR=7.7, p<0.0001), (OR=1.2, p<0.0001), (OR=1.3, p=0.01)] were also associated with higher risk of short telomeres. Higher levels of adiponectin were associated with lower risk of telomere shortening [OR=0.7, p=0.004]. Additionally, shorter telomere length were associated significantly with higher risk of T2DM [OR=7.5, p=0.003]. Higher hTERT levels though were associated with lower risk of T2DM [OR=0.8, p=0.008].

Conclusions: Our results demonstrate the link between telomere biology, cardiometabolic risk factors, and T2DM in the Kuwaiti population which has not been studied before. Metabolic changes such as the dys-regulation of adipokines (such as adiponectin), dys-lipidemia, hyper-insulinemia, IR and obesity associated inflammatory process, could play a role in mediating telomere shortening. Since, obesity and T2DM are increasing at epidemic pace in Kuwait; telomere attrition & telomerase levels could be potential cardio-metabolic risk markers of obesity and T2DM.

A-215

Associations of Common TERC Single Nucleotide Polymorphisms with Telomere Length, Human Telomerase Reverse Transcriptase and Obesity Related Factors

R. M. AlKhaldi[1], O. Mojiminiyi[1], F. Al Mulla[1], N. Abdella[2]. *[1]Pathology Department, College of Medicine, Kuwait University, Kuwait, Kuwait, [2]Medicine Department, College of Medicine, Kuwait University, Kuwait, Kuwait*

Background: Quantitative trait locus studies have mapped putative loci that probably be involved in the regulation of leukocyte telomere length [LTL] to human chromosomes 3p26.1, 10q26.13, 12q12.22 and 14q23.2. The strongest associations with LTL were reported for SNP rs12696304 and rs16847897 near TERC on 3q26. It is unclear though whether this locus identified in Europeans, American, and Chinese exerts a similar effect on LTL in other populations. Additionally, the effect of such SNPs on serum levels of human telomerase reverse transcriptase (hTERT) has not been explored before in any population. The aim of this research to: study the influence of TERC SNPs on LTL, levels of [hTERT], indices of obesity and explore the potential associations with type 2 diabetes mellitus [T2DM].

Methods: In a study on 225 T2DM patients and 245 age and sex matched controls, we used Allelic Discrimination (AD) genotyping to determine near TERC SNPs (rs12696304 and rs16847897). Fasting [hTERT], adiponectin, Insulin, Myeloperoxidase [MPO], and [LTL] were also measured. Body Mass Index (BMI), and waist circumference (WC) were also recoded and subjects were classified on the basis of the degree of obesity. Body fat percentage (BF%] was measured using Bioimpedance analysis [BIA]. Insulin resistance [IR] was assesed using [HOMA-IR] calculator.

Results: [C/C] genotype of SNP rs16847897 was significantly associated with telomere shortening [OR=1.6, p=0.004] and lower levels of hTERT [OR=0.4, p=0.006]. Nevertheless, [C/C] genotype was significantly associated with higher BMI [OR=2.2, p=0.006], WC [OR=23.4, p=0.007] and BF% [OR=2.0, p=0.005]. However, [C/C] genotype SNP rs16847897 was associated with hypo-adiponectemia [OR=0.6, p=0.006]. We found that [G/G] genotype of SNP rs12696304 was significantly associated with shorter telomeres [OR=1.5, p=0.004], lower levels of hTERT [OR=0.7, p=0.006] and hypo-adiponectemia [OR=0.5, p=0.008]. [G/G] genotype of SNP rs12696304 was associated with higher anthropometric measures such as BMI [OR=1.2, P=0.006], WC [OR=5.3, P=0.004] and BF% [OR=1.9, p=0.003]. Binary logistic regression showed that; [C/C] genotype of SNP rs16847897 and [G/G] genotype of SNP rs12696304 were significantly associated with higher T2DM risj [OR=1.7, p=0.004]. Carriers of haplotype [CG] had significantly higher ($p<0.0.0001$) BMI compared to the other two identified haplotypes [CC] and [GG] [BMI_{CG} 30.8±8.2 Kg/m^2 vs. BMI_{CC} 26.9±8.4 Kg/m^2 and BMI_{GG} 28.7±5.3 Kg/m^2]. Similar trends were observed for WC and BF%. Additionally, telomere lengths were significantly the shortest and hTERT levels were the lowest in([CG], LTL: 0.8±.01; hTERT: 21.8±5.5 ng/mL])haplotypes compared to the other haplotypes([CC], LTL: 1.03±0.1; hTERT): 23.7±6.9 ng/mL] and ([GG], LTL: 1.5±0.1; hTERT: 28.1±5.4 ng/mL]). On the other hand, levels of MPO were significantly higher in haplotype ([CG], MPO: 6.6±1.7 ng/mL) compared to other two haplotypes [CC],(MPO: 3.9±0.4 ng/mL]) and ([GG], MPO: 4.1±0.4 ng/mL]). We also found that [CG] haplotype was associated significantly with higher risk of T2DM [OR=1.5, p=0.006] and IR [OR=2.6, p=0.03].

Conclusions: We provide insights into genetic determination of a structure that is critically involved in genomic stability. Given the importance of telomeres in nuclear and cellular function and the central role of telomere length in determining telomere function; our findings could have broad relevance for both normal and pathological age associated processes.

A-216

Do anesthesia provider personnel working indoors have lower Vitamin D levels?

G. Erden[1], S. Ozdemir[1], G. Ozturk[1], A. I. Erden[2], D. Kara[2], S. Isik[3], J. Ergil[4], C. Vural[5], A. E. Arzuhal[1]. *[1]Ankara Diskapi Yildirim Beyazit Research and Training Hospital, Department of Clinical Biochemistry Ankara, Turkey, [2]Hacettepe University Faculty of Medicine, Department of Anesthesiology and Reanimation, Turkey, [3]Ankara Numune Research and Training Hospital, Department of Endocrinology Ankara, Turkey, [4]Ankara Diskapi Yildirim Beyazit Research and Training Hospital, Department of Anesthesiology and ReanimationAnkara, Turkey, [5]Ankara Numune Research and Training Hospital, Department of Anesthesiology and Reanimation Ank, Turkey*

Background: There has been an increasing awareness to vitamin D deficiency. Some of the causes of vitamin D deficiency are reduced skin synthesis (sunscreen, skin pigment etc), decreased absorption, increased catabolism, heritable disorders, decreased synthesis of 1,25 dihydroxyvitamin D, acquired disorders. One approach for clinicians to decide which patients demand screening laboratory testing is to consider serum testing in patients at high risk for vitamin D deficiency. As the most well-known source of vitamin D is known as sun exposure, anesthesia providers and anesthesia support personnel working indoors (in operating rooms) might be considered at increased risk of vitamin D deficiency due to limited sun exposure. This study aimed to investigate whether there was a higher vitamin D insufficiency or deficiency rate among anesthesia personnel working indoors when compared with personnel working outdoors.

Methods: 125 volunteered anesthesia (provider and support) personnel and 55 control (outdoor workers in marketplace) subjects were included in this study. All of the individuals were apparently healthy Turkish citizens of Ankara, Turkey (39 ° North, 32 ° East).The study was performed at the end of the winter (February 15-March 15 2013). Socioeconomic status, daily diet, vitamin D supplementation, periods of exposure to sunlight, the use of sunscreen, regular physical activities, family history of bone fractures and osteoporosis, and the clothing style in all of the individuals were asked about in a questionnaire. People with high BMI, chronic disease such as asthma, type 1 diabetes mellitus, hypertension, history of cardiac, kidney or liver disease, those taking calcium, vitamin D or multivitamin supplements were excluded from the study. Serum levels of total 25 hydroxyvitamin D (25-OHD) were measured by a chemiluminescent immunoassay (CLIA) method using an autoanalyzer (LIAISON DiaSorin, Italy). 25-OHD levels were categorized as follows: Deficient: <10 ng/

mL ; Insufficient 11-29 ng/mL and Adequate: >30 ng/mL. Data were tabulated and subjected to analysis using the Statistical Package for Social Science (SPSS) version 17.0.

Results: 74.4% of indoor anesthesia personnel and 76.6% of outdoor workers had serum 25-OHD concentrations <10 ng/mL. 20.8% of anesthesia personnel and 23.4 % of outdoor workers had serum 25-OHD concentration levels 10-20 ng/mL. 4.8 of % anesthesia personnel had serum 25-OHD concentration levels 21-30 ng/mL. There was no significant difference in the mean serum 25-OHD level between two groups (Anesthesia group: 8.98±4.89 ng/mL, Control group: 8.21±2.64 ng/mL) (p>0.05).

Conclusion: This study in Ankara suggests that significant proportions of the study populations had very low vitamin D levels at the end of winter. Vitamin D deficiency/ insufficiency is common among indoor and outdoor workers. Anesthesia personnel do not have a significant higher Vitamin D deficiency/insufficiency risk. As we have seen, UV irradiance is not the only determinant of vitamin D status. Individuals living at lower latitudes in relatively sunny environments are also at risk of vitamin D insufficiency. Vitamin D supplementation may be suggested in all groups in Ankara, including those with the highest sun exposure.

A-217

Increased Cortisol and NADPH Production in Magnesium Deficient Hepatocytes: Implicated in the Onset of Insulin Resistance and Obesity.

C. Voma[1], A. Romani[2]. *[1]Cleveland State University, Cleveland, OH, [2]Case Western Reserve University, Cleveland, OH*

Most of the clinically quantifiable liver functions take place within the hepatocytes (80% of liver cells). The current western diet is approximately 35% deficient in magnesium (Mg^{2+}). Subnormal Mg^{2+} concentrations have been reported in both diabetes and obesity, but no clear-cut cause-effect mechanism has been stated to elucidate the onset of these pathological conditions in Mg^{2+} deficiency. At the cellular level, Mg^{2+} is highly concentrated within organelles including the endoplasmic reticulum (ER), in which 15-20 mM $[Mg^{2+}]_{Total}$ has been measured. Hexose 6-phosphate dehydrogenase (H6PD), the reticular counterpart of the cytosolic G6PD, is the main NADPH generating enzyme within the ER of the hepatocyte and is regarded as an ancillary enzyme in pre-receptor glucocorticoid activation. In the present study, we report that by modulating glucose 6-phosphate entry into the ER of HepG2 cells, Mg^{2+} also regulates the oxidation of this substrate via H6PD. This regulatory effect is dynamic as glucose 6-phosphate entry and oxidation can be rapidly down-regulated by the addition of exogenous Mg^{2+}. In addition, HepG2 cells growing in low Mg^{2+} show a marked increase in H6PD mRNA and protein expression. Metabolically, these effects on H6PD are important as this enzyme increases intra-reticular NADPH production, which favors fatty acid and cholesterol synthesis. Under Mg^{2+} deficient conditions, exposure of HepG2 cells to cortisone results in a marked production of cortisol via the NADPH-dependent 11β-HSD1, thus eliciting high intra-hepatic active glucocorticoid concentrations, which in turn affects hepatocyte metabolism. Obesity is a known risk factor for type 2 diabetes. However, the degree of obesity varies greatly in people with type 2 diabetes. Not all type 2 diabetic patients are overweight or obese, and not everyone who is overweight or obese will necessarily develop type 2 diabetes, suggesting the involvement of other mechanisms in the development of the pathology. 11-β-OHSD1 has been implicated as one of these auxiliary mechanisms, as it would lead to cortisol-based insulin resistance at least in certain patients.

HepG2 cells were grown in the presence of 0.6 (deficient) or 1.0 mM (physiological) $[Mg^{2+}]o$ and analyzed for NADPH and 11β-HSD1-mediated cortisol production. The mRNA expression level of H6PD, G6Pase, and 11β-HSD1 were analyzed by RT-PCR while protein expression was assessed by Western Blot analysis. Under our experimental conditions, insulin responsiveness - assessed as pAKT level by Western Blot analysis – was decreased by approximately 25% while cortisol production was increased and associated with an increased expression of PEPCK, a key enzyme in gluconeogenesis activation. Taken together, our results indicate that the ~60% increase in NADPH production via H6PD in Mg^{2+} deficient cells resulted in increased cortisol production and a decreased insulin responsiveness. In addition, these Mg^{2+} deficient cells showed 3 to 4 fold increase in H6PD and 11β-HSD1 mRNA and protein expression.

Our results support the hypothesis that Mg^{2+} deficiency increases H6PD activity and expression, setting the conditions for increased production of cortisol and decreased hepatic insulin responsiveness.

A-218

Comparison of IFA and RIA based assays for measuring adrenal autoantibody response

O. Zhukov, J. Popov, R. Vergara, D. Keno, L. Liu, J. Nakamoto, S. J. Naides. *Quest Diagnostics Nichols Institute, San Juan Capistrano, CA*

Background: The diagnosis of autoimmune Addison's disease (ADD), a primary adrenal insufficiency, depends on demonstrating inappropriately low cortisol production and high titers of adrenal cortex autoantibodies (ACAs) or 21-hydroxylase (21-OH) autoantibodies. ACA titers are determined using an immunofluorescence assay (IFA), while 21-OH autoantibodies are detected with a radioimmunoassay (RIA). In IFAs, response against 21-hydroxylase (21-OH) accounts for majority of the immunoreactivity, but antibodies against two other steroidogenic enzymes (17α hydroxylase [17-OH] and SCC [P450cSCC]) also contribute. A sensitive and convenient RIA is available to measure anti 21-OH antibody using recombinant ^{125}I-labelled 21-OH expressed in yeast. Discrepancy between ACA IFA and 21-OH RIA test results was reported among individuals with endocrine autoimmune diseases often associated with adrenal insufficiency. We evaluated concordance between ACA IFA and 21-OH RIA results in a large set of samples received for routine adrenal antibody testing. Methods: De-identified residual specimens (n=280) originally submitted to Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) for routine adrenal antibody testing by either IFA (n=140) or RIA (n = 140) were re-tested with both assays. Also included in the analysis were 1) results for an additional 264 specimens submitted for both testing with assays (ACA IFA and 21-OH Ab RIA), and 22 sera positive for mitochondrial antibodies. The ACA IFA was performed using monkey adrenal tissue (MarDx Diagnostics, Inc., Carlsbad, CA) and FITC-labeled goat antibodies to human IgG (Inova Diagnostics, Inc., San Diego, CA). Anti 21-OH testing was performed with a commercial RIA (Kronus Inc., Star, ID) employing ^{125} I-labeled 21-OH produced in yeast. Anti-mitochondrial antibodies (AMAs) were detected by IFA using rat kidney tissue (MarDx Diagnostics) and by Quanta Lite™ ELISA (Inova Diagnostics) employing recombinant antigen (MIT3). Results: The two assays yielded concordant results in 460 (83%) of the 554 samples, including 328 with negative and 132 with positive results, 94 samples were discordant. Samples with low positive results were the main contributors for IFA/RIA discordance: among 55 RIA+/IFA- samples, 49 showed RIA values close to the cut off and only 6 samples had values >10 U/mL. This discrepancy could be due to the fact that some of 21-OH epitopes recognized in RIA were "hidden" or not present in IFA substrate. Among 29 RIA-/IFA+ samples, most (18/29) had titers of 1:10 and only 4 had titers >1:40. The presence of other antibodies, either to steroid-producing cell antibodies or anti-mitochondrial antibodies, could cause these discrepancies. Presence of mitochondrial antibodies was identified in one sample. Conclusions: Low-positive samples are the major contributors to discrepancies between IFA and RIA results on adrenal antibody testing. The presence of anti-mitochondrial antibody may interfere with adrenal antibody testing by IFA.

A-219

Extreme Physical Stress Stimulates Bone Marrow-derived Circulating Stem/ Progenitor Cells that Mediate Tissue Repair: Possible Clinical Implications

I. Papassotiriou[1], E. Goussetis[2], K. Skenderi[3], M. Tsironi[4], G. Chrousos[5]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Stem Cell Transplant Unit, "Aghia Sophia" Children's Hospital, Athens, Greece, [3]Laboratory of Nutrition and Clinical Dietetics, "Harokopio" University, Kallithea, Greece, [4]Department of Internal Medicine, University of Peloponnesus, School of Nursing, Sparta, Sparta, Greece, [5]Department of Pediatrics, University of Athens Medical School, Athens, Greece*

Background: Autologous progenitor cells represent a promising option for regenerative cell-based therapies. Endothelial progenitor cells (EPCs) participate in vascular repair and angiogenesis, while circulating bone marrow-originated fibrocytes represent multipotent cells mediating tissue repair and remodeling after injury. Aging and cardiovascular risk factors, such as diabetes, however, affect circulating endothelial and bone marrow-derived progenitor cells, limiting their therapeutic potential. The "Spartathlon" ultradistance foot race (246Km continuous, prolonged, brisk exercise for up to 36h), is associated with profound physical strain, which renders it an ideal model of prolonged severe physical stress. The runners endure dramatic systemic and inflammatory changes, as their immune system functions intensively to cope with heart and skeletal muscle and other organ damage secondary to excessive physical strain. We hypothesized that this type of exercise might stimulate release of EPCs and other bone marrow-derived cells.

Athletes and Methods: We investigated the effect of physical stress on the number of circulating EPCs and fibrocytes, along with circulating molecules indicative of endothelial dysfunction and adipose tissue-derived proteins, in 20 "Spartathlon" athletes before, at the end and at 48 h post-race. The EPCs were obtained by culturing peripheral blood mononuclear cells (PBMC) under endothelial cell conditions (EndoCult) and were measured as colony-forming units (CFUs). Circulating fibrocytes were cultured from PBMCs in IMDM medium supplemented with IL-3 and M-CSF and identified as CD(45+)CD(14+)CD(34low)Collagen-I(+) fibroblastic cells. We also determined the plasma levels of endothelial dysfunction molecules E-, L- and P-selectins, soluble Intercellular Adhesion Molecule-1 (sICAM-1), soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1), and thrombomodulin (TM), along with adipose tissue-derived proteins leptin, adiponectin (ADPN), lipocalin-2 (NGAL), Retinol Binding Protein-4 (RBP-4), Plasminogen Activator Inhibitor-1 (PAI-1), Macrophage Migration Inhibitory Factor (MIF), IL-8 and Macrophage Chemoattractant Protein 1 (MCP-1) by means of immunoenzymatic techniques.

Results: Circulating EPCs increased by nearly ten-fold in peripheral blood at the end of the "Spartathlon" race (from 48±15 cells/ml to 464±36 cells/ml) and they remained increased (420±28 cells/ml) even at 48h post-race ($p>0.5$). Plasma levels of endothelial dysfunction molecules showed different patterns of responses: E-selectin, sICAM, sVCAM and thrombomodulin were increased significantly at the end of the race and returned to pre-race levels 48 h post-race, ($p>0.6$). Similarly, the adipose tissue-derived proteins NGAL, IL-8 and MCP-1 showed significant increases at the end of the race and returned to pre-race levels 48 h post-race, ($p<0.5$).

Conclusions: Our study demonstrates that acute inflammatory tissue damage induced by exhausting exercise increases EPCs but not fibrocytes. Given the ability of EPCs to promote angiogenesis and vascular regeneration and the association of fibrocytes with tissue fibrosis after persistent inflammation, we conclude that this kind of cell mobilization may serve as a physiologic repair mechanism in acute inflammatory tissue injury and a source of potential cell therapies in the near future. Furthermore, this study shows different patterns of adipose tissue-derived protein response to the systemic effort and inflammatory changes.

A-220

CDC Standardization Programs- Testosterone, Estradiol, and Vitamin D

J. C. Botelho, H. W. Vesper. *Centers for Disease Control and Prevention, Atlanta, GA*

Laboratory measurements are critical in patient care and public health decision making. However, the accuracy and reliability of these measurements prevent appropriate detection, treatment and prevention of diseases. The aim of CDC Standardization Programs is to standardize clinical measurements which ensure that accurate and comparable measurements are obtained regardless of the measurement procedure, location, and time. To achieve this goal, the CDC Standardization Programs are providing a comprehensive range of services and programs such as Reference Services, Standardization-Certification Programs, and Accuracy-based Quality Assurance Monitoring Services for testosterone (T), estradiol (E2), and vitamin D [25(OH)D].

As part of the Reference Laboratory Services the CDC has established higher order reference measurement procedures for T, E2, 25(OH)D2 and 25(OH)D3 in serum using LC-MS/MS. These measurement procedures are traceable to primary reference materials and to JCTLM certified reference measurement procedures. Using these reference methods, CDC assigns target values to sera used in its certification programs and by outside partners such as clinical and research laboratories, assay manufacturers, and proficiency testing providers. These materials are used for method comparisons, calibration, and trueness controls. CDC Standardization

CDC Standardization-Certification Programs are operating for T and E2 with the Hormone Standardization (HoSt) Program and total 25(OH)D with the Vitamin D Standardization-Certification Program (VDSCP). In both of these programs, quarterly blinded challenges are performed. Bias and imprecision assessments using established protocols and final assessment are made using criteria derived from biological variability. At present, 17 participants are enrolled in the HoSt-T Program (established in 2010) and 23 in VDSCP (established in 2013). Participants include clinical, academic, and pharmaceutical laboratories as well as manufacturers. Approximately 85% of participants have met the established criteria. Successful laboratories are published on the CDC website (http://www.cdc.gov/labstandards/hs.html). Over the past 4 years the CDC has provided 97 calibration verification serum sets to requestors and has had 85 enrollments in HoSt and VDSCP, which include many reenrollments. While participation has increased the success rate of participants has continued to improve as well. The testosterone HoSt Program has increased success rates by participants over the past 3 years from 79% in cycle 1 to 100% in cycle 3.

CDC Hormone Standardization Programs are endorsed and supported by key stakeholders such as the Partnership for Accuracy in Hormone Testing (PATH) and its affiliated organizations (i.e., AACC, The Endocrine Society, and American Urology Association). Furthermore, it collaborates with these organizations to further improve testing for other hormones such as thyroid hormones.

A-221

Quantifying Insulin-like Growth Factor-1: Inter-assay Variation Remains an Issue

H. Li[1], D. Bailey[2], L. Martin[2], T. Randell[2], K. Garen[2], G. Waite[2], A. S. Ptolemy[2]. *[1]Gamma-Dynacare Medical Laboratories, Brampton, ON, Canada, [2]Gamma-Dynacare Medical Laboratories, London, ON, Canada*

Background: As the main mediator of the somatotropic effects of growth hormone (GH), an accurate measurement of human insulin-like growth factor-1 (IGF-1) is required for the diagnosis and management of GH secretion disorders. However, the standardized measurement of IGF-1 continues to suffer from inter-assay variability, which may lead to inaccurate patient case decision making. In early 2013, the receipt of a vendor notification stating that the IGF-1 reagent lots deployed in our laboratory positively shifted patient median values prompted us to validate and ultimately deploy an alternate vendor's IGF-1 platform for patient testing. Recently, the primary vendor resumed IGF-1 reagent supply, triggering a secondary validation of these reformulated lots. These two studies specifically examined the inter-assay variability of IGF-1 measurements and the relative analytical performance of each test.

Methods: Linearity, intra- and inter-day precision, accuracy and sample carry-over were validated for IGF-1 measurements using the IDS-iSYS (Immunodiagnostic Systems) and the reformulated Immulite 2000 (Siemens Healthcare Diagnostics) assays, respectively. Patient correlation studies between the IDS-iSYS and the original and reformulated Immulite 2000 reagents were also respectively performed.

Results: The IDS-iSYS and reformulated Immulite 2000 assays had linear ranges of 10 to 1200 ng/mL ($R^2 = 0.998$, slope= 0.973) and 20 to 1700 ng/mL ($R^2= 0.999$, slope=1.02), respectively. At IGF-1 concentrations of 30.8, 249.1, 830.3 ng/mL and 45.0, 67.5 and 227.0 ng/mL the intra- and inter-day precision (%CV, N=20) of the IDS-iSYS and reformulated Immulite 2000 assays did not exceed 4.6% and 7.5%, respectively. The relative error (%RE) of the IDS-iSYS and Immulite 2000 methods respectively ranged from -8.4% to 1.5% and -3.5% to 7.5% for these precision studies. No significant carry-over was observed on either platform. Patient sample comparisons between the IDS-iSYS and the original Immulite 2000 formation showed significant bias (Deming regression: $y = 0.739x+35.87$, N=94, $R^2=0.988$). This method bias was exacerbated at IDS-iSYS derived IGF-1 concentrations >300 ng/mL (Deming regression: $y = 0.616x+114.88$, N=15, $R^2=0.981$, IDS-iSYS range= 256.3 to 770.1 ng/mL, Immulite 2000 range = 307.0 to 1128.0 ng/mL), relative to lower IGF-1 concentrations (Deming regression: $y = 0.854x+14.73$, N=79, $R^2=0.976$, IDS-iSYS range= 42.8 to 290.7 ng/mL, Immulite 2000 range = 31.5 to 351.0 ng/mL). Interestingly, this bias was less significant when patient results obtained with the reformulated Immulite 2000 reagents were correlated to those obtained with the IDS-iSYS (Deming regression: $y = 1.082x-9.9$, N=60, $R^2=0.989$, Immulite 2000 range = 25.0 to 352.0 ng/mL, IDS-iSYS range=31.0 to 352.0 ng/mL), although fewer samples with IGF-1 concentrations >300 ng/mL were included in this cohort.

Conclusion: Although the reformulated Immulite 2000 and IDS-iSYS IGF-1 assays offer acceptable analytical and clinical performance, a significant bias was noted with the original Immulite 2000 formulation. This difference was observed despite both Immulite formulations being traceable to the reference standard NIBSC 1st IRR 87/518. The IDS-iSYS assay is traceable to NIBSC 02/254. Laboratories should be aware that inter-assay IGF-1 variability must be carefully examined and its impact on the diagnosis and management of GH deficiency and acromegaly considered when testing platforms are changed.

A-222

Development of a Biochip Based Immunoassay for Quantification of Total Beta hCG in Serum

R. Duncan, A. McFall, S. Brockbank, A. Chacko, C. Richardson, P. Lowry, R. McConnell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: Human chorionic gonadotropin (hCG) is a member of the glycoprotein hormone family. It is heterodimeric and the alpha-subunit of hCG (92 amino acids) is identical to that of LH, FSH and TSH. The beta-subunit of hCG (145 amino acids) comprises the unique component of hCG and accounts

for the biological activity of this hormone. hCG interacts with the LH/hCG receptor and stimulates and maintains the corpus luteum after fertilization so it will not degenerate. The corpus luteum of pregnancy produces increasingly greater amounts of estrogen and progesterone for an additional ten weeks until the placenta takes over the secretion of these steroid hormones.This study reports the development of a biochip based immunoassay for the determination of total beta hCG in serum. This represents a new analytical tool for the detection of pregnancy. Methods: A sandwich chemiluminescent biochip based immunoassay applied to the Evidence Investigator analyser was employed. The capture antibody was immobilised and stabilized on the surface of the biochip and detector antibody was conjugated to HRP. Chemiluminescent signal was detected by digital imaging technology. The intensity of the signal is proportional to the analyte concentration in the sample. A correlation study was conducted using a commercially available immunoassay. Results: The assay was target specific showing <1% cross reactivity with FSH, LH and prolactin and <1% recovery of hCGα. The limit of detection was 0.914 mIU/ml for an assay range 0-2500 mIU/ml and the limit of blank was 0.388 mIU/ml. The intra-assay precision (n=23), expressed as %CV, was <7.5%. In the correlation study 80 serum samples were tested and the following linear regression equation was achieved versus another available *i*mmunoassay: y=1.5138x-185.07; r=0.984. Conclusion: This evaluation indicates applicability of the developed biochip based immunoassay for the detection of total beta hCG in serum. This represents a new analytical tool for the detection of pregnancy in test settings.

A-223

Performance Characteristics of Six Automated 25-Hydroxyvitamin D Assays: Mind Your 3's and 2's

S. P. Wyness[1], J. A. Straseski[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]University of Utah School of Medicine, Salt Lake City, UT*

Objective: Analyze the performance of 6 automated total 25-hydroxyvitamin D assays using 25(OH)D2/D3 and 25(OH)D3 only samples.

Methods: Access 2 and DxI 800 (Beckman Coulter*), ARCHITECT *i*2000$_{SR}$ (Abbott Diagnostics), ADVIA Centaur XP (Siemens), Liaison XL (DiaSorin) and Modular E170 (Roche Diagnostics) assays were evaluated for imprecision, method comparison and concordance. Imprecision used commercial control material tested in duplicate twice daily for 5 days. Method comparisons used residual serum samples with endogenous D2 and D3 (n=50) or D3 only (n=86). Comparisons with all 136 samples were intended to simulate real-world laboratory testing. Results were compared to an in-house LC-MS/MS method (traceable to NIST SRM 972) using Passing-Bablok regression and Bland-Altman bias plots. Acceptability criteria were coefficient of variation (CV) <10% and bias <15.8%.

Results: Imprecision was acceptable for all assays except E170 and Centaur (both CV 11%). Regression analysis of all samples in comparison to LC-MS/MS demonstrated under-recovery for ARCHITECT, DxI, E170 and Liaison assays (slopes 0.868, 0.983, 0.912, 0.834) while Access and Centaur over-recovered (slopes 1.013, 1.030). All correlation coefficients were below 0.95. Compared to D2/D3 samples, E170 and Centaur showed the greatest improvement in slope without D2 while Liaison was unaffected. Also, E170 under-recovered with D2/D3 and over-recovered in the absence of D2. Access, Centaur and DxI assays exhibited the opposite effect. Constant bias for all samples ranged from -3.3 (Centaur) to 1.7 ng/mL (ARCHITECT). Intercepts improved without D2 present for all assays except ARCHITECT and E170. Centaur constant bias improved the most in the absence of D2. Testing all samples, Centaur had the lowest overall bias (2%) and E170 (20%) and Liaison (22%) exceeded acceptable criteria. Testing D2/D3 samples, DxI and Access had the lowest bias (4%); ARCHITECT (26%), E170 (36%) and Liaison (29%) exceeded acceptable criteria with these samples. In the absence of D2 the Liaison still exceeded this limit (18%), the ARCHITECT had the lowest bias (1%) and E170, Centaur and Access were comparable to each other (8-9%). All assays over-recovered when analyzing vitamin D deficient samples (<20 ng/mL, n=31), with E170 (20%) and Liaison (19%) exceeding bias criteria. Concordance with LC-MS/MS at 20 ng/mL ranged from 77% (Centaur) to 89% (DxI). ARCHITECT, E170 and Liaison concordance improved without D2.

Overall, Access and DxI had slopes close to 1 and acceptable bias for all sample groups. Liaison had the lowest slopes and was not affected by D2. While ARCHITECT slope and intercept were not greatly affected by D2, bias and concordance improved without D2 present. E170 and Centaur assays were most affected by D2, based on improvements in slope, intercept or bias when D2 was absent.

Conclusions: It is important to consider the effects of D2 and D3 on individual assay performance. Assessing performance using total vitamin D may mask possible interferences in supplemented populations.

*Assays pending US FDA approval

A-224

Quantitation of Anti-Müllerian Hormone by the AnshLabs picoAMH ELISA Assay

J. Lu[1], C. Holt[2], M. Choi[2], K. Kalp[2], J. Straseski[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT*

Background: Anti-Müllerian hormone (AMH) is responsible for regression of the female ductal system during embryonic development. It is produced by the male testes until puberty and the female granulosa cells until menopause. A highly sensitive AMH assay is ideal for investigation of infertility, menopause, ovarian reserve or monitoring granulosa cell tumors post-therapy. The picoAMH ELISA kit (Ansh Labs, Webster, TX, USA) is a new quantitative immunoassay that detects ultra-low concentrations of AMH in human serum. Here, we describe the analytical performance of the picoAMH assay.

Methods: Imprecision studies used manufacturer's controls (65 and 185 pg/mL) and patient pools (201 and 404 pg/mL) assayed in duplicate or triplicate once daily for 10 days. Dilution imprecision was tested using 1:10 and 1:100 dilutions of serum pools assayed in triplicate once daily for 5 days. Limit of blank (LOB) and limit of detection (LOD) were assessed using the blank and 6.3 pg/mL calibrators. Linearity was determined by serially diluting a high AMH serum sample in blank calibrator to create 5 samples tested in duplicate. Recovery was evaluated by adding the highest 2 calibrators to patient samples (84 and 202 pg/mL). Temperature stability was determined by storing 2 specimens (99 and 301 pg/mL) ambient for 24 hr, 4C for 7 days and -20C for 3 weeks. Effects of up to 3 freeze/thaw cycles were studied. Method comparison of 57 samples in the range of 80-181,000 pg/mL was performed using the Beckman AMH Gen II ELISA as the comparator method. Gender/age-specific reference intervals were established using fresh or biorepository serum specimens (6 mos-71 yrs, n=1,273).

Results: Imprecision and dilution imprecision studies showed total CVs ≤ 6.3 and ≤ 8.7%, respectively. LOB and LOD were 0.81 and 3.11 pg/mL, respectively. The assay was linear to 696 pg/mL. Recovery ranged 76-101%. AMH differed <18% at all storage temperatures and <5% after 3 freeze/thaw cycles. Deming regression of the method comparison yielded $y = 0.999 x - 0.226$, $R^2 = 0.99$. Eleven gender/age-specific reference intervals were established using non-parametric and robust statistics.

Conclusions: The AnshLabs picoAMH ELISA demonstrated performance close to the manufacturer claims and excellent correlation with the comparator method. This method lowered the LOD from the current 80 pg/mL to 3 pg/mL. We report gender/age-specific reference intervals for this assay that will be useful for clinical practice.

A-225

Insulin and leptin signaling in placenta from gestational diabetic subjects

A. Pérez-Pérez[1], P. Guadix[1], J. Maymó[2], J. L. Dueñas[1], C. Varone[2], C. González-Rodríguez[1], M. Fernández-Sánchez[3], V. SANCHEZ-MARGALET[1]. *[1]VIRGEN MACARENA UNIVERSITY HOSPITAL, SEVILLE, Spain, [2]University of Buenos Aires, Buenos Aires, Argentina, [3]IVI, SEVILLE, Spain*

Background: Insulin and leptin receptors are known to share signaling pathways, such as JAK2/STAT-3 (Janus kinase 2/signal transduction and activator of transcription 3), MAPK (Mitogen activated protein kinase) and PI3K (phosphoinositide 3-kinase). Both positive and negative cross-talk have been previously found in different cellular systems. Gestational diabetes (GDM) is a pathophysiological state with high circulating levels of both insulin and leptin. We have previously found that these three signaling pathways are activated in placenta from GDM patients to promote translation, involving the activation of leptin receptor. Now, we tested the hypothesis that both leptin and insulin receptors might contribute to this activation in a positive way that may become negative when the system is overactivated.

Methods: To answer this question we studied the activation of leptin and insulin receptors in placenta from GDM and normal pregnancies by Western blot. Besides, we performed in vitro studies with insulin and leptin stimulation of trophoblast explants to study PI3K and MAPK signal transduction pathways by Western blot using specific antibodies of phosphorylated proteins. Bands were scanned and data analyzed by Anova followed by Bonferroni´s post test.

Results: We have found that both leptin and insulin receptors are activated in placenta from GDM. In vitro stimulation of trophoblast explants with both leptin and insulin at submaximal doses (0.1 nM) potentiated the activation of PI3K and MAPK signaling, whereas preincubation with maximal concentrations of insulin (10 nM) and further

stimulation with leptin showed negative effect. Similarly, trophoblastic explants from GDM placenta, which presented high signaling levels, had a negative signaling effect when further incubated in vitro with leptin.

Conclusions: Insulin and leptin receptors have positive effects on signaling, contributing to high signaling levels in placenta from GDM, but insulin and leptin have negative effects upon overstimulation.

A-226

Ultrasensitive Luteinizing Hormone Assay on the MesoScale Discovery Platform

N. Vidal-Folch, S. K. Grebe, R. J. Singh, A. Algeciras-Schimnich. *Mayo Foundation, Rochester, MN*

Background: Luteinizing Hormone (LH) or Lutropin is a glycoprotein composed of α and β subunits secreted by the anterior pituitary gland after stimulation by gonadotropin-releasing hormone (GnRH). In children, LH is measured as an aid in the diagnosis of gonadal disorders such as central precocious puberty (CPP) and delayed puberty. Current automated immunoassays are sensitive to about 0.1 IU/L. These levels are appropriate for adults, but clinical studies suggest that basal LH reference ranges in pre-pubertal children are below the lower limit of detection of automated assays and a more sensitive assay is needed. **Objective:** Develop an ultrasensitive LH assay for pediatric patients using the electrochemiluminescence multi-array technology from MesoScale Discovery (MSD). **Methods:** The LH assay is a sequential two-site electrochemiluminescence laboratory developed test. A monoclonal biotinylated LH capture antibody is added to a streptavidin coated plate, incubated for 30 min and washed to remove unbound antibody. Sample is added and incubated overnight at 4ºC. After washing, a SULFO-TAG™ labeled detection antibody is added. After 2 h the plate is washed and counted on the MSD sector imager reader. The assay is calibrated against the WHO International Standard reference material from NIBSC (2nd IS 80/552). The performance characteristics of the assay were established over two different reagent lots and included determination of imprecision, limits of quantification and detection, linear measurement range and dilution linearity, spike recovery, interferences, sample stability and a method comparison with the Beckman Access LH assay. **Results:** Intra-assay and intra-assay imprecision on patient samples (0.03-25 IU/L) ranged from 1.3% to 3.1% and from 5.8% to 7.9%, respectively. The limit of detection was 0.004 IU/L and the limit of quantitation was 0.02 IU/L (15% CV). The linear measurement range was 0.02 to 28 IU/L. Average dilution linearity and spike recoveries were 92.9% (range 86-122%) and 101% (range 94-104%), respectively. The assay is not affected by hemolysis (up to 1000 mg/dL hemoglobin), lipemia (up to 1000mg/dL triglycerides) or bilirubin (up to 5mg/dL bilirubin). Repeat measurements showed <20% variability for serum and serum-separator tubes up to 14 days ambient or refrigerated and through 3 freeze/thaw cycles. Method correlation using Passing-Bablok regression fit against the Beckman Access LH assay was y=0.9119x-0.04042 and r=0.982 (N=200) in the concentration range of 0.3 - 28 IU/L. **Conclusion:** We have developed an ultrasensitive LH assay useful in the diagnosis of gonadal disorders in pediatric patients. The assay provides accurate results with a 10-fold improvement in functional sensitivity over existing automated assays.

A-227

Preanalytical validation of a serum normetanephrine, metanephrine and 3-methoxytyramine assay

O. M. Itkonen, U. Turpeinen, N. Tohmola, E. Hämäläinen. *Helsinki University Central Hospital, HUS Helsinki, Finland*

Background: Analysis of metanephrine (MN), normetanephrine (NMN) and 3-methoxytyramine (3MT) in plasma or serum has recently replaced the urinary assay in many laboratories for the diagnosis of pheochromocytoma. The aim of this study was to validate preanalytical factors of serum MN, NMN and 3MT assays.

Methods: We used samples from apparently healthy adult volunteers to study sample stability (n=25), sampling device (n=13), postprandial effect (n=7), intra-individual within-day variation and diurnal variation (n=7). Samples (200µL) with [2H3]-labeled internal standards were extracted with Oasis® WCX µElution plates (Waters), washed with water, methanol and 0.1% formic acid in acetonitrile and eluted with 2 x 50 µL of 2% formic acid in 95% acetonitrile-5% water. The eluent (25 µL) was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) employing an Agilent 1200 liquid chromatograph (Agilent Technologies), a 4000 QTRAP mass spectrometer (AB Sciex), and an Atlantis HILIC Silica 50x2.10 mm column (Waters). The LOQ of the assay was 0.025 nmol/L, the intra-assay CV was <7.2%, the inter-assay CV was <8.3%, and the linear range 0.025-5 nmol/L for MN, NMN and 3MT. Paired t-test was performed by Analyse-it for Microsoft® Excel 2003.

Results: Serum NMN and MN were stable (concentration changed <20%) for at least 7 days at room temperature and at +4°C, for 12 weeks at -20°C. NMN was stable during 1 and MN at least during 4 freeze-thaw cycles. No valid stability data of serum 3MT could be obtained because the concentrations were below the detection limit in the majority of our samples. NMN and 3MT concentrations were lower ($p \leq 0.032$) in samples drawn into Li-heparin plasma tubes (mean 0.41 and 0.03 nmol/L, respectively, Venosafe 60 USP U Lithium Heparin tube, Terumo) than in samples drawn into glass tubes (0.49 and 0.05 nmol/L, respectively), clotting catalyzator tubes (0.47 and 0.04 nmol/L, respectively) and SST™ II Advance gel tubes (0.47 and 0.04 nmol/L, respectively). All serum tubes were from Vacutainer. On contrary, MN was the highest in Li-heparin plasma (0.18 nmol/L), but the difference was significant only as compared to serum drawn into catalyzator tubes (0.17 nmol/L, $p=0.0165$). A regular breakfast meal had no effect on serum NMN, MN or 3MT concentrations ($p<0.075$ for all). There was no difference ($p>0.066$) in NMN and 3MT concentrations in samples drawn at 8 a.m. (0.48 and 0.03 nmol/L, respectively), noon (0.51 and 0.04 nmol/L, respectively) and 4 p.m. (0.45 and 0.04 nmol/L, respectively). However, MN concentration was 0.16 nmol/L at 8 a.m., 0.17 nmol/L at noon and 0.19 nmol/L at 4 p.m. ($p=0.0304$). The mean intra-individual within-day variation of NMN, MN and 3MT was 13% (range 7%-23%), 13% (range 3%-13%) and 22% (range 9%-36%), respectively.

Conclusions: To minimize assay variation due to preanalytical factors, we suggest that samples be transported to the laboratory at room temperature but stored frozen. Only 1 freeze-thaw cycle should be allowed before analysis, serum instead of Li-heparin plasma should be used, sampling should occur before noon and no fasting before sampling is required.

A-228

Functional Sensitivity of Five Automated Estradiol Immunoassays

W. E. Owen[1], J. A. Straseski[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT*

Background: Estradiol (E2) is a steroid hormone produced primarily by the ovaries with small amounts produced in the testes and adrenal cortex. E2 measurement is used for assessing sexual development, fertility disorders, gynecomastia, estrogen-producing tumors and hyperplasia in the adrenal cortex. E2 is also used in monitoring fertility therapy for patients undergoing in vitro fertilization. Imprecision and method-to-method differences, especially at clinically important low concentrations, continue to be problematic for E2 immunoassays. We studied the functional sensitivity (FS) of five automated E2 immunoassays.

Methods: We evaluated the ARCHITECT i2000 (Abbott), DxI 800 (Beckman), ELECSYS E170 (Roche), and ADVIA Centaur and IMMULITE 2000 (Siemens) immunoassays. Five pools of different concentrations were each prepared by combining serum samples with comparable E2 concentrations as determined by LC-MS/MS. Pools were aliquotted and stored frozen (-70 ^{0}C) until testing. Imprecision was evaluated over 12 days using two lots of reagent and two calibrations. Five aliquots per pool (one per method) were thawed per day and assayed once per run, one run per day, two days per week, and three weeks per reagent lot (total=12 replicates). FS was determined by fitting a power function to the imprecision data using Excel.

Results: The FS's (ng/L) for ARCHITECT i2000, DxI 800, ELECSYS E170, ADVIA Centaur, and IMMULITE 2000 were determined to be 3, 39, 11, 30, and 22 respectively. All methods met manufacturer's claims except ADVIA Centaur (12.5 ng/L). Mean concentrations per pool are summarized in Table 1.

Conclusions: The ARCHITECT i2000 and ELECSYS E170 showed the best performance with FS's below 20 ng/L. However, it has been suggested that FS of 5 ng/L or lower are needed for clinical usefulness. Additionally, these immunoassays did not provide comparable mean E2 concentrations for the serum pools tested. Further harmonization of E2 immunoassays is required, particularly at lower concentrations.

Comparison of pool E2 cencentrations (ng/L) and FS (ng/L) by method

Pool		LC-MS /MS	ARCHITECT i2000	DxI 800	ELECSYS E170	ADVIA Centaur	IMMULITE 2000
1		15.4	25.8	19.1	15.5	29.5	25.5
2		36.0	38.0	39.3	33.6	41.0	41.6
3		101.0	86.1	93.1	92.3	89.8	88.8
4		202.0	160.3	215.6	207.2	183.3	210.3
5		651.0	524.8	738.8	782.3	631.1	634.9
FS		0.5	3	39	11	30	22

A-229

Development of a sensitive Dried Blood Spot Anti-Mullerian Hormone (AMH) ELISA

A. Kumar[1], B. Kalra[1], A. S. Patel[1], G. Savjani[1], L. Lechtenberg[2], C. R. Garcia[2]. *[1]Ansh Labs, Webster, TX, [2]University of Pennsylvania, Philadelphia, PA*

Background: The aim of this study was to develop a highly sensitive and simple dried blood spot human AMH ELISA to assess ovarian reserve.

Relevance: AMH has been reported to be strongly associated with age, antral follicle counts (AFC), FSH and has emerged as a clinically useful biomarker of ovarian reserve. Recently, there have been concerns related to AMH stability in serum/plasma and complement interferences affecting the end result. This has generated numerous debates and publications related to reproducibility of AMH measurements and impact of pre-analytical sample handling. Dried blood spot specimens stability makes it a practicable alternative to venous blood. It opens new possibilities in AMH testing, such as comparison of historical to current patient results; simplified blood sampling for patients in remote locations or for those who are homebound. Instead of traveling to a clinic to get blood drawn, a blood spot sample can be taken at a convenient site and mailed to a laboratory. This technology will be especially useful for monitoring ovarian function of physically challenged cancer patients undergoing chemotherapy.

Methods: A three-step, sandwich-type enzymatic microplate assay has been developed to measure AMH levels in two 7.9 mm dried blood spot disc in less than 6 hours. The assay measures human AMH and uses stabilized recombinant human AMH as calibrators (7-1000 pg/mL). This method uses a drop of whole blood collected on filter paper from a simple finger stick. The sample is eluted from the dried blood spot in an extraction solution and is added directly to the well. The assay measures the bio-essential AMH and does not exhibit interference by hematocrit in the extracted spot.

Results: Ansh Labs DBS AMH ELISA (AL-129), when compared to Ansh Labs US AMH ELISA (AL-105) using 56 matched serum and dried blood spot samples in the range of 62-18443 pg/mL yielded a correlation coefficient of 0.98 ($p < 0.0001$) and a slope of 0.96 with an intercept of -7.56 pg/mL. DBS AMH ELISA (AL-129) when compared to Ansh Labs picoAMH ELISA (AL-124) using 65 matched serum and dried blood spot samples in the range of 5-5240 pg/mL yielded a correlation coefficient of 0.99 ($p<0.0001$) and a slope of 1.02 with an intercept of -4.7 pg/mL. Serial dilution of seven extracted dried blood specimens (5000-11000 pg/mL) in the sample diluent showed an average recovery of 87-105%. Total imprecision, calculated on 3 controls over 40 runs, 2 replicates per run, was 5.84% at 22.58 pg/mL, 3.15% at 86.51 pg/mL and 4.34% at 373.18 pg/mL. The functional sensitivity of the assay calculated at 20% CV was 3.9 pg/mL.

Conclusion: A highly simplified, sensitive, specific and reproducible dried blood spot AMH assay has been developed to assess ovarian reserve in females of reproductive age. The DBS results are comparable to serum based assays. The specimen stability, ease and low cost of collection and transportation makes it a very attractive sample type for epidemiologic and other research studies.

A-230

Nonalcholic Steato-hepatitis (NASH) in Type 2 Diabetes: Serum Body Fat-normalized Plasma Leptin Level is a Predictor of Serum Alanine Aminotransferase Level

H. S. Chaudhury[1], S. Akter[2], S. Murshed[2], M. K. Rahman[2], L. Ali[2]. *[1]International Medical College, Dhaka, Bangladesh, [2]Biomedical Research Group, BIRDEM, Dhaka, Bangladesh*

Background: Leptin is a multifunctional hormone which may be involved in the pathogenesis of type 2 diabetes mellitus (T2DM) and its complications. Anti-steatotic function of leptin is well demonstrated in animal studies. Nonalcholic steato hepatitis (NASH) is common complication of metabolic syndrome and T2DM. In present study we investigated the role of leptin in the development of NASH in type 2 diabetes.

Methods: A total of 119 subjects were included. In Group I (Case), 59 newly diagnosed T2DM and in Group II (Comparison), 60 age (year, 36±4 vs. 35±4, M±SD) and Body Mass Index (BMI)-matched (kg/m2, 24.0±3.1 in Group I vs. 23.6±2.0 in Group II) healthy control subjects were included in this observational study. Plasma insulin (fasting and 30 min post-glucose) and leptin were estimated by Enzyme Immunoassay. Insulin secretory capacity (HOMA-B%) and insulin sensitivity (HOMA-S%) were calculated by Homeostasis Model Assessment using HOMA-CIGMA software. Fasting serum non-esterified fatty acid (NEFA) was measured by enzymatic-colorimetric method. Hepatocellular damage was assesed by serum alanine aminotransferase (ALT) which was measured by enzymatic colorimetric method. Serum ALT level > 30 u/l was considered elevated.

Results: The diabetic subjects showed highly significant β-cell dysfunction and also insulin resistance as evident from HOMA B% [20.2(4.2-89.6) in diabetic vs. 78.4(35.5-365.7) in control, $p<0.001$] and HOMA S% [84.6(39.1-226.4) vs. 118.8(22.0-3573.0), $p<0.004$]. Serum fat normalized leptin level was found significantly lower in diabetic subjects [ng/ml, 5.44 (0.65-34.7)] compared to controls [8.35 (1.36-55), p=0.012]. Diabetic subjects had higher prevalence of elevated serum ALT compared to control subjects (33% vs 62%, p <0/01). The fat-normalized serum leptin level inversely correlated with insulin secretory dysfunction. The serum ALT level was correlated with fat-normalized serum leptin level (r= -0.224, p=0.016), serum triacylglycerol (r= 0.372, $p<0.001$) and phase 1 insulin secretion (r= -0.213, p=0.024). The total NEFA level in the diabetic subjects was higher than control (mmol/l, 0.652±0.196 vs. 0.42±0.15, $p<0.001$).

Conclusion: The data suggest that a) Low plasma leptin in type 2 diabetes mellitus subjects is associated with insulin secretory dysfunction; and b) Elevated serum ALT in diabetic subjects is associated with lower level of fat-normalized leptin and decrease in phase 1 insulin secreation. c) The fat-normalized serum leptin is a predictor serum ALT level.

A-231

Predicted decrease of plasma 1,5-anhydroglucitol (AG) in presence of inhibitors of glucose reabsorption (SGLT2 inhibitors): potential utility of AG as a primary marker of drug effect

D. Fortuna, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Renal reabsorption of glucose under conditions of normoglycemia is essentially 100%. Drugs that inhibit renal glucose reabsorption, via inhibition of the main renal glucose transporter (sodium-glucose transporter-2, or SGLT2), have recently been approved for use in the U.S. for treatment of Type 2 diabetes (e.g., Invokana). Renal reabsorption of plasma 1,5-anhydroglucitol (AG, 1-deoxyglucose), an unregulated, non-metabolizable glucose analogue derived from diet, is normally >99%; normal plasma AG represents a balance between slow rates of input (5 mg/day) and excretion. In diabetes, plasma AG is often substantially decreased due to accelerated renal loss that occurs when glucose concentration is high enough to saturate reabsorption capacity (viz., under conditions of glucosuria). Correspondingly, plasma AG is likely to be directly affected by drugs that inhibit glucose reabsorption. Our objective was to examine this potential effect using an established mass balance model for AG and varying the AG reabsorption fraction according to the same degree of the effect on glucose reabsorption caused by the new reabsorption inhibitors.

Methods: We used a two-compartment AG mass balance model previously described (*Am J Physiol Endocrinol Metab* 1997;273:E821-E830). If displaced from steady-state, changes in body total AG (T) are given by $dT/dt = ki - T (GFR\ fe)/(1+K)/V$, where ki = AG input rate (5 mg/day), GFR = glomerular filtration rate (nominally 100 mL/min), K is the ratio between tissue and plasma compartments (K = 2.1), V is the plasma volume (nominally 3 L), and fe is the fractional excretion (0 to 1) of filtered AG. In normoglycemic steady-state, fe is <0.01 (= fe(ss)). According to literature, fractional excretion of glucose in presence of target blood concentrations of the SGLT2 inhibitor dapagliflozin is 35%-50%. We assumed an equivalent effect, fe = 0.35, for AG in the presence of inhibitor. Using normal, steady-state plasma AG = 21 ug/mL as an intial condition, we calculated the model-predicted time course of changes in plasma AG following a step increase in fe from fe(ss) to fe = 0.35.

Results: The model predicts an exponential decrease in plasma AG when fe is increased. For fe = 0.35, plasma [AG] transitions rapidly from a normal value (AG = 21 ug/mL) to a new steady-state (AG = 0.9 ug/mL) within approximately 24 hours, with t1/2 = 3.1 hours. According to the model, the same t1/2 would be operative for any starting plasma AG upon initiation of the same degree of inhibition of AG reabsorption. Low GFR will slow this effect (e.g., t1/2 = 6.8 hours for GFR = 45 mL/min) but will not affect the eventual net % change in plasma AG.

Conclusions: SGLT2 inhibitors are predicted to produce a rapid and substantial decrease in plasma AG. The effects of SGLT2 inhibitors would obviate the usual intent of AG measurement, which is to verify increasing plasma AG as a marker of improvement in glycemic control. Conversely, however, plasma AG measurement might potentially be useful in SGLT2 therapy precisely because it might (is predicted to) act as a direct marker for successful inhibition of reabsorption of hexoses.

A-232

Rapid and Cost-effective Determination of Plasma Renin Activity in Human EDTA Plasma by Two Dimensional Liquid Chromatography-Tandem Mass Spectrometry

T. Guo, B. Yue. *NMS Labs, Willow Grove, PA*

Background: Determination of plasma rennin activity (PRA) is a critical part in evaluation of primary aldosteronism, which accounts for at least 2% of hypertensive patients. PRA assay is used to quantitatively assess the capacity of renin to generate angiotensin I (Ang I) from angiotensinogen. PRA has traditionally been measured by radioimmunoassay (RIA) with fair sensitivity and consistency when optimal sample handling and incubation conditions were applied. However, RIAs lack specificity due to potential cross-reactivity between the Ang I antibody and other endogenous peptides, have a limited dynamic range, and require laborious sample handling. Several liquid chromatography tandem mass spectrometry (LC-MS/MS) based assays were reported to measure PRA using either off-line or on-line solid phase extraction (SPE). The off-line SPE based cleanup is labor-intensive and time consuming; while on-line SPE is not cost effective. We developed a rapid and cost-effective two dimensional LC-MS/MS assay to measure PRA in human plasma.

Methods: An API-5000 triple-quadrupole mass spectrometer (AB-Sciex) coupled with electrospray ionization (ESI) source and Shimadzu HPLC system was used to quantify Ang I in human plasma after incubation. Plasma samples with double-labeled degradation standard (DS) were incubated in a water bath at 37 ºC for three hours. Labeled internal standard (IS) were spiked prior to protein precipitation with acetonitrile. After centrifugation, the supernatants were transferred into injection vials. 70 µL extracted sample were injected onto a ZORBAX-C8 columns as the first dimension and a Synergi Polar-RP column with a water/acetonitrile/formic acid gradient as the second dimension. The ESI source was operated in positive ion mode with ionspray voltage at 4000 V and heater temperature at 400 ºC. Quantitation by multiple reaction monitoring (MRM) analysis was performed. Two ion pair transitions were monitored for the analyte and its IS/DS.

Results: The assay was validated as linear over the range from 0.15 to 150 ng/mL for Ang I. The lower limit of quantiation (LLOQ) was 0.15 ng/mL for Ang I. Within-run CVs were < 3.0% for all three levels of QC samples tested. Between-run CVs were 4.14% for low QC, 3.59% for mid QC, and 4.3% for high QC Samples, respectively. Recoveries ranged from 69% to 81% for Ang I. Mean carryover was <0.13% for Ang I in 3 runs. No interference was observed. Preliminary comparison with a validated LC-MS/MS method for PRA was assessed as follows: y = 1.06x + 0.03 (r=0.999, n=6).

Conclusion: LC-MS/MS method offers specificity superior to that of RIAs. This 2D LC-MS/MS method can rapidly measure PRA in human plasma within 3.5 minute. Compared to off-line SPE, the advantages of this method include simplicity, high throughput, and low cost. Thus, it can be routinely employed in a clinical environment.

A-233

Impact of angiotensin II receptor blockade on the renal cortical tissue renin-angiotensin system during the normoalbuminuric stage of diabetic mellitus in the rat.

N. Ishii[1], Y. Kurosaki[1], P. K. Carmines[2], Y. Yamada[3], T. Tsukushi[4], A. Imoto[1], T. Ichikawa[3], M. Yokoba[1], H. Suzuki[5], H. Ikenaga[6], Y. Aoki[7], T. Takenaka[8], T. Ichikawa[1], M. Katagiri[1]. *[1]Kitasato University School of Allied Health Sciences, Sagamihara, Japan, [2]University of Nebraska College of Medicine, Omaha, NE, [3]Kitasato University Graduate School of Medical Sciences, Sagamihara, Japan, [4]Kitasato University Hospita, Sagamihara, Japan, [5]Kitasato junior college of health and hygienic sciences, Minamiuonuma, Japan, [6]Ikenaga Clinic, Otawara, Japan, [7]Kanagawa Health Service Association, Yokohama, Japan, [8]International University of Health and Welfare, Sanno Hospital, Minato-ku, Japan*

Background: The circulating renin-angiotensin system (RAS) produces changes in plasma angiotensin II (AngII) levels as a mechanism for regulating blood pressure and maintaining fluid and electrolyte homeostasis. The renal tissue RAS can function independent of the circulating RAS. One key component of the renal tissue RAS is the (pro)renin receptor ((P)RR), which is able to bind either renin or prorenin. When bound to the (P)RR, prorenin catalyzes formation of angiotensin I from angiotensinogen (similar to the action of renin). Increased renal (P)RR expression has been reported to contribute to development of diabetic nephropathy (DN) through a pro-inflammatory mechanism (*Clin Exp Pharmacol Physiol*37:277-82,2010). Renal oxidative stress has also been implicated in DN, and RAS inhibition suppresses renal cortical oxidant production even during the early, normoalbuminuric stage of DM (*Clin Sci*124:543-52,2013).

Objective: The goal of this study was to evaluate the impact of systemic RAS inhibition (AngII receptor blocker;ARB), and the attendant suppression of renal oxidative stress, on the renal tissue RAS during the normoalbuminuric stage of DM.

Methods: Four groups of rats (n=5 per group) were examined: 1) STZ group: rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg,*i.p.*), 2) Sham group: rats receiving the STZ vehicle, 3) STZ+TLM group: STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day in chow for 2 wks), and 4) Sham+TLM group: TLM-treated Sham rats. In each rat, blood glucose, blood pressure and glomerular filtration rate (GFR) were measured. We quantified the following parameters in renal cortex: 3-nitrotyrosine (3-NT) production (an oxidative stress marker; by HPLC), AngII levels (by RIA), (P)RR expression, and expression of both angiotensin type-1 and type-2 receptors (AT_1R and AT_2R by western blotting).

Results: Similar to previous reports, blood glucose levels were higher in STZ and STZ+TLM groups than in Sham and Sham+TLM groups. Blood pressure did not differ among groups. GFR was increased in STZ group compared with Sham (P<0.05), and this was prevented by TLM-treatment (P<0.05 vs.STZ+TLM). Renal cortical 3-NT production was increased in STZ compared with Sham (P<0.05); however, TLM suppressed this phenomenon (P<0.05vs.STZ+TLM). Renal cortical AngII levels did not differ among groups. In contrast, STZ rats showed significant increases in renal cortical (P)RR (323±52% of Sham;P<0.05) and both of 42 and 58kDa AT_1R expression (286±6% and 228±5% of Sham, respectively;P<0.05). These changes were prevented by TLM treatment (P<0.05vs.STZ+TLM), although TLM did not alter either parameter in the Sham group. Renal cortex AT_2R expression was elevated in the STZ group (155±6% of Sham;P<0.05), and further increased by TLM-treatment (182±10% of Sham;P<0.05).

Conclusions: During the normoalbuminuric stage of DM in the rat, the renal cortex exhibits upregulation of major components of the intrarenal RAS (AT_1R, AT_2R and (P)RR) without a change in tissue AngII levels. The DM-induced changes in AT_1R and (P)RR expression are prevented by systemic AT_1R blockade, and may arise via oxidative stress. These observations indicate that the renoprotective effects of ARB may involve not only an antioxidant effect but also effects that rely on suppression of the intrarenal RAS.

A-234

Comparison of Immunoassays to Mass Spectrometry for Free and Total Testosterone in Men, Women, and Children

S. L. La'ulu[1], J. A. Straseski[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT*

Background: Circulating testosterone may be bound to albumin, sex hormone binding globulin (SHBG) or remain free. Measurement of these various forms of testosterone provides an overall assessment of androgen status and aids diagnosis of several conditions in men, women, and children. Study objectives were to compare 5 commercially available immunoassays to mass spectrometry for free and total testosterone in adults and children.

Methods: Residual serum samples from men (n=150), women (n=100), boys (n=25), and girls (n=25) were obtained after completion of clinical testing for total testosterone (TT) by liquid chromatography tandem mass spectrometry (LC-MS/MS). Free testosterone (FT) was determined in men using equilibrium dialysis (ED)/LC-MS/MS. All samples were further tested for TT and SHBG by the Abbott ARCHITECT *ci*8200, SIEMENS ADVIA Centaur and IMMULITE 2000, Beckman Coulter DxI, and Roche Modular E170. Albumin was measured using the Abbott ARCHITECT *ci*8200 and Roche c702. FT was calculated using the Vermeulen equation. For women, boys, and girls, calculated FT by immunoassays was compared to calculated FT using TT by LC-MS/MS.

Results: Comparisons using Deming regression for TT and FT in men and women are provided (Table). For boys and girls, slopes for TT ranged from 0.72 (IMMULITE) to 1.14 (ARCHITECT) and 0.84 (IMMULITE) to 1.76 (DxI), and slopes for FT ranged from 0.82 (IMMULITE) to 1.25 (ARCHITECT) and 1.06 (ARCHITECT) to 1.18 (DxI), respectively. Overall, more immunoassays under-recovered in men and women and over-recovered in boys and girls for both TT and FT. The average of absolute percent bias was highest in boys for both TT (92.4%) and FT (98.1%) compared to men (8.7% and 10.8%, respectively).

Conclusions: Consistent biases were not observed amongst methods and populations evaluated. Challenges with accurately measuring testosterone appear to remain in some immunoassays, but not all.

Total Testosterone					Free Testosterone				
Method	Slope	Intercept	R	% Bias	Method	Slope	Intercept	R	% Bias
Men (comparison to LC-MS/MS)					Men (comparison of calculated FT to ED/LC-MS/MS				
ARCHITECT	1.07	-0.87	0.985	8.6	ARCHITECT	0.98	0.66	0.954	8.2
Centaur	0.99	-41.0	0.906	-12.3	Centaur	1.00	-0.85	0.831	-12.5
DxI	0.76	34.3	0.970	-8.8	DxI	0.82	0.25	0.970	-14.1
E170	0.95	-7.83	0.985	-7.6	E170	0.86	0.45	0.959	-8.4
IMMULITE	0.81	25.3	0.922	-6.2	IMMULITE	0.82	0.42	0.906	-10.9
Women (comparison to LC-MS/MS)					Women (comparison to calculated FT using TT by LC-MS/MS)				
ARCHITECT	1.04	0.64	0.995	20.8	ARCHITECT	1.06	0.00	0.990	21.0
Centaur	0.89	7.15	0.943	22.0	Centaur	0.93	0.10	0.933	22.7
DxI	0.73	23.9	0.927	81.4	DxI	0.85	0.30	0.917	82.6
E170	0.93	-1.87	0.985	-9.9	E170	0.94	-0.02	0.990	-9.9
IMMULITE	0.71	10.2	0.970	-3.3	IMMULITE	0.76	0.12	0.970	34.3