Endocrinology
T068

SEASONAL VARIATION OF 25- HIDROKSY VITAMIN D LEVELS IN DIYARBAKIR

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BACKGROUND-AIM
Vitamin D is an important micronutrient for health. The main vitamin D source is cutaneous production involving the conversion of 7-dehydrocholesterol into previtamin D3 by solar ultraviolet radiation. Hypovitaminosis D is thought to play a role in the seasonality of a number of diseases and adverse health conditions such as infectious disease, chronic obstructive pulmonary disease, cancer, fractures, healthy pregnancy, and other diseases. We aimed to investigate seasonal variation of vitamin D levels according to sex and age in 3468 serum samples during a year time period in Diyarbakir in Turkey.

METHODS
25-OHD (25-hidroxy vitamin D) results were extracted from the laboratory information system without patient identification. 3468 results were included to study. 25-OHD assay testing by high pressure liquid chromatography was performed during 2013 and separated by season. The seasonal winter period consisted of the months of 21st December through 21st March; spring, 21st March through 21th June; summer, 21th June through 23 th September; and fall, 21th September through 21th December. SPSS software version 15 was used for the statistical analysis. All data were expressed as mean ± SD. Statistical significance at p <0.05 was accepted as the cut-off value.

RESULTS
25-OHD concentration in winter was 15.4±13 ng/ml (n:752), in spring was 16.7±15 ng/ml (n:878), in summer was 18.9±13 ng/ml (n:804), and in fall was18.1±16 ng/ml (n:1034) and the mean serum 25-OHD levels in summer and fall, were significantly higher than from in winter and spring. A total of 3468 results (2746 females, 722 males) with the mean age of 41± 20.5 years entered into the study. The mean age of women and men were 44±18.4 and 34.3±23.3 years, respectively. The mean serum 25-hidroxy vitamin D in the study population was 17.3±14 ng/ ml and the mean serum 25-OHD level in females (16.8±14 ng/ml) was significantly lower than in males(19.5±14 ng/ml).

CONCLUSION
This retrospective epidemiological study indicates that seasonal changes lead to significant serum 25-OHD variations with the lowest values in the winter and in the spring and the highest values in the summer and in the fall. Although, seasonal change in itself does not cause significant reduction of serum 25-OHD in geographic region of this study, but may lead to serum 25-OHD reduction in subjects who are at risk of vitamin D deficiency especially women. Our findings implicate that vitamin D supplementation becomes more important in risk groups and during wintertime.
Endocrinology

T069

MEASUREMENT OF ANTI MULLERIAN HORMONE BY A NEW AUTOMATED CHEMILUMINESCENT IMMUNOASSAY

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BACKGROUND-AIM

Anti-Müllerian hormone (AMH) is primarily used in the evaluation of ovarian reserve and to predict an infertile woman’s response to controlled ovarian stimulation. Considering the wide use of AMH measurement in daily clinical practice and the large number of conditions in which it may be used, it is essential for the clinician to have accurate and reproducible results. Currently the most widely used method is enzyme linked immunoassay (ELISA) but this method has intrinsic limitations of sensitivity and of throughput. Recently a new automated chemiluminescent immunoassay method is available. As laboratory tests performed on automated platforms are more accurate and less time costing, we compared results of our traditional method ELISA with the new automated one.

METHODS

A total of 107 archived serum samples from women with subfertility or reproductive endocrine disorders (aged from 22 to 52) were assayed using the AMH Gen II ELISA manual assay (Beckman Coulter) and Access AMH assay, a paramagnetic particle chemiluminescent immunoassay (Beckman Coulter) using the DxI600 instrument. The samples covered a wide range of AMH concentrations (0.0-22 ng/ml).

RESULTS

Total imprecision of the AMH Gen II ELISA and the Access AMH assays was ±12.0 and ±10.0%, respectively, over a range of concentrations from 0.16 to 22 ng/ml. The detection limit of the assays was 0.08 ng/ml and 0.02 ng/ml.

For the AMH Gen II and the Access AMH assays, the median (interquartile range) was 1.51(0.08-20.0) ng/ml and 1.03 (0.02 – 25.4) ng/ml respectively (P<0.0001). The Passing-Bablok regression equation (in ng/ml) was: y (AMH Access) = -0.0195+0.7312 x (AMH Gen II ELISA) and the regression coefficient R=0.988.

CONCLUSION

AMH concentrations using the Access AMH assay are slightly lower than those from the AMH Gen II ELISA kit, but well correlated. The worldwide standardization of the assay is required and this study can facilitate a comparison between the old results and those which will be obtained in the future, using any of the 2 assays considered. Meanwhile, adapting clinical cut-offs from previously published works by direct conversion is not still recommended, but it is important a critical clinical evaluation together with other diagnostic and ecographic parameters.
Endocrinology
T070
CORRELATION OF FREE \( \beta \)-HUMAN CHORIONIC GONADOTROPIN AND PREGNANCY-ASSOCIATED PLASMA PROTEIN-A WITH BODY MASS INDEX AND MATERNAL AGE IN THE FIRST TRIMESTER OF PREGNANCY

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BACKGROUND-AIM
Biochemical markers of serum free \( \beta \)-human chorionic gonadotropin (free \( \beta \)-hCG) and pregnancy-associated plasma protein-A (PAPP-A) have been shown to be an effective approach to screening for fetal trisomies in the first trimester of pregnancy. The aim of our study was to determine whether free \( \beta \)-hCG and PAPP-A were associated with body mass index (BMI) and maternal age as well as among themselves in the first trimester of pregnancy.

METHODS
This study included 73 women of non-invasive prenatal screening for fetal trisomy 21 in the first trimester pregnancy. Subjects were classified in two groups. Group A (n = 40) had low- risk of Down syndrome, and group B (n = 33) had high risk (cut-off 1:250). Free \( \beta \)-hCG and PAPP-A concentrations were measured by electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany). The biochemical markers were converted to multiples of the expected normal median for a pregnancy of the same gestation (MoM).

RESULTS
In group A median of free \( \beta \)-hCG (IU/L) and PAPP-A (mIU/L) were: 27 (16/35), 0.99 (0.57/1.24) MoM and 3385 (2635/5131), 1.14 (0.81/1.58) MoM; BMI was 23.60 (21.00/26.05) kg/m2 and age 28.27 ± 3.31 years.
In group B mean values of free \( \beta \)-hCG (mIU/L) and PAPP-A (mIU/L) were: 84 (40/139), 2.71 (1.35/4.14) MoM and 1969 (1097/2832), 0.69 (0.43/1.23) MoM; BMI was 24.63 (22.64/25.40) kg/m2 and age 32.80 ± 4.36 years.
There was a correlation of PAPP-A with BMI (r = -0.373, p = 0.025) in group A and correlation with age (r = 0.419, p = 0.015) in group B. There was no correlation between the free \( \beta \)-hCG and PAPP-A. There was no differences between the two analysed groups concerning BMI (p=0.148), but there were differences for other parameters, as expected.

CONCLUSION
Our results show that the maintenance of good body mass index can contribute to expected normal PAPP-A values in the first trimester of pregnancy.
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T071

GLUCOSE TOLERANCE TESTING AS PREDICTOR FOR EARLY DIABETES MELLITUS AND OTHER ALTERATIONS IN CARBOHYDRATE METABOLISM IN WOMEN WITH PREVIOUS GESTATIONAL DIABETES MELLITUS.

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BACKGROUND-AIM

Gestational diabetes mellitus (GDM) is an important risk factor associated to the development of type 2 diabetes mellitus (DM2) later. Identifying women with previous GDM at the highest risk of progressing to alterations in carbohydrate metabolism can reduce incidence of DM. In this study, we evaluated the usefulness of maternal characteristics and measures of glucose tolerance to predict early alterations in carbohydrate metabolism in women with previous GDM.

METHODS

Women with previous GDM attended in our laboratory for postpartum metabolic classification were included. Exclusion criteria were: women with known IFG and/or IGT before pregnancy or with overt diabetes, defined as fasting glucose ≥ 126 mg/dL in 100 g 3-h OGTT, and women without information about pre-pregnancy body mass index (BMI).

The following data were recorded for all women: age and gestational week at diagnosis of GDM, pre-pregnancy BMI, family history of DM and glycemic parameters diagnostic 3-h 100g OGTT.

RESULTS

Finally, 77 women (age 34,6 years (4,8), BMI 24,2 kg/m2 (6,9) were included in the study. Final diagnosis was alteration in carbohydrate metabolism in 19 women (24,7%) (DM in 3, Impaired Fasting Glucose (IFG) in 10, Impaired Glucose Tolerance (IGT) in 1 and IGT + IFG in 5) and Normal Glucose Tolerance (NGT) in 58 (75,3%).

Only BMI and fasting glucose (Glucose 0) were higher in women with alterations in postpartum than in NGT women (BMI: 29 kg/m2 (6,5) vs 23.6 kg/m2 (5,8), p=0,007; 89.9 mg/dL vs 81.7 mg/dL (7.2) (11.3), p=0.007). There were not differences for other evaluated variables (age, family history of DM, AUC 3-h 100g OGTT and glucose at 60, 120 and 180 min).

In univariate analysis, BMI ≥ 25 kg/m2 and Glucose 0 ≥ 89 mg/dL, corresponding a quartile 3, were independent predictors of alterations in carbohydrate metabolism (Odd ratio (OR) BMI ≥ 25 Kg/m2: 3.8 (CI95%: 1.3-11.5; p=0,028); OR Glucose 0 ≥ 89 mg/dL: 5.3 (CI95%: 1.7-16.5; p=0,002). In multivariate analysis, Glucose 0 ≥ 89 mg/dL, adjusted by BMI, was an independent predictor for DMG (OR: 4.6 (CI95%: 1.4-14.7; p=0,011).

CONCLUSION

Glucose 0 and pregestational BMI could be useful tools to identify a subgroup of women with DMG at highest risk for alterations in carbohydrate metabolism after delivery.
Endocrinology

T072

ANTI-MULLERIAN HORMONE - IMMUNOASSAY METHOD COMPARISON

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BACKGROUND-AIM

Anti-Mullerian Hormone (AMH) is a dimeric glycoprotein produced in the gonad exclusively. It is used as marker for assessing the ovarian reserve and as an initial predictor of ovarian response to gonadotropin stimulation. The National Institute for Health and Care Excellence (NICE-UK) recommends a 3 class approach when aiming at in vitro fertilisation (IVF) ovarian gonadotrophin stimulation response prediction (Low <0,8 ng/mL; Moderate 0,8-3,6 ng/mL; High >3,6 ng/mL). The objective of this study was to evaluate the performance of two different AMH immunoassays (CLIA and ECLIA), and compare them with the long standing standardized ELISA method.

METHODS

78 patients were enrolled (convenience sample). Serum AMH levels were simultaneously assayed using three distinct analytical methods: ELISA (AMH Gen II ELISA, Beckman Coulter; Werfen Best® 2000), CLIA (Access AMH Paramagnetic-Particle CLIA Beckman Coulter; Beckman Coulter Access® 2) and ECLIA (Elecsys® AMH Roche; Roche Cobas® e411). SPSS® 20V software was used for statistical analysis.

RESULTS

After removal of 3 outliers >15 ng/mL, the Correlation Coefficient showed a very strong positive correlation between ELISA/CLIA assays (r=0,977) (p<0,001)(Pearson’s test)(y=0,93x), and between ELISA/ECLIA assays (r=0,980)(p<0,001) (Pearson’s test)(y=0,81x-0,01). The Bland-Altman dispersion plot pointed that, despite the very strong correlation, the values obtained when using the ELISA assay were almost always higher than values obtained by CLIA or ECLIA. This difference was more obvious with the ELISA/ECLIA comparison. The Fleiss’ test showed a strong class (3 classes) agreement between ELISA/CLIA (κ=0,846)(p<0,001)and ELISA/ECLIA (κ=0,750)(p<0,001) which was stronger between ELISA/CLIA.

CONCLUSION

A strong correlation has been shown between the ELISA/CLIA and ELISA/ECLIA assays. When compared with the standardized ELISA assay, the CLIA assay had a better class agreement, when using the above described prognostic groups. Clinical studies should address the prognostic importance of class allocation and class inclusion cut-off values regarding AMH, since small interassay differences, in highly correlated assays, can mean different class allocation and different prognosis.
Endocrinology

T073

PRIMARY HYPERALDOSTERONISM SCREENING WITH NEW AUTOMATED CHEMILUMINESCENCE METHOD LIAISON DIASORIN® FOR RENIN AND ALDOSTERONE DETERMINATIONS

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BACKGROUND-AIM

Primary hyperaldosteronism (PA) is the most common form of secondary hypertension. Plasma and urinary aldosterone concentrations are commonly used to screen PA but aldosterone/renin ratio (ARR) has the best diagnosis performance. Aldosterone concentrations largely change with the method, and PA screening criteria are variable with these methods. In the present study, we defined the biological criteria for PA screening with a new automated chemiluminescence method Liaison® (DiaSorin®) for aldosterone measurement.

METHODS

Samples from patients received at Georges Pompidou European Hospital without interfering treatments (angiotensin converting enzyme inhibitor, mineralocorticoid receptor antagonist, angiotensin receptor blocker, diuretic, beta-blocker) were selected. The initial biological diagnosis were made with aldosterone measurements by DPC Siemens® radio-immuno assay. 222 plasma EDTA samples basal conditions were analysed: 29 were from confirmed PA patients, 110 from normotensive subjects and 83 from essential hypertension patients. 190 of these samples were obtained on standardized conditions after 30 minute sitting. 119 samples from 24-hour urine collection were used; out of them, 96 collections were considered complete (based on 24-hour creatinine measurement), including 34 from patients with PA or secondary hyperaldosteronism.

Active renin, plasma and urine aldosterone were measured with DiaSorin® methods on automat Liaison® in accordance with manufacturer instructions.

RESULTS

Analysis of ROC curve of ARR shows that a cut-off value of 64 pmol/mU yielded 97 % specificity and 91 % specificity for PA screening. When sitting, a cut-off value for plasma aldosterone of 500 pmol/L yielded 78% sensitivity and 93% specificity. For 24-hour urine collection, a cut-off value of 50 nmol/24 hours yielded 66% sensitivity and 89% specificity.

CONCLUSION

Our data indicate that the new automated method for aldosterone measurement is suitable for PA screening using the defined cut-off values for the three common criteria.
Endocrinology

T074

ULTRA-SENSITIVE ANALYSIS OF ALDOSTERONE IN SERUM BY HPLC-MS/MS

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BACKGROUND-AIM

LC-MS/MS has become an important tool for the measurement of steroid hormones in clinical research studies. Historically, these analytes have been measured using GC-MS or immunoassays. However it is generally accepted that the measurement of steroids by immunoassay suffers from a lack of specificity due to cross-reactivity, resulting in overestimation of serum concentrations for these analytes. Furthermore, immunoassay measurements tend to exhibit high variability at low concentrations that can provide erroneous and misleading results. The trend is to move towards LC-MS/MS for the analysis of steroid hormones due to its many advantages, including sensitivity, selectivity, and ease of sample preparation.

METHODS

The sample preparation consisted of a liquid-liquid extraction, using methyl tert-butyl ether (MTBE), followed by dry-down and reconstitution of the sample. HPLC was carried out on a Phenomenex Gemini column and samples were analysed on the AB SCIEX Triple Quad™ 6500 LC/MS/MS system equipped with IonDrive™ Turbo V source, in negative electrospray mode.

RESULTS

The method described here was used to analyze a series of human serum samples containing concentrations of aldosterone ranging from 14 pg/mL to 300 pg/mL. The LC/MS/MS method enabled quantification of aldosterone at concentrations as low as 1 pg/mL in human serum.

CONCLUSION

A sensitive, robust and reliable method has been demonstrated for the analysis of aldosterone in serum, using a simple liquid-liquid extraction sample preparation. The use of the new AB SCIEX Triple Quad™ 6500 system, featuring IonDrive™ technology, has enabled improved limits of quantitation (LLOQ = 1 pg/mL), and provided larger dynamic range compared to earlier high performance MS/MS systems.
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T075

RELATIONSHIP BETWEEN SERUM MELATONIN AND SOME HORMONAL PARAMETERS IN WOMEN WITH POLYCYSTIC Ovary SYNDROME.

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BACKGROUND-AIM

Background: The role of melatonin in human reproductive physiology and pathology has not been well defined. In the last years, a possible relationship between changes in serum melatonin levels and disorders, associated with insulin resistance such as diabetes mellitus type 2 and polycystic ovary syndrome (PCOS), was hypothesized. The aim of this study was to assess the relationship of serum melatonin in 3:00 a.m. and 8:00 a.m. with luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (T) and immunoreactive insulin (IRI) in women with polycystic ovary syndrome.

METHODS

Methods: Serum samples were collected from 30 women with PCOS. All hormonal measurements were carried out between days 3 and 5 counted from the beginning of the last regular menstrual cycle. Serum melatonin concentration was calculated in Sirio Microplate reader (SEAC, Italy) using ELISA kit (IBL-Hamburg, Germany). Concentrations of LH, FSH, T, DHEA-S and IRI were measured on AxSYM™ system (Abbott, USA). We analyzed the correlation of melatonin at 3:00 a.m. and 8:00 a.m. and these hormonal parameters at 3:00 a.m. and 8:00 a.m. using variation and correlation analysis.

RESULTS

Results: The women with PCOS were between 18 and 40 years of age (mean age: 25.07 ± 1.10 years). We found statistically significant positive correlation between serum melatonin levels in 3:00 a.m. and FSH at 3:00 a.m. (r = 0.456, P = 0.049). Serum melatonin at 8:00 a.m. correlated negatively with DHEA-S (r = - 0.396 P = 0.031) and IRI at 8:00 a.m. (r = - 0.460, P = 0.011). The serum levels of LH and T did not show significant correlation with melatonin.

CONCLUSION

Conclusions: Our data showed an interesting association between serum melatonin and some hormonal parameters in women with PCOS. The results are consistent with possible role of melatonin in complex pathogenesis of PCOS.
Endocrinology
T076

DETERMINATION OF SALIVARY CORTISOL ON ROCHE COBAS E411 IN SERBIAN POPULATION

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BACKGROUND-AIM
For decades, research on the acute and chronic effects of stress has employed cortisol levels as index of the individual response to stress. Determination of salivary cortisol has become very popular in the early 80s last century. Salivary cortisol is a realistic measurement of an active free cortisol that represents diurnal rhythm of serum or plasma cortisol. The purpose of this pilot study was to investigate the reference range of morning and midnight salivary cortisol in healthy population in Serbia using Cortisol reagent kit (Roche Diagnostics GmbH, Germany).

METHODS
The subjects included in the study were between 20 and 67 years of age. Seventy-four healthy individuals (21 males, 53 females) provided one morning and one evening saliva sample. Samples were immediately frozen upon the arrival to the laboratory. Following thawing minimum 3 hours after freezing and centrifugation, cortisol was measured on the automated electrochemiluminescence immunoassay (ECLIA) analyzer Roche Cobas e411.

RESULTS
42 subjects (56,7%) had high morning cortisol (28,64±9,81 nmol/L), where as the midnight cortisol levels were above the reference range only in 6 subjects (8,1%) (19,90±3,87 nmol/L). All of the 6 subjects also had increased morning cortisol levels (34,97±16,23 nmol/L): 86% of the subjects with high morning cortisol had normal midnight cortisol levels (morning cortisol: 27,58±8,18 nmol/L, midnight cortisol: 8,97±2,25 nmol/L).

CONCLUSION
Based on the results obtained it could be concluded that 48,6% of the subjects had increased levels of morning cortisol possibly as a result of everyday stress exposure. Nevertheless, the literature available shows significantly higher morning cortisol reference ranges reaching up to 30 nmol/L. The reference ranges given by the manufacturer (morning cortisol: <19,2 nmol/L, midnight cortisol: < 14,2 nmol/L) should therefore be accepted with care when applied to the Serbian population. In order to establish reliable reference ranges and to minimize the effects of variables (such as the procedure of specimen collection, lifestyle, geographical location and possible errors during the performance of the test) our own reference values need to be created. Further studies are needed.
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T077
ESTABLISHMENT OF REFERENCE VALUES FOR URINARY ALDOSTERONE BY LC-MS/MS
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BACKGROUND-AIM
The aldosterone dosage is critical to the screening and diagnosis of primary hyperaldosteronism, location of aldosterone producing tumors, and investigation of other disorders of renin-angiotensin system. The aim of our study was to establish new reference values for urinary aldosterone analyzed by liquid chromatography-tandem mass spectrometry (LCMS-MS) on the Triple Quad TQ 5500 from AB SCIEX.

METHODS
We enrolled 37 healthy Caucasian volunteers (13 M, 24W) aged between 25 and 61 years(mean 36 years) for a 24 hours collect of urine. In urine, we measured the sodium, so we calculated the sodium excretion with the formula 60 X UV(L)Na(mEq)=mg NaCl/L. A normal sodium intake must be <12g/24h. Exclusion criteria were: not on any medications, including contraceptives, hypertension, and abnormal sodium. Aldosterone was measured by LC-MS/MS (TQ5500, ABSciex, Framingham, Massachusetts, USA) and urinary sodium on the c501 (Cobas6000, Roche Diagnostic, Manheim, Germany). The samples were centrifuged; a acid hydrolysis of 18 hours was performed, after deuterium labelled aldosterone was added as internal standard and injected in LC. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for sample and internal standard. In negative ion mode, aldosterone can be quantified using the MRM transition at 359.2>189 (quantifier ion) and 359.2>331.1 (qualifier ion). We calculated the reference values with the robust method CLSI C28-A3 with the MedCalc software (Mariakerke, Belgium).

RESULTS
For urinary aldosterone, the data had a normal distribution; the cut-off at 95th percentile was 32µg/24 hours with a calculated sodium intake of 8.9 ±3.2 g/ 24hours.

CONCLUSION
Our study confirms the results reported in literature. We have redefined our reference values with our new urinary aldosterone measurement by LC-MS/MS for our Belgian population with a normal sodium intake.
Endocrinology
T078

ADENOSINE DEAMINASE ACTIVITY IN DIABETES MELLITUS

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BACKGROUND-AIM
Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia. It is a major worldwide health problem leading to increased mortality and serious morbidity. Adenosine Deaminase (ADA) is a polymorphic enzyme that catalyses the irreversible deamination of adenosine to inosine. ADA is considered as a good marker of the cell mediated immunity and it has been a established screening test for Tuberculosis. Literature suggests that the Serum ADA activity is significantly raised in patients with Type 2 DM. Diabetic patients are prone to opportunistic infection, thus serum ADA levels in these patients is very important as a screening test for Tuberculosis and autoimmune diseases.

Objective: To correlate the serum ADA level with HbA1c, Fasting and Postprandial Blood Glucose level in Patients with Diabetes Mellitus.

METHODS
This is a Hospital based cross-sectional study done in B.P.Koirala Institute of Health Sciences. 150 diagnosed patients (72 males and 78 females) with DM was enrolled in the study from April 2014 to August 2014. Fasting, Postprandial and HbA1c blood sample was analysed in an Autoanalyser (cobas c311). Serum ADA was done by Giusti and Galanti method. Data were analysed using SPSS version 20, p value <0.05 was considered significant.

RESULTS
Mean age group in the study was 56 ± 11.95. Mean value of HbA1c, fasting and postprandial blood glucose and serum ADA level was 6.54 ± 2.49; 153.45 ±94.40, 239.56 ± 139.38 and 41.30 ± 19.99 respectively. Serum ADA level was significantly correlated with HbA1c levels (r= 0.426, p=0.0001), fasting blood glucose(r=0.297, p=0.0001) and Postprandial Blood Glucose(r=0.278, p value= 0.001).

CONCLUSION
There is a significant increase in Serum ADA activity in DM with increase in HbA1c levels which may play an important role in predicting the glycemic status in these patients.
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T079

OXIDATIVE STRESS MARKERS IN GESTATIONAL DIABETES MELLITUS

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BACKGROUND-AIM

Oxidative stress is typically the result of an imbalance between the reactive oxygen species (ROS) and the antioxidant. The increased production of ROS could lead to some serious physiological problems of the cell, such as damage to DNA and peroxidation of lipids and proteins. Normal pregnancy is characterized by an increase in free radical production and lipoperoxidation towards the end of the pregnancy compared to healthy non-pregnant women. It is expected that pregnant women with gestational diabetes mellitus (GDM) are highly susceptible to having some imbalance between ROS and antioxidant that could be proved by measuring the level of oxidative stress markers.

METHODS

A cross-sectional study was performed on 61 pregnant women between 24-28 weeks of gestation; of which 21 were diagnosed with GDM and 40 were healthy control pregnant women. They were recruited from antenatal care- women hospital at HMC, Qatar.

ELISA technique was performed on the following markers; lipoperoxidation(MDA) and the antioxidant buffer system including the total antioxidant capacity (TAC), antioxidant enzymes: superoxide dismutase (SOD), glutathione reductase (GPx) and myeloperoxidase MPO). Anthropometric analysis was performed on newborns such as height, weight, head circumference, Ponderal index and Apgar score.

RESULTS

Control and GDM pregnant women were matched for age, BMI, and blood pressure. The median and the interquartile (25%-75%) plasma concentrations of TAC in GDM was 11.19(9.32- 12.49) mol/\(\mu\)l and in control of 11.83(10.27- 12.40) mol/\(\mu\)l, \((p=0.5085)\). GPx activity of GDM was 1.74(0.78- 3.94) mU/ml and in control was 3.59(1.97-6.78), \((p =0.0790)\). SOD activity of GDM 90.24(74.73- 113.02) and control 86.12(79.83- 98.05) and \((p=0.8665)\). MPO of GDM 7324.57(2160.18- 9836.98) pg/ml and control 8162.04(844.104- 15140.94) pg/ml \((P =0.4916)\). A significant increase of MDA was present in GDM 14.00(11.62- 28.31) nmol/ml than in control 14.167(11.83- 17.67) nmol/ml \(p\) value of 0.048. No significant associations of the neonatal birth weight with ROS markers in both GDM and control.

CONCLUSION

These data suggest an imbalance in pro-oxidant-antioxidant balance among GDM in late gestation. GDM is characterized by an increase in lipoperoxidation (MDA), without a corresponding increase in the anti-oxidant buffer system (TCA, SOD, Glutathione reductase and MPO). Further longitudinal studies are needed to highlight the long-term effects on GDM subjects, as they are pre-diabetic to type 2 DM.
Endocrinology
T080

BIOCHEMICAL TESTS PREDICTING THE OCCURRENCE OF THE LOW T3 SYNDROME AMONG CRITICALLY ILL PATIENTS

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BACKGROUND-AIM

The NTIS or “non thyroidal illness syndrome”, also called “LOW T3 SYNDROME” is defined by the occurrence of disturbances at the thyroid function tests apart from any thyroid’s morphological or functional anomaly. Biologically, the diagnosis criteria are a low triiodothyronine level (T3) with variable levels of tetraiodothyronine (T4) and thyroid stimulating hormone (TSH).

Several studies have proven its correlation to a bad prognosis.

The aim of our study was to compare the biochemical assessments of the patients carrying and non carrying NTIS (NTIS+ and NTIS−).

METHODS

It is a prospective study related to a population of 54 patients hospitalized in intensive care unit of H.Bourguiba hospital at Sfax. Blood samples was tested for: TSH, FT4, T3, urea, creatinin, glycemia, protidemia, albuminemia, ASAT, ALAT and bilirubin at the first day of hospitalization. The thyroid assessment was repeated the 3rd and the 7th Day of hospitalization. The comparison of the biological tests results found at the admission day was made by software SPSS 20.0.

RESULTS

The albuminemia was significantly lower at the admission for the NTIS+ group. The rate of plasmatic urea and the glycemia at the admission was significantly higher for the NTIS+ group. However, no significant difference was noted with the other studied tests.

CONCLUSION

The hyperglycemia, dehydration, and denutrition reflected by the hypoalbuminemia would be predictive factors of NTIS occurrence.

A strict glycemic balance with a sufficient addition of nutrients and electrolytes would play a significant role in this syndrome prevention in intensive care units.
Endocrinology
T081
FREQUENCY AND TYING OF NON THYROIDAL ILLNESS SYNDROME IN CRITICALLY ILL PATIENTS
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BACKGROUND-AIM
Endocrine disorders are often suspected in intensive care units but rarely studied. The non thyroidal illness syndrome (NTIS) is a recent and probably underestimated entity in the critically ill patients. It is defined by a low T3 (triiodothyronin) level, variable levels of TSH (thyroid stimulating hormone) and FT4 (free thyroxin). Its typing is based on the FT4 level (normal in type1, low in type2 and high in type 3).

The aim of this study is to determine the frequency and the types of NTIS in an intensive care unit.

METHODS
It is a prospective study related to 54 patients hospitalized in the intensive care unit of the Habib Bourguiba hospital. This study was conducted in a period of 2 months and a half. We excluded from the admitted patients whose suffering from chronic renal failure, pediatric and aged patients.
Thyroid function tests including the measurements of Total T3, FT4 and TSH were carried out in all patients at the admission day, the third and the seventh day of hospitalization.
These measurements were done with the ELECSYS 2010(ROCHE) by a sandwich immunoassay for TSH and competitive immunoassays for TT3 and FT4.

RESULTS
The mean age of our patients was 41, 5+/–17, 1 years. The extreme ages were 17 and 73 years.
The occurrence of NTIS was detected at the admission in 37 patients (68,5%), at J3 in 8 patients(73,8%)and at J7 in 1 patient.
In the NTIS +group, the type 1 was the most frequent type observed at the admission (26 patients (70%)), at J3 (17 patients (55%)) and at J7 (4 patients (50%)).
The NTIS type was unchanged for 32 patients overall the study period but it was different between the times of testing in 14 patients.

CONCLUSION
The NTIS is a very frequent entity in intensive care units as demonstrated in several studies. The type1 is the most frequent type observed in our study and even in the literature. It is necessary to study the impact of this syndrome on the outcome and the mortality of critically ill patients.
The identification and the correction of this disorder can be a valuable contribution to the management of critically ill patients.
Endocrinology
T082
THE EFFECT OF ORAL CONTRACEPTIVES ON HEMOSTATIC STATUS
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BACKGROUND-AIM
Haemostatic status and thrombovascular disease are associated with oral contraceptives. The effect of oral contraceptives on the process of coagulation is often in healthy women taking oral contraceptives. The incidence of thrombovascular disease is increased, too. The role of hormones in development of higher risk of thromboembolic complications is well-known through their application for therapeutic reasons or as oral contraceptives.

METHODS
The study included 50 healthy women, 25 to 40 years old, taking different oral contraceptives for more than 3 years (experimental group) and 30 women (25 - 40 years old) who had never used oral contraceptives (control group). The concentration of fibrinogen, plasminogen, antithrombin III (AT III) and prothrombin time (PT) were measured by using coagulometry analyzers in plasma examples.

RESULTS
The concentration of antithrombin III, plasminogen and prothrombin time were significantly lower (p<0.01) in experimental group of women with history of oral contraceptive compared with control group of women who had never used oral contraceptives. On the other hand, the concentration of fibrinogen was statistically significant increased in experimental group of women compared to the control group (p<0.01).

CONCLUSION
Based on the obtained results we can conclude that decreased levels of antithrombin III, plasminogen and prothrombin time and elevated level of fibrinogen, are associated with the oral contraceptive. That is relevant factor for increased risk of thrombovascular disease.
Endocrinology

T083

MULTICENTER PERFORMANCE EVALUATION OF A SECOND GENERATION CORTISOL IMMUNOASSAY ON ROCHE DIAGNOSTICS COBAS SYSTEMS

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BACKGROUND-AIM

Objective of the multicenter study was to assess the analytical performance of a new Elecsys Cortisol Generation II (Cort II) assay (Roche Diagnostics) and to generate descriptive data comparing this Gen II to Elecsys Cortisol Gen I assay, to LC-MS/MS and other cortisol immunometric methods.

METHODS

The new cortisol II assay is a fully-automated competitive electrochemiluminescence immunoassay using 10μl serum/plasma or saliva and is traceable to IFCC 451 Panel (ID-GCMS). The analytical run time is 18 min. To characterize QC data PriciControl Cortisol Universal and -Saliva were used. For the precision experiments, each study site used an identical set of native and spiked samples covering major part of the measuring range between 1.70–1735 nmol/L cortisol.

RESULTS

For the intermediate precision study, 5 different sample pools were assayed on 21 days on cobas e 411 analyzers, 2 runs per day according to CLSI EP05-A3. Standard deviations (SD’s) for intermediate precision were found to be ≤ 1.42 nmol/L at cortisol concentrations between 7.03–8.55 nmol/L and coefficients of variation (CV) were ≤ 5.75 % for cortisol concentrations between 94.0 – 1660 nmol/L.

For the reproducibility study 5 different sample pools were assayed at four sites on 5 days on cobas e 411 analyzers with 2 runs per day in 5 aliquots each using 3 different reagent lots according to CLSI EP05-A3. Total standard deviation over all sites (SD’s) was 0.96 nmol/L at 8.44 nmol/L, CV’s were found between 6.8 and 9.5% at serum cortisol concentrations of 99.7, 482, 966 and 1611 nmol/L.

Method comparison based on Passing/Bablok regression analysis yielded the following results using the cobas e 411 analyzer (n=256–541):

- Elecsys Cort II(y)\(2\) vs. Cortisol Gen I(x)\(1\):
  - y = 0.76x + 10.27, \(r=0.968\) for serum and \(y=1.21x-5.50, r=0.992\) for saliva samples;
- Elecsys Cort II(y) vs. LC-MS/MS y = 1.02x+4.47, \(r=0.986\) for serum and \(y=1.13x+0.83, r=0.993\) for saliva samples;
- Elecsys Cort II(y) vs. Abbott Architect y = 1.16x-24.50, \(r=0.971\) for serum;
- Elecsys Cort II(y) vs. Siemens Centaur y = 0.92x-4.06, \(r=0.832\) for serum.

CONCLUSION

The Elecsys Cortisol II assay offers good precision over the entire measuring range for both serum and saliva as the sample matrix, as well as an excellent correlation of the results to LC-MS/MS. The test was found to be suited for routine diagnostic application.
Endocrinology
T084

ACCURATE THYROGLOBULIN QUANTITATION IN THE PRESENCE OF ANTI-THYROGLOBULIN AUTOANTIBODIES: EVALUATION OF FOUR AUTOMATED THYROGLOBULIN (TG) AND ANTI-TG ANTIBODIES (TGAb) ASSAYS

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BACKGROUND-AIM

TgAb can lead to falsely low Tg results in immunometric assays (IA). However, TgAb-presence alone is not a good predictor of interference. The goal of this study was to: (1) assess the magnitude of TgAb interference in four automated Tg IAs (Beckman Access, Roche Elecsys, Siemens Immulite and Thermo Kryptor) by comparison with a Tg-mass spectrometry (MS) assay, and (2) determine the effectiveness of TgAb IAs in detecting interfering antibodies.

METHODS

Samples from 589 thyroid cancer patients were tested with four Tg and TgAb IAs and one Tg-MS assay. The limits of quantification (LOQ) of the respective assays were used to define Tg status (Tg+ or Tg-) and TgAb status (TgAb+ or TgAb-) status. The manufacturers’ reference intervals (RI) were used as an alternative measure of TgAb status.

RESULTS

TgAbs were >LOQ in 339 (58%, Roche), 241(41%, Beckman), 121(21%, Immulite) and 227 (39%, Kryptor) of samples. Tg was detectable by MS, but undetectable by the Immulite IA in 36 samples; 23 (64%) were TgAb- and 13 (36%) TgAb+ by the Immulite TgAb IA using either LOQ or RI cut-offs. The Roche IA had 19 samples with undetectable Tg that were detectable by MS; 3 (16%) were TgAb- and 16 (84%) TgAb+ by the Roche TgAb IA using the LOQ cut-off; 14 (74%) were TgAb- and 5 TgAb+ (26%) by the RI cut-off. The Beckman IA had 19 samples with undetectable Tg, but detectable by MS; 7 (37%) were TgAb- and 12 (63%) TgAb+ by the Beckman TgAb IA using either LOQ or RI cut-offs. The Kryptor IA had 15 undetectable Tg samples, but detectable by MS; 8 (53%) were TgAb- and 7 (47%) TgAb+ by the Kryptor TgAb IA using either LOQ or RI cut-offs. In TgAb+ samples with detectable Tg by both MS and IAs, Tg underestimation averaged 40% (Kryptor and Beckman), 50% (Roche) and 86% (Immulite).

CONCLUSION

None of the TgAb assays identified all samples that resulted in false negative Tg measurements. The Roche TgAb assay detected the largest number of interfering TgAb antibodies, but only when the LOQ cut-off was used. The Immulite Tg and TgAb IAs showed the greatest underestimation of Tg in TgAb+ samples and missed the greatest number of samples with interfering TgAb. The Roche, Beckman and Kryptor assay showed similar performance in respect to Tg underestimation in TgAb+ samples.
Endocrinology
T085

NEONATAL THYROID SCREENING AS AN INDICATOR FOR MONITORING IODINE STATUS IN MACEDONIAN POPULATION

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BACKGROUND-AIM

Iodine deficiency is the most common cause of preventable brain damage in the newborn. The indicators for assessing population iodine status include urinary iodine excretion, thyroid size, frequency of neonatal thyroid-stimulating hormone (TSH) above 5 mU/L and blood thyroglobulin concentration. A frequency of neonatal TSH concentrations above 5mU/L below 3% has been proposed as threshold indicating iodine sufficiency. The objective of this study was to evaluate feasibility and usefulness of nation-wide neonatal TSH screening results to assess iodine status in the Republic of Macedonia. All neonates born in Macedonia during the period 2002-2014 were included in this study, except those suffering from congenital hypothyroidism, premature neonates and neonates screened before 48 hours of age.

METHODS

Using the time-resolved fluoroimmunometric assay we have performed screening for neonatal thyroid-stimulating hormone (DELFIA neonatal TSH, LKB) from blood spots on filter paper Schleicher&Schull 903, obtained on the day 2-5 after birth, during the period 2002-2014.

RESULTS

Out of 250,893 newborns, a total of 238,623 (95.1%) have been screened, of which 198,213 (83.1%) have been evaluated for TSH values above 5mU/L. The rest of screened neonates (16.9%) were not included in this study because of early or inadequate sampling, and prematurity. Total of 6105 newborns (3.08%) had TSH values above 5mU/L.

CONCLUSION

A 3.08% frequency of TSH concentrations above 5mU/L indicates reasonable iodine sufficiency in the Macedonian population. Neonatal screening for thyroid-stimulating hormone is a sensitive and reliable tool for monitoring iodine status in populations.
Endocrinology
T086

DEVELOPMENT OF A NEW KIT FOR FREE PLASMA METANEPHRINES

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BACKGROUND-AIM
Determination of metanephrines from blood plasma plays an important role in the diagnosis of chromaffin cell tumors pheochromocytoma (PHEO) and paraganglioma (PGL). It is highly preferable to use this method for the diagnosis as it is more sensitive than the other methods.
This study aims to develop a new kit for the determination of metanephrine (MN), normetanephrine (NMN) and 3-methoxytyramine (3-MT).
In order to simplify the pre-treatment of the samples and the chromatographic analysis, the solid face extraction (SPE) and all measurement conditions have been thoroughly optimized.

METHODS
We tested patients with and without diagnosis of PHEO and/or PGL. All selected patients were fasting overnight and on a special diet before blood taking. Heparin was used as an anticoagulant. The blood corpuscles were separated by centrifugation. Metanephrines from plasma matrix were extracted by SPE and subsequently determined by high performance liquid chromatography with electrochemical detection.

RESULTS
To optimize the SPE method, eight of the commercially available SPE ion-exchange sorbents were tested. We found a mixture of Discovery DSC-SCX and Discovery DSC-SAX (m/m 4:1) as the most suitable sorbent for metanephrines.
As for the HPLC separation, six analytical columns were tested. According to our results, Kinetex XB-C18 100 x 4.6 mm (5 μm) is the most convenient solution to perform a short and sensitive analysis.
For the cell potentials optimization, the current-voltage curves of MN, NMN, 3-MT and internal standard have been measured. As the result, +100 mV (1st cell), -350 mV (2nd cell) and +400 mV (conditioning cell) have been determined for further measurements.

CONCLUSION
The new kit for free plasma metanephrines has been developed. We found the suitable SPE sorbent for the sample preparation and the analytical column for HPLC analysis. The conditions of the measurement have been successfully optimized.
Endocrinology
T087

CORRELATION OF SALIVARY STRESS MARKERS AND PHYSICAL ACTIVITY IN STUDENT POPULATION

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BACKGROUND-AIM
Stress is a condition which disturbs inner (psychophysiological) balance of the organism activating the hypothalamic-pituitary-adrenal axis. Salivary cortisol is used as an indicator of free cortisol, correlates well with serum free value and reflects biologically active fraction. At the same time, stressful situation stimulates the sympathetic neural system which causes a change in the secretion of salivary alpha amylase.

The aim of the study was to investigate correlation of salivary cortisol and alpha amylase (sAA) with physical activity induced stress and psychological indicators in student population.

METHODS
The study included 54 healthy volunteers, 27 (15 males, 12 females) physically active volunteers from Faculty of Kinesiology (FK) and 27 (14 males, 13 females) physically less active volunteers from other faculties (OF), aged 19 - 26 years. All participants were subject to psychological testing (approved Croatian version of COPE and WHOQOL-BREF). Saliva samples were taken in Salivetta system (Sarsted, Germany) between 10 -12 am. Both, salivary cortisol and alpha amylase were determined by ELISA method (Euroimmun, Luebeck, Germany).

RESULTS
The results have shown statistically significant higher sAA concentration in males in FK subgroup (P=0.008) and in all males (P=0.033). Concentrations of salivary cortisol did not differ between subgroups (P=0.426) or gender (females, P=0.241; males P=0.930). Psychological testing showed difference between studied subgroups only in the focused problem-coping (FK, P=0.023). The results did not show correlation between sAA and cortisol and the level of the physical activity (r=-0.225, P=0.102). Statistically significant, but weak negative correlation between sAA and cortisol was found in OS subgroup (r=-0.410, P=0.034). No correlation was found between psychological testing results and investigated salivary markers.

CONCLUSION
According to our results, there is no correlation between concentrations of sAA and salivary cortisol with the level of physical activity or with the psychological indicators in students.
Endocrinology
T088
INVESTIGATION OF RELATIONSHIP BETWEEN GLYCATED ALBUMIN, HBA1C, AND THEIR RATIO IN KOREAN PATIENTS WITH IMPAIRED GLUCOSE METABOLISM

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BACKGROUND-AIM

Glycated albumin (GA) is a useful laboratory tool for monitoring blood glucose status during proceeding 2-3 weeks. It reflects the more rapid change of blood glucose status than HbA1c. However, the quantitative analysis for relationship between GA and HbA1c levels has not been fully investigated yet. Therefore, we plan to study the relationship between these two laboratory tests for providing more precise interpretations in glucose monitoring in patients with impaired glucose metabolism.

METHODS

A total 573 patients were recruited in this study in Kyung Hee University Hospital in October, 2014. GA was measured with Lucica GA (Asahi Kasei Pharma Co., Japan) using TBA-200FR (Toshiba, Japan). HbA1c was tested using HPLC method (HLC-723 analyser, Tosoh, Belgium). The relationship between GA and HbA1c was analyzed with segmented regression analysis.

RESULTS

The mean value of GA and HbA1c were 21.98 (range: 11.5-56.2) % and 7.9 (range: 4.6-14.6) %, respectively. The regression equation from the segmented regression analysis was \( Y = 3.55X - 6.24 \), and the optimal break-point for HbA1c and GA were 6.4 % and 16.5 %, respectively. The number of patients who were satisfied the above equation are 498 (86.9 %), in whom the mean value of GA and HbA1c is 22.85 %, and 8.2 %, respectively. Above this optimal break point value, the two variables revealed a clear positive correlation. The ratio of GA/HbA1c was slightly increased in patients above the break point (2.7697) than below (2.7079).

CONCLUSION

The break-point for HbA1c found in the study was similar with the lower reference limit for the diagnosis of diabetes mellitus, namely, 6.5 %. This study showed the similar regression pattern with the previous study. However, the break-point value of HbA1c in this study was slightly higher than the previous study (5.868 %), and very close to the lower reference limit for the disease cut-off level of HbA1c, 6.5 %. Therefore, in this study, these two test results presented the significant positive correlation in the abnormal range of HbA1c. Meanwhile, in the range of normal HbA1c levels, GA showed a relatively high value than HbA1c reflecting short term glucose fluctuation. This finding suggests that GA play a role as a useful marker for short term glucose monitoring in patients with normal HbA1c level. In the future, further studies should be needed to investigate the clinical meanings of high GA but normal HbA1c levels, and changes in GA/HbA1c ratio in patients with impaired glucose metabolism.
Endocrinology
T089
MACROPROLACTIN: A REACTIVITY COMPARISON IN THREE IMMUNOASSAY ANALYZERS

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BACKGROUND-AIM

High blood prolactin (PRL) concentration (hyperprolactinaemia - hyperPRL) is the most common endocrine disorder of the hypothalamic-pituitary axis. Diagnosis depends on circulating prolactin measurement in appropriate clinical settings. Macroprolactin (big big prolactin), a nonbioactive immunoglobulin complexed monomeric prolactin, is measured in most available immunoassay platforms. Macroprolactin presence may be the reason of false hyperPRL which may lead to misdiagnosis, inappropriate investigations and unnecessary treatment.

METHODS

55 stored hyperPRL sera, routinely measured in Immulite 2000 XPi (Siemens) solid-phase chemiluminescent PRL immunometric assay, during a period of one year, were re-tested in 2 other immunoassay platforms - the Kryptor (Thermo Scientific) and the Centaur Xp (Siemens). The polyethylene glycol (PEG) precipitation method was used in all immunoassay platforms. For that purpose, we mixed equal volumes of patient’s sera with 25% (w/v) PEG6000, in phosphate buffered saline, centrifuged and measured the PRL in the supernatant. Percent recovery (%R) post-PEG PRL was determined and a cut-off of <40% indicated the macroprolactin predominance.

RESULTS

This study included 55 patients, 7 men and 48 women. The mean\(\pm\)SD age was 46\(\pm\)13.1 years. The median (25th-75th percentile, range) [total-PRL] results, from Imm2000, Kryptor and Centaur were respectively 44.50 (30-57.6) ng/mL, 32.55 (17.8-53.4) ng/mL and 38.10 (23.2-50.6) ng/mL.

We found a macroprolactin predominance in the Imm2000 post-PEG PRL measurements (%R of <40%) in 3 sera (5.5%), but undetermined levels in 11 (20%) (%R of >40 but less than 60%).

Mann-Whitney test analysis showed statistically significant differences between Imm2000 assay and Imm2000 after PEG precipitation (p\#0.012).

Comparison of Kryptor and Centaur pre-PEG PRL with Imm2000 results, after PEG precipitation, were not significantly different (p\#0.75 and p\#0.65, respectively). Similar results were obtained for post-PEG Kryptor and Centaur vs post-PEG Imm2000 (p\#0.90 and p\#0.62, respectively).

CONCLUSION

The results obtained with Kryptor and Centaur immunoassay analysers confirmed the reduced reactivity for most forms of macroprolactin when compared to the widely used Immulite 2000 PRL assay.
Endocrinology

T090

IS YOUR THYROID FUNCTION TESTS IN TUNE?: ESTABLISHMENT OF POPULATION BASEDREFERENCE INTERVAL FOR
THYROID FUNCTION TESTS

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BACKGROUND-AIM

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.

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. Different statistical procedures
were applied for reference interval study, i.e., a non-parametric procedure (bootstrap) and a parametric one (after
transformation of the data).

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. The reference interval (90% Confidence interval)
for TSH by non-parametric procedure (bootstrap) is 0.48-4.52, and by parametric one (after transformation of the data)
is 0.45-4.27.

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.

Being a member of International Federation of Clinical Chemistry's (IFCC) Committee for Standardization of Thyroid
Function Test (C-STFT), we have realized that variability in TSH results in different platforms can create a lot of
collision to clinicians and the general population; harmonization of procedures is therefore the need of the hour.
Endocrinology

T091

IMPACT OF A NEW STRATEGY TO MAKE PHYSICIANS TAKE IN CONSIDERATION SERUM SODIUM CONCENTRATION BELOW 126 MMOL/L IN HOSPITALIZED PATIENTS: A RETROSPECTIVE STUDY.

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BACKGROUND-AIM

Hyponatremia is a common electrolyte abnormality among hospitalized patients. It is associated with increased mortality, morbidity and length of hospital stay in patients. Some Clinical Practice Guidelines have been developed in 2014 to outline the importance of this clinical problem. The aim of this study is to describe the relevance of a strategy to make physicians to take in consideration severe hyponatremia.

METHODS

A retrospective analysis of 40 hospitalized patients with severe hyponatremia (defined as a serum sodium concentration < 126 mmol/L) during a 6-month period was assessed to describe this disorder in hospitalized patients. In 2013 December, Laboratory Department included a commentary in the analysis report when serum sodium concentration was < 126 mmol/L in order to encourage physicians to take in consideration the abnormality and recommend doing a serum or/and urine osmolality measurement. The condition to include the patients in the study was to develop an episode of hyponatremia during their stay at the hospital. Details of all serum sodium results with accompanying patient demographics for 6 months were downloaded from the laboratory database Servolab.

RESULTS

Data from 120528 samples were available for analysis. Prevalence of sodium concentration < 126 mmol/L were: 0.69% for acute hospital care patients, 0.04% for ambulatory hospital care; 0.01% for community care. The mean of serum sodium concentration was 139.38 mmol/L in all sodium serum determinations during this period. It has been observed that 72% of the 40 studied patients acquired the disorder during the course of their hospital stay. Studied patients were hospitalized 18.85 days on average. The average age was 70.49 years (43.98 to 93.08 years), including 50% women and 50% men. Two of them died during the stay at the hospital. 40% of the patients had sodium serum levels < 126 mmol/L at the moment of leaving the hospital. Only 20% (8) of these patients had serum or urinary osmolality measurements, as the commentary recommended; and 75% (6) of them had serum sodium >126 mmol/L when they left hospital.

CONCLUSION

The commentary was not as well accepted by physicians as Laboratory Department expected, according to the osmolality measurements. However, at the point of leaving Hospital, patients who had osmolality measurements done, had higher serum sodium concentration than patients without them.
Endocrinology
T092

FIRST TRIMESTER-SPECIFIC REFERENCE INTERVALS FOR THYROID HORMONES DURING PREGNANCY IN A SPANISH POPULATION BY ADVIA CENTAUR METHOD.

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BACKGROUND-AIM
Determining serum thyrotropin (TSH) is a basic test for the diagnosis of thyroid function in the general population. Recent guidelines recommend to use method and trimester-specific reference intervals for TSH. The aim of this work was obtain reference values for TSH and thyroid hormones in first trimester pregnant women living in North Spain area population (Asturias) using the ADVIA Centaur analyzer technology

METHODS
Serum samples were collected from 907 pregnant women (9-13 weeks' gestation) attending in Cabueñes Hospital (Gijón, Asturias) for first-trimester screening during 2014. Levels of serum TSH, free thyroxine (FT4) and free triiodothyronine (FT3) were measured by chemiluminescent immunoassay in ADVIA Centaur analyser (Siemens). Exclusion criteria were previous thyroid disease, thyroid Ab(+), TSH>5 μIU/mL, major health problems and multiple gestations. After the application of these criteria, 840 women were included. Statistical analyses were performed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium).

RESULTS
Reference intervals for thyroid function tests during the first trimester of pregnancy were: TSH 0.11–4.67 μIU/mL; FT4 0.85–1.48 ng/dL; and FT3 2.43–4.27 pg/mL. The medians for these parameters were 2.02 (95% CI: 1.93-2.12), 1.09 (1.08-1.11) and 3.13 (3.07-3.22), respectively.

CONCLUSION
Recent guidelines recommend to use trimester-specific reference intervals for TSH. When these are not available, the reference range usually accepted in the first trimester for the TSH is 0.1–2.5 μIU/mL. There are notable differences in the quantification of these hormones between different analysis methods, so it is very important that each laboratory establish its own normal values.
Endocrinology
T093
VITAMIN D SUPPLEMENTATION IN OBSTRUCTIVE SLEEP APNOEA SYNDROME.
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BACKGROUND-AIM
Our group and others have reported a high rate of vitamin D deficiency in obstructive sleep apnoea syndrome (OSAS), where vitamin D levels (25(OH)D) correlate negatively with apnea–hypopnea index (AHI) and glucose metabolism.

METHODS
In Autum/Winter 2013 we recruited 26 adults (20 male), aged 55.2y ± 12, BMI: 30.4 kg/m² ± 5.6) with nocturnal polysomnogram (PSG) proven OSAS.
70% were stable, long term continuous positive airway pressure (CPAP) users.
At baseline we assessed: Quality of life (QoL) with the Epworth Sleepiness Scale (ESS) and the Sleep Apnoea Quality of Life Inventory (SAQLI), neuropsychological function with trail making tests and Connor’s Continuous Performance Test II.
25(OH)D, calcium, PTH, phosphate, hsCRP, Cholesterol, LDL, HDL and fasting glucose were measured using an Abbott Architect ci8200.
The intervention was 15 weeks of 4,000iu vitamin D3/day or matching placebo.

RESULTS
There were no CPAP or medication changes. CPAP compliance was high (~93%).
There were 7 dropouts, leaving 19 subjects who completed all assessments.
There were no differences between the vitamin D and placebo groups at baseline.
Mean baseline 25(OH)D was 37.2nmol/L (range: 15-87). According to the Institute of Medicine guidelines, 17 (89%) were vitamin D deficient (25(OH)D <50nmol/L), while 2 (11%) were vitamin D sufficient (25(OH)D >50nmol/L).

CONCLUSION
In conjunction with a significant increase in 25(OH)D levels ( p=0.00001), vitamin D supplementation was associated with improved quality of life, as well as metabolic and neuropsychological indices compared to placebo.
Vitamin D replenishment warrants further investigation as an adjunct therapeutic strategy in OSAS.
Endocrinology

T094

COMPARISON BETWEEN SERUM AND PLASMA ALDOSTERONE BY LC-MS/MS

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BACKGROUND-AIM

The aldosterone measurement is important to the screening and diagnosis of primary aldosteronism, location of aldosterone producing tumors, and investigation of other disorders of the renin-angiotensin system. The aim of our study was to compare EDTA plasma and serum samples for aldosterone measurement with our new LC-MS/MS method.

METHODS

All the samples were treated according to our preanalytical procedure: after sampling, they were spun at +4°C at 3500G, aliquoted and kept frozen at -80°C until determination. A comparison was assessed between serum and plasma to check if we could either use one or the other sample type indifferently for aldosterone measurement. We selected 87 remnant samples of EDTA and serum with aldosterone levels ranging from 20 to 700 ng/L to cover the range of usual values. Slope and intercept were calculated using Passing and Bablok linear regression and we compared the methods with the Bland and Altman plots (Medcalc, Mariakerke, Belgium).

RESULTS

On the whole measuring range (n=87), the regression equation was Aldosterone serum = -1.05 + 0.97 Aldosterone Plasma (95%CI of the intercept: (-3.5078 to 1.1625) and 95% CI of the slope (0.9413 to 0.9938). The Bland and Altman plot showed a mean bias of 4.7 ng/L between the two matrix and the standard deviation of the mean was 18.7 ng/ml.

CONCLUSION

The aldosterone results were a little bit lower for the plasma than for serum. After results discussion with the clinicians and the collaborators, despite the small difference between them, we decided to worked indifferently on EDTA plasma or serum with a preference for the EDTA plasma to simplify the preanalytical phase; as we also measure plasma renin activity in EDTA plasma, an analysis which is always asked in the same time for the hyperaldosteronism diagnosis.
Endocrinology

T095

THE INFLUENCE OF CENTRALLY APPLIED GHERLIN ON METABOLIC HORMONS RESPONSE IN YOUNG RATS

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BACKGROUND-AIM

Ghrelin is a peptide that is predominantly produced by the stomach that has a unique structure with 28 amino-acids and an n-octanoyl ester at its third serine residue, which is essential for its potent stimulatory activity on endocrine secretion. The aim of our study was to investigate the effects of centrally applied ghrelin on the blood concentrations of adrenocorticotropic hormone (ACTH), corticosterone, insulin and leptin.

METHODS

Five daily ICV injections of rat acylated ghrelin or solvent were administered once per day (n=10/group, 0.15 nmol of ghrelin in 5 μL) into lateral cerebral ventricle (ICV) of free feeding peripubertal Wistar rats. Two hours after the last injection of ghrelin, rats were decapitated in deep ether anesthesia, and their blood was taken for hormonal analyses.

RESULTS

Serum concentrations of ACTH, corticosterone in ghrelin treated rats were significantly increased by 81.5% and 87.6%, respectively in comparison with controls. Concentration of insulin and leptin were significantly decreased by 53.8% and 46.2% in comparison with controls.

CONCLUSION

These results clearly demonstrate that daily sub-nanomolar doses of ICV ghrelin during five consecutive days significantly increased serum ACTH, corticosterone while decreased insulin and leptin levels. Modulation of central ghrelin receptors may represent a pharmacological approach for controlling hormonal factors involved in energy balance. A further investigations related to central ghrelin effects in the energy balance regulation will hopefully lead to a better understanding of this complex system and provide a new approaches for obesity treatment in young.
Endocrinology

T096

ACCESS® AMH IMMUNOASSAY: PERFORMANCE OF A NEW HIGHLY SENSITIVE AUTOMATED ASSAY

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BACKGROUND-AIM

Anti-Müllerian hormone (AMH) measurement is useful as an aid in the evaluation of the ovarian reserve and in prediction of the outcome of assisted reproductive technology. A number of manual AMH enzyme linked immunosorbent assays (ELISA) are available to determine the AMH level in serum or plasma. However, with the development of automated assays that provide increased sensitivity and lower imprecision compared to ELISA, the use of AMH in routine clinical practice can be expanded. The aim of our study was to evaluate the performance of a new, fully automated AMH assay on the Access family of immunoassay systems.

METHODS

Access AMH is a simultaneous one-step immunoenzymatic assay that uses two AMH-specific monoclonal antibodies in a sandwich format using serum or lithium heparin plasma. The Access AMH assay detects 140 kDa total AMH (cleaved and uncleaved) and does not bind to the other related members of transforming growth factor-\(\beta\) superfamily. Calibrators are prepared with recombinant human AMH. Twenty microliters of sample volume is needed and the quantitative result is available after approximately 40 minutes. Within run and total imprecision were calculated based on 4 serum samples. Method comparison was performed with the Beckman Coulter AMH Gen II assay in 104 patient sera and with the Ansh Labs and Immunotech AMH ELISA assays in 47 patient sera.

RESULTS

The Access AMH assay was standardized against the Beckman Coulter AMH Gen II assay covering a measuring range from 0.02 to 24 ng/mL. The calibration curve and open vial calibrator stability are 31 and 90 days, respectively. Within run and total imprecision ranged from 1.5 to 1.7% and 3.0 to 3.1%, respectively. In this study, the limit of detection (LoD) was 0.0049 ng/mL and limit of quantitation (LoQ) was 0.010 ng/mL. Access AMH, when compared to the AMH Gen II, Ansh Labs, and Immunotech AMH ELISA kits yielded a correlation coefficient of 0.99, 0.99, and 1.00, and a slope of 0.91, 0.79, and 0.87, respectively.

CONCLUSION

The fully automated Access AMH immunoassay demonstrates excellent analytical performance. As a consequence, the availability of the fully automated Access AMH assay will represent a fast and precise alternative to manual AMH assay testing.
Endocrinology
T097

PREVALENCE OF SUBCLINICAL HYPOTHYROIDISM IN ADULTS WITHOUT KNOWN THYROID DISEASE: AN EPIDEMIOLOGICAL STUDY IN FOUR PROVINCES IN WESTERN CHINA

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BACKGROUND-AIM
Subclinical hypothyroidism has previously been associated with an increased risk for many serious diseases such as coronary heart disease (CHD) and metabolic syndrome (MS), etc. In addition, of patients with SH, approximately 2% to 5% per year will progress to overt hypothyroidism (OH) which is another serious threat to human health. However, there is a paucity of data on the prevalence of SH in healthy adult population of western China. This multi-center epidemiological study was conducted in four major provinces (Sichuan, Shanxi, Qinghai and Xinjiang) to estimate prevalence of subclinical hypothyroidism among healthy adults.

METHODS
All participants answered a questionnaire that included demographic data, reproductive history, smoking history, previous thyroid disease, family history of thyroid disease, etc. and had a blood sample collected to assess levels of thyrotropin, free-thyroxine and free triiodothyronine when enrolled. SH were diagnosed on the basis of laboratory results.

RESULTS
(1)The prevalence of SH in the overall study population was 15.8% (11143/70540) and in Sichuan, Shanxi, Qinghai and Xinjiang, were 15.7% (8373/53499), 15.7% (10896/69579), 27.3% (204/748) and 20.7% (44/213), respectively. 
(2)Prevalence of SH increased gradually with age both in males and females(P<0.05). (3)No matter in which age strata, SH prevalence in females was higher than that in males(P<0.05).

CONCLUSION
The prevalence of SH in western China was high, affecting approximately 2 in 10 adults in the study population. Female gender and older age were found to have significant association with SH. Adults in western China, especially females over 40 years old, should regularly check thyroid function and take timely corresponding intervention.
Endocrinology
T098
A POTENTIAL RELATION BETWEEN 25 HYDROXYVITAMIN D AND VITAMIN B12 IN OBESE WOMEN
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BACKGROUND-AIM
It is known that obesity is associated with low circulating concentrations of 25-hydroxyvitamin D (25OH-D). Also, it is observed that level of serum vitamin B12 could be altered in individuals with a high bodyweight. The aim of this study was to evaluate serum concentration and potential relation between vitamin B12 and 25OH-D in group of obese middle aged women.

METHODS
The study included 50 obese women with body mass index (BMI) mean 39.36±6.07 kg/m² and 30 healthy aged matched, lean control subjects, (BMI 22.34±1.81kg/m²). Blood was drawn in order to determine serum concentration of 25OH-D and vitamin B12 assayed on automated system Elecsys 2010 and Abbott Architect ci4000, respectively. Also, after assessing anthropometric measurements (body weight (BW, kg), body height (BH, m) and waist circumference (WC)), we calculated anthropometric indexes (body mass index (BMI) and waist to stature ratio (WSR)). Fat mass (kg), fat percentage and total body water (TBW) were determined by the bioelectrical impedance method, extracellular volume (ECV) was calculated using Peters formula. Results were processed by Data Analysis statistical package.

RESULTS
The vitamin B12 level in obese women was significantly lower than of lean women (median 190.15 (144.0-250.2) vs. 252.95 (227.0-417.7), pmol/L p<0.01). Also, concentration of 25OH-D was statistically lower in obese group (median 27(16-38) vs. 74.5(55-80), nmol/L, p<0.01). Level of 25OH-D inversely correlated with BMI, WSR and ECV, r=-0.685, r=-0.370; r=-0.600, p<0.01. Vitamin B12 negatively correlated to identical parameters, r=-0.359, r=-0.557, r=-0.253, p<0.01, respectively.

We found statistically significant negative correlation between level of 25OH-D and TBW, however didn’t established equivalent relation for vitamin B12. We determined significant positive correlation between 25OH-D and vitamin B12 (r=0.412, p<0.01).

CONCLUSION
25OH-D and vitamin B12 was lower in obese women. According to results, there is apparent relation between low circulating levels of 25OH-D and vitamin B12. 25OH-D dependent calcium level and absorption of vitamin B12 or/and enhancement of extracellular volume in study group could be responsible for low vitamin B12 status in obesity.
Endocrinology
T099

PROTOTYPE OF THE FIRST ACCURATE IMMUNOASSAY FOR LOW ESTRADIOL CONCENTRATION DETERMINATION.

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BACKGROUND-AIM
Estradiol immunoassays are prone to inaccuracy at low estradiol concentrations which is detrimental when addressing the clinical status of children, men, postmenopausal women, and women receiving aromatase inhibitors. The aim of the abstract is to present the first immunoassay that is able to accurately and precisely measure estradiol concentrations from 15 pg/mL to 5000 pg/mL.

METHODS
75 samples covering the physiological variability (male, female, post-menopausal) and the range of estradiol concentrations (1.19-4368 pg/mL) were assayed on a JCTLM approved reference measurement procedure (ID-GC/MS), Abbott Architect (A), Siemens Centaur XP (S), Roche Cobas (R) and Beckman Coulter Access current (B) and prototype (P) immunoassays. Method comparison was assessed using Passing Bablok linear regression and Bland-Altman percentage bias. For bias, a criterion of 35% was used from German RLiBak recommendations for accuracy. Correlation was assessed using Spearman rank correlation. For all analysis, ID-GC/MS estradiol measurement is used as reference.

RESULTS
The Beckman Coulter Access prototype assay exhibits the most accurate performance compared to the reference method. The prototype assay correlated with a 95% confidence interval (95% CI) lower limit of 0.98 while methods B and S had a lower limit below 0.97 and methods R and A below 0.93. The 95% CI of the linear regression slope is within 0.9-1.1 for the prototype assay and methods A and B while it is up to 1.15 and 1.24 for methods S and R respectively. The intercept is 3.3 pg/mL and is not statistically different from 0 at 95% CI for the prototype assay while it is statistically different and goes up to 10-15 pg/mL for the other methods. For samples approximately 15 pg/mL, the Bland-Altman percentage difference is below 35% for the prototype assay while it is up to 150-400% for all other methods.

CONCLUSION
Beckman Coulter Access prototype assay is able to accurately measure low estradiol concentrations which represents more than 50% of routine clinical measurements in general laboratories. In this study the prototype assay is the only immunoassay that is accurate and precise down to 15 pg/mL while other immunoassays are efficient down to 30-50 pg/mL in the best case.
Endocrinology

T100

IMPROVING SURVEILLANCE IN MEN WITH ANDROGEN-DEPENDENT TUMORS: CALCULATED OR MEASURED FREE TESTOSTERONE?

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BACKGROUND-AIM

Background. Recent studies showed the usefulness of total testosterone (TT), sex hormones binding globulin (SHBG), free testosterone (FT) and bioavailable testosterone (BT) in the improved assessment of tumor aggressiveness in prostate cancer (PK).

Aim. To assess the correlations between TT, SHBG, FT and BT in men, in order to evaluate the utility of these markers in post-surgery PK patients’ monitoring.

METHODS

We selected 32 sera from randomized patients (men) without major endocrine disorders. Patients with renal or hepatic diseases were excluded. Mean age of the studied group was 36.4 years (± 11.4). TT and SHBG were measured by immunochemiluminiscence, FT by ELISA (mFT). Analytical performance was controlled with internal controls and external quality participation scheme. Calculated FT (cFT) and BT values were obtained with an online calculator (Vermeulen et. al, www.issam.ch), considering an albumin value of 4.3 g/dl.

The study was approved by Ethics Committee of the Institute.

RESULTS

We found a positive, significant correlation between TT and SHBG (r=0.51, p<0.0001), TT and BT (r=0.59, p<0.0001), TT and cFT (r=0.59, p<0.0001), and a lower correlation between TT and mFT (r=0.36, p<0.0001). There is a slightly negative, but significant correlation between TT and FT(%) (r=-0.39, p<0.0001) and no correlation between TT or mFT and age, respectively. mFT concentrations were lower than cFT (0.01 ± 0.0059 ng/ml vs 0.078 ± 0.033ng/ml).

CONCLUSION

As post-surgery PK patients usually undergo a hormonal therapy (Luteinizing hormone-releasing hormone (LH-RH) agonist therapy, antiandrogen therapy, androgen deprivation therapy) we suggest that, behind the routinely measured biomarkers such as total and/or free prostate-antigen specific antibody (PSA), TT, cFT and SHBG should be measured before surgery and monitored during the specific treatment. From our preliminary results we estimate that cFT is a better monitoring biomarker in these patients.

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Endocrinology

T101

SELECTION OF SUITABLE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS TESTS FOR DIAGNOSTIC OF HYPOCORTISOLISM

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BACKGROUND-AIM

Hypothalamic-pituitary-adrenal axis disorders (HPA axis) manifested as adrenal insufficiency are related to higher morbidity and mortality rates. In contrast, the substitution therapy, when inappropriately indicated, has also vital metabolic risks. Nowadays, a range of diagnostic methods of HPA axis testing is used. A gold standard for HPA axis evaluation is a hypoglycemia test (ITT) which has, however, several restrictions in use. The aims of the study were a comparison of four HPA axis tests and design the new diagnostic algorithms of hypocortisolism.

METHODS

Firstly we estimated reference intervals for salivary cortisol in healthy volunteers. In our study we investigated 20 healthy volunteers by insulin tolerance test (ITT), high (HDST - 250 μg), low dose (LDST - 1 μg) and 10 μg Synacthen test (MDST) as well as 20 patients with adrenal insufficiency (primary and secondary). We evaluated serum cortisol, cortisone, and other metabolites during dynamic tests by LCMS-MS. Serum cortisol, salivary cortisol were determined also by chemiluminiscent immunoassay (ADVIA:Centaur Siemens) and basal levels of cortisol binding globulin, aldosterone and ACTH were determined by radioimmunoassay.

RESULTS

All healthy volunteers reached the normal response of cortisol (>500nmol/L) in all tests. The levels of cortisol metabolites were significantly lower in LDST comparing to remaining tests and the peak was observed at the 60 minutes after the stimulation. The levels of salivary cortisol were significantly higher (45 ± 10.5 nmol/L) in the HDST and ITT compared to LDST and MDST (32 ± 2.5 nmol/L). Serum cortisol levels were significantly lower after stimulation in these tests.

CONCLUSION

In healthy volunteers, four different HPA axis tests gave sufficient response of cortisol. MDST test gave the similar response as LDST test. Salivary cortisol reached similar response as serum cortisol. In patients, the HDST test gave also sufficient response in immunoassay analysis compared to LCMS/MS. Serum cortisol reached in MDST and LDST levels of adrenal insufficiency. Salivary cortisol was significantly lower in all tests. The MDST test may replace the LDST test since it gave the similar response in cortisol.

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Endocrinology

T102

EFFECTS OF SUBCLINICAL HYPOTHYROIDISM ON LIPID PROFILE

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BACKGROUND-AIM

Hypothyroidism is associated with abnormalities of lipid metabolism, but there are conflicting results regarding the values of lipid profile in subclinical hypothyroidism (SCH). The aim of the study was to assess differences in lipid profile parameters between subjects with and without SCH.

METHODS

Serum lipid parameters of 40 patients with subclinical hypothyroidism were evaluated in this study.

RESULTS

Mean serum total cholesterol (TC) (5.72+/−1.15 vs 4.93+/−0.81 mmol/L) and triglycerides (TG) (1.97+/−1.12 vs. 1.55+/−0.62) were significantly higher in patients with SCH (P<0.05). Mean TC, TG and low-density cholesterol (LDL-C) concentrations were higher in patients with serum thyroid stimulating hormone (TSH) greater than 10mIU/L than those with serum TSH equal to or less than 10mIU/L, but this difference was not statistically significant. There was no association between serum high-density cholesterol (HDL-C) concentration and serum TSH level.

CONCLUSION

Thyroid dysfunction has a great impact on serum lipid profile. High TC and TG were found in our patients with subclinical hypothyroidism.
Endocrinology  
T103  
CHROMOGRAIN A AND WE-14 PEPTIDE Importance AS BIOCHEMICAL MARKERS IN CARCINOID SYNDROME DIAGNOSIS  
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BACKGROUND-AIM  
Carcinoid syndrome occurs in 20% of cases of well-differentiated endocrine tumors of the jejunum or ileum. Chromogranin A (CgA) is a general marker for neuroendocrine tumors. CgA level is increased in 85%-100% of patients with carcinoid regardless the tumour is functional or non-functional. The specificity has been found to be over 95% and the sensitivity over 60%. Some authors revealed that false negative or positive CgA results could affect a correct diagnosis and raised the question if it is not the time to find another reliable marker in such cases. Chromogranin A appears to undergo a process of fragmentation and the fragments detected by particular tests influence the resulting sensitivity. In our study besides CgA, we tried to measure another peptide: WE-14 as a result of post-translational processing of CgA. We considered WE-14 as a new tool in carcinoid diagnosis.

METHODS  
A group of 10 patients suspected of carcinoid: 5 women (27-74 years) and 5 men (35-69 years) and a matched control group of 10 subjects (6 women and 4 men, without no endocrine dysfunction) were included in this retrospective study (2013-2014). 
Plasma CgA was assayed by an Elisa kit and plasma WE-14 by an EIA research kit. Serum serotonin (5-HT) was also assayed by an Elisa method. Paired t-test were used for geometric means comparison and for two-tailed probability. Sensitivity and specificity of all parameters were tested by Receiver Operating Curves (ROC analysis).

RESULTS  
In tumor cases all 3 parameters were increased. As expected, geometric means for all 3 parameters differed significantly in carcinoid group vs. control group: CgA: 115.87 ng/mL vs. 41.28 ng/mL (mean difference: -0.44; standard error: 0.1254; P=0.006); 
WE-14: 1.21 ng/mL vs. 0.39 ng/mL (mean difference: -0.48; standard error: 0.1288; P=0.0044); 5-HT: 487.10 ng/mL vs. 163.84 ng/mL (mean difference: -0.4732; standard error: 0.077; P=0.0002). ROC analysis established for CgA: 70% sensitivity and 90% specificity (associated criterion >55 ng/mL); for WE-14: 80% sensitivity and 90% specificity (associated criterion >0.70 ng/mL). Comparison of ROC curves: WE-14>CgA, revealed no significant difference between their areas.

CONCLUSION  
In our study both CgA and WE-14 have the same specificity in carcinoid syndrome diagnosis but WE-14 sensitivity is greater.
Endocrinology
T104

THYROID-STIMULATING HORMONE CONCENTRATION IN EUTHYROID WOMEN WITH AND WITHOUT METFORMIN TREATMENT

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BACKGROUND-AIM

Recent studies have suggested that metformin, a first-line oral hypoglycemic agent, may lower thyroid-stimulating hormone (TSH) concentration in patients with diabetes and hypothyroidism, while thyroid hormone concentrations remain unaltered. This often makes the results difficult to interpret. The clinical consequences of this effect is still incompletely understood. Our objective was to determine whether the use of metformin monotherapy in euthyroid women with type 2 diabetes, when compared with normoglycemic and hyperglycemic euthyroid women without treatment, is associated with decreased TSH concentration.

METHODS

In this study, we examined three groups of women First group (I): 40 euthyroid women (53 ± 5 yrs old) with type 2 diabetes treated with metformin at least 1 year; Second group (II): 45 euthyroid women (52.5 ± 8 yrs old) with fasting plasma glucose concentration below 100 mg/dl; Third group (III) 35 euthyroid women (54 ± 5 yrs old) with fasting plasma glucose concentration greater than or equal 126 mg/dl (pharmacologically untreated diabetes type 2). All groups were adjusted for BMI (BMI values 39.4 ± 7.1; 37.1 ± 8.0; 37.0 ± 7.5, respectively), smoking status and alcohol consumption. All women were recruited during the first day of sanatorium treatment at the Department of Balneology of Nicholas Copernicus University in Bydgoszcz, Poland. Serum TSH and plasma glucose were measured on the Architect ci8200 (Abbott Diagnostics).

RESULTS

Medians and interquartile ranges of TSH concentration were as follows: group I 1,105 μIU/ml (0,96-1,67), group II 1,29 μIU/ml (0,91-1,89), group III 1,23 μIU/ml (0,93 – 1,89) p = 0.21. The prevalence of TSH results were as follows: I 30%, II 30%, III 30% (TSH range: 0,35-1,0 μIU/ml); I 50%, II 50%, III 46% (TSH range: 1,1 – 2,0 μIU/ml); I 20%, II 20%, III 11,5% (TSH range: 2,1 -3,0 μIU/ml); I 0%, II 0%, III 7,5% (TSH range: 3,1 – 4,0 μIU/ml).

CONCLUSION

Metformin appeared to have no effect on TSH levels in euthyroid women with diabetes type 2.
Endocrinology

T105

PROLACTIN AND REPRODUCTIVE HORMONE STATUS IN OLIGOMENORRHEIC AND INFERTILE FEMALES

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BACKGROUND-AIM

Oligomenorrhea is one of the significant problems of women these days. Oligomenorrhea during reproductive age group may lead to infertility which may cause matrimonial disharmony which is taken as serious problem in Asian sub-continent. The present study was designed to assess the Prolactin, Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in oligomenorrheic and infertile patients in Eastern region of Nepal.

METHODS

A total of 126 patients came to the immunoassay laboratory of Department of Biochemistry for the testing of Prolactin, LH and FSH from Department of the Obstetrics and Gynecology with complain of oligomenorrhea and primary and secondary infertility were enrolled in this study. Five milliliters venous blood samples were collected in plain vials and transported to the laboratory maintaining cold chains. Serum Prolactin, FSH and LH were measured by ELISA method (Eliscan, India). Kolmogorov-Smirnov test was used to test the normality of the data. Man-Whitney test was used to test the significance of hormone level between the groups at p value <0.05.

RESULTS

The mean age of patients was 24.33±5.91 ranges from 15-45 years. Majority (96, 76.2%) of them had complain of oligomenorrhea and 30 (23.8%) of them had either primary or secondary infertility in whom pregnancy test was ruled out and kept under single category. Out of 96 oligomenorrhic patients elevated level of FSH, LH and Prolactin were found in 17 (17.7%), 16 (16.67%) and 40 (41.66%) respectively. Similarly, in 30 patients with primary or secondary infertility, elevated level of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. The median and interquartile range of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. The median and interquartile range of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. The median and interquartile range of FSH, LH and Prolactin were found in 6 (20.00%), 3 (10.00%) and 16 (53.33%) respectively. There was no statistical difference between the median values of LH (p=0.665) and Prolactin (p=0.229) in oligomenorrhea and infertile group.

CONCLUSION

Our study showed that there was no remarkable difference of serum LH and Prolactin between oligomenorrheic and infertile women.
Endocrinology

T106

THE EFFECT OF 25-OH VITAMINE D LEVELS IN CORD BLOOD ON FETAL MALNUTRITION AND NEONATAL ANTHROPOMETRY MEASUREMENT

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BACKGROUND-AIM

We aimed to investigate 25-OH vitamine D levels in cord blood with Fetal ratio of fetal malnutrition (FM), distribution of FM among Appropriate for Gestational Age (AGA) and Small Gestational Age (SGA) neonates.

METHODS

Our study 112 neonates were included and carried out between March 1, 2012 and 01 April 2012 in Bakirkoy Dr. Sadi Konuk Research and Training Hospital. We established correlation between FM and socioeconomical status of the families by using CAN Score (Clinical Assessment of Nutritional Status) and then to compare. Cord blood is collected from the umbilical cord vein attached to the placenta after the umbilical cord has been detached from the neonate, tubes were protected from sun light during for clotting about 45 minutes at room temperature before centrifuged at 4000 rpm for 10 minutes and then 25 OH vitamin D levels (ng/mL) were measured immediately using an Electrochemiluminescence immuno-assay (Liasion hormon analyzer). Our patients group was choosen 58 neonates according their FM condition (n=22 Small Gestational Age (SGA) and N=36 Appropriate Gestational Age (AGA)) and who has not FM included control group (n=1 SGA and N=53 AGA)

RESULTS

In control and study groups 25 OH vitamin D levels were analyzed. There was no statistical correlation for 25-OH vitamin D levels between birth height and weight (p=0.05). But statistically correlation was observed between head circumference measurement, CAN Score points and D vitamine levels (respectively, r =0.219 p=0.021, r=0.290 p=0.002).

CONCLUSION

Low 25-OH vitamine D level in cord blood can a risk factor for intrauterine brain development. Further investigations with larger patient groups are required to confirm our results.
Endocrinology
T107

THE EFFECT OF GLUCOSE LOADING ON SERUM METHYLATED ARGININES BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

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BACKGROUND-AIM

Methylated L-arginine analogs are involved in nitric oxide synthase activity regulation. Impaired glucose tolerance (IGT) represents a state that increases the risk not only for type 2 diabetes mellitus but also for cardiovascular diseases. Endothelial dysfunction and low-grade inflammation are important abnormalities in people with IGT that may contribute to this dual risk. Our aim was to determine the acute effect of glucose loading on serum methylarginine levels.

METHODS

For serum methylated arginines measurement, 78 (32 men, 46 women) serum samples were collected from 75 g oral glucose tolerance testing (OGTT) and 100 µL of internal standard (d7-ADMA) in methanol were added to 200 µL of serum and centrifuged at 13,000 rpm for 10 minutes to remove the precipitated proteins. The supernatant was collected and dried under a nitrogen gas flow at 60 ºC. Derivatisation step was performed dissolving the dried extract in 200 µL of a freshly prepared butanol solution containing 5% (v v−1) acetyl chloride and kept at 60 ºC for 20 minutes. The solvent was removed by evaporation under nitrogen flow at 60 ºC. The derivatised samples were dissolved in 100 µL of water–methanol (90:10, v v−1) containing 0.1% (v v−1) formic acid and 40 µL was injected into the UPLC analytical column for chromatography.

RESULTS

According to statistical analysis, monomethylarginine (L-NMMA) was found to be higher in men compared to women for all 0., 60. and 120. minutes. There was no statistically significant change for 0, 60 and 120. minutes for ADMA values (p=0.686) but for arginine and citrulline as higher at 0. minute (p=0.002 and p=0.011, respectively).

CONCLUSION

Serum methylated arginine levels are considered to be a risk factor for chronic processes as atherosclerosis. Glucose loading leads to lower arginine and citrulline levels. According to this study’s results, glucose seems not to affect serum ADMA in acute processes.
Endocrinology
T108

CHANGES OF C-AMP LEVEL DURING OESTRUS CYCLE IN NORMOTENSIVE AND SPONTANEOUS HYPERTENSIVE RATS

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BACKGROUND-AIM
The mammalian pineal gland is under adrenergic control; however, the physiological oscillations of gonadal steroids could strongly affect the melatonin synthesis and secretion by acting on the pre- and postsynaptic levels and by modulation of the target cells replay. The aim of this study was to determine the basal levels of cAMP in the pineal gland during the various phases of oestrous cycle in normotensive (NTR), Wistar rats and spontaneously hypertensive (SHR) Okamoto and Aoki rats and to describe the histological finding of the pineal gland tissues.

METHODS
Two hundred female mature rats (100NTR and 100SHR) were investigated. They were divided in 4 groups according to the phases of the oestrous cycle (diestrus, proestrus, estrus and metaestrus). The phase of oestrous cycle has been determined by microscopic analysis of the vaginal smears.

RESULTS
The level of cAMP (RIA) in the pineal gland was the parameter of its intracellular activity. The pineal gland tissues were stained on HaEo. In SHR there is a slight shortening of the oestrous cycle. In NTR there was an increase of the cAMP level from proestrus to metaestrus, contrary to the dramatic decrease in SHR. Histological findings of pineal glands showed the presence of many changed pinealocytes with piconotic nucleuses, while the neuroepithelial cells, in the upper parts of the glands, were separated in gland-like islets. There was a normal pineal histology in NTR.

CONCLUSION
This study indicated significant neurohormonal differences between NTR and SHR. The changed adrenal activity in SHR correlated with histological findings in the pineal gland.
Endocrinology

T109

ANALYTICAL EVALUATION OF A NOVEL AUTOMATED ANTI-MÜLLERIAN HORMONE IMMUNOASSAY

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BACKGROUND-AIM

Anti-Müllerian hormone (AMH) is a glycoprotein produced by the granulosa cells of the growing ovarian follicles and by immature Sertoli cells. Measurement of AMH levels is relevant for the evaluation of primary ovarian insufficiency, success of assisted reproductive therapies, and for the diagnosis of polycystic ovary syndrome. However, the methods for measuring AMH concentrations remain mostly enzyme linked immunosorbent assays (ELISA) and are far from fully automated. The objective of our study was the evaluation of a new fully automated AMH assay.

METHODS

We determined the limit of detection, within-run and between-run imprecision, and linearity of Elecsys® AMH assay performed on the Cobas 8000 platform (Roche Diagnostics). The Elecsys AMH assay is a fully automated method based on the ruthenium electrochemiluminescence technology. The capture and detection antibodies bind preferentially to the AMH mature region (Mab F2B/12H) and to the AMH pro-region (Mab F2B/7A), respectively. Method comparison was performed with the Ansh ELISA (AnshLabs) using serum samples of 65 patients.

RESULTS

The limit of detection of the Elecsys AMH immunoassay was calculated to be 0.013 ng/ml (n=10). Intra-assay and inter-assay coefficients of variation were 2%. The linearity of the assay between 0.14 to 11.8 ng/ml was confirmed through serial dilutions of a high concentration sample. The median AMH levels were 1.26 ng/ml (range: 0-12.98 ng/ml) with Elecsys assay and 1.7 ng/ml with the Ansh assay (range: 0-17.9 ng/ml). The correlation between the AMH assays was excellent (r=0.97, p<0.001). Passing-Bablok regression analysis showed a slope of 0.73 and an intercept of 0.05. Bland-Altman plot evidence a strong bias between the methods with a mean bias of 0.9 ng/ml.

CONCLUSION

Our results demonstrated excellent analytical performances of the Elecsys AMH assay as well as a significant relationship with an established ELISA assay. Furthermore, this automated format can provide benefits for shorter turn around time of analysis and assays consolidation. However, AMH assays are not standardized and commutable as confirmed by our study and a transition to routine needs careful evaluation of reference values and strong communication with physicians.
Endocrinology
T110

ENDOCRINE RESPONSE IN RUGBY PLAYERS BEFORE COMPETITION AND DURING SIX DAYS OF RECOVERY PERIOD

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BACKGROUND-AIM
Physical exercise can lead to activation of hypothalamic-pituitary-adrenal (HPA) axis, but also adversely affect reproductive hormones. Rugby match is a form of exhaustive physical activity that induces change in concentration of different hormones as well of interleukin 6 (IL-6) which synthesis is a part of normal physiological response to exercise. It is also known IL-6 ability to activate HPA axis at its various levels. The objective of this study was to investigate time course changes in concentrations of IL-6, ACTH, cortisol (C), as parameters of HPA axis activity, as well the change of FSH, LH and testosterone (T) in amateur male rugby players before the game and during six days of recovery period.

METHODS
Blood samples were collected from 13 rugby players (22.92±4.59 years) at a day before match (Day0), on the morning of competition (Game) and during recovery period: 24 hours after the game (Day1), at the third day (Day3) and the sixth day following competition (Day6). ACTH was determined with ECLIAs method (Elecsys, Roche Diagnostics), while other hormones and IL-6 were measured using Access® 2 analyzer (Beckman Coulter, Inc., USA). One-way repeated measures ANOVA with Bonferroni post hoc correction was used to compare results at specified time points and p<0.05 was consider to be statistically significant.

RESULTS
Our results revealed statistically significant decrease in ACTH (p<0.05) at Day1 and increase of IL-6 (p<0.05) at Day3 compared to their basal levels (Day0). Cortisol reached its maximal concentration (509.86±20.39 nmol/L) immediately before competition (Game) and showed progressive decline during recovery period. There was no statistically significant effect of time on FSH and LH levels, but testosterone showed statistically significant increase (p<0.05) at Day3 compared to its minimal value reached at Day1.

CONCLUSION
The results of presented study suggest that intensive physical exercise, such as rugby match, influences the dynamic of the change of parameters of HPA axis activity, as well of the reproductive hormones. The pattern of change of analyzed hormones, corroborates anabolic hormonal profile during recovery period and altogether may suggest that period of six days is required to their return to baseline measures.
Endocrinology

T111

DEVELOPMENT AND PERFORMANCE OF THE DIMENSION VISTA® TOTAL TESTOSTERONE ASSAY WITH LOCI® TECHNOLOGY

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BACKGROUND-AIM
The Dimension Vista® System Total Testosterone* assay is a homogenous, chemiluminescent immunoassay incorporating LOCI® technology, which enables high sensitivity immunoassay formats. We describe the development and performance of the Total Testosterone method.

METHODS
The Total Testosterone assay has three components and follows a competitive format. One component (Sensibeads) has latex particles coated with streptavidin and contains a photosensitive dye. A second component (Chemibeads) has latex beads coated with a Testosterone analog and contains a chemiluminescent dye as the signal generating component. During an assay the sensibead and chemibead form a bead-aggregated immunocomplex in the presence of biotinylated antibody reactive with analog on chemibeads. Illumination of the complex by light at 680 nm generates singlet oxygen from sensibeads, which diffuses into chemibeads to trigger a chemiluminescent reaction that is measured at 612 nm. The resulting signal is inversely proportional to the concentration of analyte in the sample.

RESULTS
The assay uses a 10 µL sample volume of serum or plasma and has an analytical range of 8-1000 ng/dL undiluted. With dilution, samples up to 2000 ng/dL can be tested. Results are traceable to the CDC ID-LC-MS/MS reference method. Time to first result is 23 minutes. Precision was evaluated per CLSI EP5 using serum pools and commercial quality control materials. Repeatability and within-lab precision were < 4.9 %CV and < 7.0 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus two different systems: Dimension Vista = 0.93 * ID-LC-MS/MS + 3.9 ng/dL (r = 0.99, n = 38), Dimension Vista = 0.90 * Roche ELECSYS® – 2.60 ng/dL (r = 0.99, n = 215). Minimal cross reactivity (< 10%) was observed with key compounds including: androstenedione, androsterone, 5α-dihydrotestosterone, corticosterone, 11-deoxycortisol, DHEA, DHEA-sulfate, 17b-estradiol, progesterone, cortisol, dexamethazone, danazol, 17a-methyltestosterone, 11b-hydroxytestosterone, and 11-ketotestosterone.

CONCLUSION
The Dimension Vista Total Testosterone assay exhibits excellent performance characteristics and shows a high level of agreement with the Testosterone assays on Roche Elecsys and ID-LC-MS/MS.

*product under development – not available for sale
Endocrinology

T112

THE IMPACT OF GENETIC POLYMORPHISM OF AROMATASE (CYP 19) ENZYME ON THE SERUM LEVEL OF TESTOSTERONE AND THE SUSCEPTIBILITY TO POLYCYSTIC OVARY SYNDROME

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BACKGROUND-AIM

Ovarian androgen overproduction is the key physiopathologic feature of polycystic ovary syndrome (PCOS). Aromatase is a key steroidogenic enzyme that catalyzes the conversion of androgens to estrogen. Several studies reported the association of the SNP 50 (rs2414096) in aromatase enzyme with hyper-androgenism. The aim of the present study was to investigate the association of genetic polymorphism of aromatase enzyme with hyper-androgenism and the susceptibility to polycystic ovary syndrome (PCOS).

METHODS

The study consisted of 124 women diagnosed with PCOS and 112 healthy women as a control group. Individuals were genotyped for rs2414096 of aromatase enzyme by using polymerase chain reaction- restriction fragment length polymorphism. Statistical analysis was done by SPSS program.

RESULTS

Mean serum level of testosterone was significantly higher among carrier of XA (GA & AA) genotype compared to carriers of GG (P< 0.05). Frequency of (GG) and (GA&AA) genotypes were 64.5% and 35.5% in PCOS group compared to 82.1% and 17.9% in control group. Statistical analysis demonstrated that carriers of (GA&AA) genotype were at significant higher risk for PCOS compared to carriers of (GG) genotype (OR= 2.5, 95%CI= 1.4-4.6).

CONCLUSION

Polymorphism of rs2414096 in CYP19 is associated with the pathogenesis of PCOS.
THE INTERFERENCE OF FOOD CAPSAICIN ON THE TOTAL METANEPHRINES URINE ASSAY

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BACKGROUND-AIM

Urine metanephrines are widely used to screen for catecholamine producing tumors. Routine use of a high-pressure liquid chromatography (HPLC) assay for (nor)-metanephrines in urine comprises the use of an internal standard for concentration calculation. The peak of the internal standard, in samples from presumably patients of Hindu origin appeared to be sporadically increased by an unknown interference, giving rise to false negative (nor)-metanephrines concentrations. The aim of this study was to explore whether extensive use of paprika and chili peppers (capsaicin sources) might be related to interference with the internal standard.

METHODS

Chemicals for total urine metanephrine analysis were purchased from Instruchemie (Delfzijl, The Netherlands). Dried chili peppers have been extracted and derivatised as described earlier (1). The used extraction is a two-step procedure: acid extraction with toluene-isoamylalcohol, followed twice by an alkaline extraction at pH 10-12 with ethylacetate of the first watery fraction. The combined and dried ethylacetate fractions were derivatised with pentafluoropropionic anhydride (PFPA). The extracted and derivatised pepper samples were analysed with GC-MS-MS and compared to derivatised vanillylamin samples.

RESULTS

Vanillylamin was found in extracts of dried chilli peppers, after derivatisation with PFPA. Retention time and MS-transitions of the peak in dried chili peppers are identical to those of internal standard vanillylamin used in HPLC analysis of Metanephrines.

CONCLUSION

We proved that disturbing elevation of vanillylamin peak in human urinary samples is dietary related and caused by capsaicin after consumption of chilli peppers. One should be aware that in some groups of patients where extensive dietary or pain management use of capsaicin in chronic diseases may interfere in the assays where vanillylamin is used as internal standard.

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T114

SWITCHING FROM RIA TO LC-MS/MS FOR PLASMA AND URINARY ALDOSTERONE

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BACKGROUND-AIM

Aldosterone measurement is critical for screening and diagnosis of primary aldosteronism, location of aldosterone producing tumors, and investigation of other disorders of the renin-angiotensin system. Liquid chromatography triple quadrupole mass spectrometry (LCMS2) has become an essential tool for small molecule quantitation due to its high sensitivity, specificity and its excellent reproducibility. We aimed to compare RIA and our new LCMS2 method for plasma and urinary aldosterone measurement.

METHODS

Until 2014 October we used Radio-Immunoassays (RIA) (Diasorin). From October 2014, we used a LCMS2 (TQ5500, ABSciex). The accuracy profile was determined in triplicate during 3 days with 5 plasma and 5 urine pool levels. A total of 68 plasma and 22 urine samples were assayed for method comparison. Slope and intercept were calculated using Passing and Bablok linear regression and we compared the methods with the Bland and Altman plots (Medcalc software).

RESULTS

CV intra-assay were 5.1% and 7.3%, total precision 5.1% and 8.6% (range: 5-1000 ng/L for plasma and 7-110 µg/L for urine respectively). LOQ were at 20 ng/L for plasma and 2.7 µg/L for urine. Linearity was good between 5 and 1000 ng/L for plasma and between 2.7 and 112.5 µg/L for urine. Recovery is 100±4.7% (95%CI for the mean: 98.3-101.7%) for urine and 100±1.9% (95%CI for the mean: 98.9-101.1%) for plasma. For the comparison between RIA and LCMS2 in plasma, the regression equation was RIA=40.6+1.6 LCMS2 (95% CI of the intercept: (30.3; 52) and 95% CI of the slope: (1.5; 1.7)). In urine, the regression equation was RIA=2.4+0.8 LCMS2 (95% CI of the intercept: (1.2; 3) and 95% CI of the slope: (0.7; 0.9)). The Bland and Altman showed that results were in mean 59% higher in RIA than in LCMS2 for plasma and 26% lower in RIA than in LCMS2.

CONCLUSION

We noted a significant bias between results by RIA and LCMS2. Compared to LCMS2, RIA didn’t differentiate aldosterone glucuronide (in CKD patients) from native aldosterone. After the comparison with 2 others laboratories using this method and results discussion with the clinicians, we switched from the RIA to LCMS2 for the aldosterone on the basis of its improved sensitivity and specificity.
Endocrinology

T115

SIMULTANEOUS MEASUREMENT OF SERUM CORTISOL AND ALDOSTERONE BY LC-MS/MS TQ5500

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BACKGROUND-AIM

Obtain an accurate and precise dosage of steroid hormones is important for the clinicians. The liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) is become an essential tool for small molecule quantification due to its high sensitivity and specificity, excellent reproducibility and the ability to perform simultaneous analysis. As there is a structure similitude between aldosterone (ALDO) and cortisol (COR), the aim of our evaluation was to test if it is possible to associate both in a same run with a same sample preparation.

METHODS

Our LC-MS/MS included a UFLC XR (Shimadzu) and a triple quadrupole mass spectrometry TQ5500 (ABSciex). The samples were centrifuged; deuterium labelled aldosterone and cortisol was added as internal standard and a liquid-liquid extraction (LLE) was performed. The supernatant was evaporated, dissolved in a mix water/methanol (50/50) and analyzed by LC-MS/MS. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each compound and internal standard. In negative ion mode, aldosterone can be quantified using the MRM transition at 359.2>189 (quantifier ion) and 359.2>331.1 (qualifier ion). In positive ion mode, cortisol can be quantified using the MRM transition at 363.3>97 (quantifier ion) and at 363.3>121.1 (qualifier ion). We performed validation with the Enoval software (Arlenda, Belgium) on 3 and 5 levels in triplicate that we analysed during 3 days for COR and ALDO respectively. For the validation, we used remnant samples with measured levels of compound by other method for the COR and ALDO respectively.

RESULTS

For the COR, the with-in run and the between-run did not exceed 6.1% in the concentration range 5-500 µg/L. The limit of quantification was 5µg/L. The linearity was good between 5 and 500 µg/L. The recovery is 99.7±2.4% (95%CI for the mean: 98.1-101.8%). For the ALDO, the with-in run and between run did not exceed 5.1% in the concentration range 20-1000 ng/L. The limit of quantification was 20ng/L. The analyse presents a good linearity between 20 and 1000 ng/L. The recovery is 100±1.9% (95%CI for the mean: 98.9-101.1%).

CONCLUSION

Our method is available for the simultaneous measurement of cortisol and aldosterone in the serum. It is a big saving of time. The advantage of using mass spectrometry consist not only better specificity, but also capability of quantifying multiple compounds.
Endocrinology
T116
ADRENO-CORTICAL DYSFUNCTION IN CRITICALLY ILL PATIENTS – DEVELOPMENT OF A SENSITIVE 2D-UHPLC-MS/MS-METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF SIX CORTICOSTEROIDS

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BACKGROUND-AIM
Since relative adreno-cortical dysfunction is recognized as a potential complication in critically ill patients, it is of interest to study the metabolic pathways of adrenal steroids in respective patients. Immunoassays offer limited specificity for this aim. We therefore decided to develop a semi-automated isotope-dilution mass spectrometry method for the simultaneous quantification of cortisol, cortison (a product of cortisol inactivation), and corticosterone, 11-desoxycortisol, 17-OH-progesterone, 11-desoxycorticosterone (precursor molecules).

METHODS
After spiking with stable isotope labelled internal standards samples were deproteinized and fractionated by two-dimensional UPLC with column switching prior to MS/MS analysis. The run time was 7 minutes and baseline separation of isomeric analytes was achieved. In a preliminary study the impact of ACTH stimulation on the corticosteroid pattern was investigated.

RESULTS
Performance evaluation demonstrated acceptable accuracy (94 – 98.4%) and reproducibility (CV 3.1% - 8.5%) as well as good sensitivity for all target analytes. Upon stimulation with ACTH, complex and substantial changes were observed in the serum corticosteroid patterns. A substantially more pronounced increase of corticosterone compared to cortisol in serum was observed (median increase of cortisol 2.7-fold, of corticosterone 16.5-fold, in healthy individuals, n=15).

CONCLUSION
Our preliminary results suggest that profiling of serum corticosteroids by a convenient and highly specific mass spectrometric multi-method – instead of mere immunometric quantification of serum cortisol - might enable important insights into the functional status of the adrenal cortex.
Endocrinology
T117
THE IMPACT OF TIME OF SAMPLE COLLECTION ON THE MEASUREMENT OF THYROID-STIMULATING HORMONE VALUES IN THE SERUM
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BACKGROUND-AIM
Taking blood samples for the measurement of serum thyroid-stimulating hormone (TSH) is commonly performed in laboratories in the morning (7:00 a.m. - 10:00 a.m.), but sometimes in the afternoon too, with no recommendation that patients should be fasting. The aim of our research is to determine whether the time of blood sampling and fasting of patients have an impact on TSH values.

METHODS
A total of 76 participants were enrolled in this study and classified in two groups. Group A (n= 46) had their first TSH samples collection between 7:00 a.m. and 8:00 a.m. at fasting and the second one after 140 min with food intake. Group B (n=30) had their first TSH samples collection between 7:00 a.m. and 8:00 a.m. at fasting and the second one after 140 min without food intake, i.e. again fasting. Serum TSH concentration was measured by electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics, Mannheim, Germany). The reference range for the TSH assay was 0.27 to 4.2 mIU/ L, and the functional sensitivity provided by the manufacturer was 0.014 mIU/ L.

RESULTS
Mean values of TSH (mIU/L) in group A were: baseline 2.107#0.943, after 140 min 1.460#0.563; mean difference: absolute -0.647, relative -30.72%, p<0.001.
Mean values of TSH (mIU/L) in group B were: baseline 2.447#0.980, after 140 min 1.760#0.693; mean difference: absolute -0.687, relative -28.07%, p<0.001. Roche TSH assays showed an excellent repeatability from the same sample (CV = 0.30%). Baseline, there was no difference between two groups in TSH values, but there was a difference after 140 min (p= 0.042).

CONCLUSION
Obtained TSH values were extremely different between the first and the second sample collection in both groups. Our results are strong evidence that time of day when the samples are collected have an impact on the TSH testing and results. The time of sample collection must be standardized for the purpose of standardization and harmonization of TSH measurements.
Endocrinology
T118

HIGH FRUCTOSE CONSUMPTION ALTERS DNA METHYLATION IN RAT LIVER.

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BACKGROUND-AIM

DNA methylation is the most extensively studied mechanism of epigenetic gene regulation. Increasing evidence indicates that DNA methylation is affected by environmental factors such as nutrition. Alterations in DNA methylation can lead to change in gene expression, resulting in diverse phenotypes with the potential for increased disease. Recently, there has been much concern regarding excess fructose intake to develop non-alcoholic fatty liver disease and hyperlipidemia. However, the pathogenetic mechanism is still unclear. We hypothesized that excess fructose intake alter the DNA methylation, resulting to the development of hyperlipidemia. The aim of this study is to investigate DNA methylation in the liver of rats feeding high fructose water.

METHODS

The male SD rats aged 6 weeks divided into two groups (n=6 per each). One group received normal water and other group received 20% fructose water for 14 weeks. At the end of 14 weeks, blood and liver tissue were collected. The triglyceride level in blood serum and liver tissue were analyzed. The RNA extracted from liver tissue was quantitatively analyzed expression levels of peroxisome proliferator-activated receptor alpha (PPARA) and carnitine palmitoyltransferase 1A (CPT1A) mRNA by real-time PCR. Genomic DNA from liver tissue was analyzed methylation status of PPARA and CPT1A promoter regions by restriction digestion and real-time PCR (qAMP).

RESULTS

The rats with feeding fructose induced more weight gain and serum triglyceride level. And also liver triglyceride accumulation was observed. These results indicated that high fructose consumption induce typical hyperlipidemia. The mRNA levels of PPARA and CPT1A were significantly reduced in fructose water group than water groups. The global methylation level of hepatic DNA was increased by fructose consumption. The qAMP analysis demonstrated the hypermethylation of promoter regions of PPARA and CPT1A.

CONCLUSION

Fructose-mediated attenuated hepatic gene expressions may be mediated by alterations of DNA methylation status. And pathogenesis of dyslipidemia induced by fructose is relevant to DNA methylation status.
Endocrinology

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CHRONIC, LONG TERM ADMINISTRATION OF VARDENAFIL IMPROVES ENDOTHELIAL FUNCTION AND IMPROVES TESTOSTERONE LEVELS IN HYPOGONADIC PATIENTS WITH TYPE 2 DIABETES MELLITUS

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BACKGROUND-AIM

Phosphodiesterase-5 inhibitors (PDE5i) have hemodynamic beneficial effects, improving endothelial-nitric oxide (NO) levels. The chronic use of PDE5i is supposed to control endothelial dysfunction in men with erectile dysfunction, but a complete understanding of PDE5i effects on endothelial function is far to be reached. The reduced bioavailability of NO, together with increased synthesis of mediators of vasoconstriction and inflammation, seems to be the first step of the vascular complications in diabetes mellitus (DM). Objective: to investigate if long term, chronic treatment with the PDE5i Vardenafil, improves systemic endothelial function in men with type 2 DM (T2DM). In particular we report the effects of this drug on the gonadic function.

METHODS

A longitudinal, prospective, randomized, placebo-controlled, double blind, clinical trial, was performed. 54 male patients, diagnosed with T2DM in the last 5 years, were enrolled and assigned by permuted block randomization to the verum (26 patients) and placebo group (28 patients). Patients were treated with 10 mg Vardenafil or placebo twice a day for 24 weeks and further followed-up for 12 weeks. Parameters evaluated included International Index of Erectile Function (IIEF-15), flow mediated dilation (FMD), intima media thickness (IMT), serum markers of inflammation, hemologic analysis. Testosterone (T) and its precursors were quantified by high specificity and sensitivity liquid chromatography–tandem mass spectrometry.

RESULTS

The erectile function domain of IIEF-15 improved after 6 months of drug administration (p=0.049). At the end of the treatment phase FMD (p=0.002) and IMT (p=0.003) significantly increased. FMD was significantly related to T serum levels (p=0.002). 24% of our patients were hypogonadic at baseline (T<10.4 nmol/L). Total T significantly improved in this subgroup of hypogonadal men (p=0.023), whereas no changes were observed in the placebo group.

CONCLUSION

Chronically administered Vardenafil in T2DM men improves both tissue oxygenation and inflammatory markers but the effect is lost after therapy withdrawal. In the hypogonadal group PDE5i seems to restore normal serum T levels, but this effect possibly due to improved microcirculation in the testis, is not preserved after withdrawal. However these results need further investigations since they derived from only 13 subjects.
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THE IMPORTANCE OF SERUM BONE ALKALINE PHOSPHATASE IN METABOLIC SYNDROME

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BACKGROUND-AIM
Serum alkaline phosphatase plays a role in vascular calcification. It is found in various tissues, whereas bone-speciﬁc alkaline phosphatase (BAP) more speciﬁcally reﬂects mineral metabolism. The relationship of BAP with metabolic syndrome (MetS) is largely unknown. The aim of our study was to determine the optimal cut off level for BAP assess whether BAP could represent a novel, sensitive marker of bone mineral disease (BMD) in MetS patients.

METHODS
80 metabolic syndrome patients (57 female and 23 male) and 50 healthy individuals (33 female,17 male) were included in this study. BAP levels were measured using on Ostease Kit (Beckman Coulter, California, USA). The BAP concentration was reported as the microgram per liter (µg/L). Other variables; Serum glucose, urea, creatinine, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride were determined by AU 5800 otoanalyzer system and insulin was detected DXI 800 Beckman Coulter System and using commercial kits (Beckman Coulter, USA).

RESULTS
There was no significant difference in age, gender, height, hyperlipidemia, smoking ratio between the MetS group and control group (p>0.05 for all). Weight, BMI, waist circumference, hypertension, family history, blood pressure were significant higher in MetS group compared to the control group (p=0.0001 for all).
When the laboratory parameters compared between the patients and control groups ; there was no significant differences for T. Chol, LDL, Urea, Creatinine (p>0.05 for all), HDL, fasting blood glucose, TG, Insulin, HOMA-IR and BAP were significant higher in patients than control group (p=0.001, p=0.0001 respectively for all) The Receiver Operating Characteristics (ROC) analysis is used to measure the per-formance of BAP,Insuline and HOMA-IR in detecting bone mineral disease in metabolic syndrome. The cut off value of BAP was ≤ 15.1 µg/L. Area under the ROC curve was 0.839 (95% CI : 0.764-0.890, SE;0.038) ( sensitivity; 83.75, spesifity; 76, PPV,84.4, NPV,74.5, +LR,3.49 )

CONCLUSION
BAP may be a clinically useful bone formation marker to predict the BMD reduction in MetS patients. Further investigations with larger patient groups are required to confirm our results.
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THE VISFATIN LEVELS IN THYROID DYSFUNCTION

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BACKGROUND-AIM

Visfatin, an adipose tissue–derived protein is reported that may play a role in cholesterol homeostasis however, the literature about visfatin’s physiology remains controversial. Recent studies have shown multiple roles of hormones on visfatin expression and downregulation of visfatin expression by T3. We aimed to investigate the relationship between thyroid functions and visfatin in this study.

METHODS

Twenty-seven patients with hyperthyroidism, 27 patients with hypothyroidism and 31 euthyroid subjects as control group were selected from patients referred to the hospital of Gazi University Medical Faculty, Ankara. Serum TSH, FT3, FT4, fasting glucose, lipid profile and visfatin levels were determined. Fasting glucose, triglycerides, total cholesterol, HDL-C levels were measured by enzymatic colorimetric method with auto analyzer(Architect c-16000, Abbott Laboratories). TSH, FT3, FT4 levels were established by directly-chemiluminescent method with double sandwich immunoassay.(ADVIA Centaur-XP,Siemens-Healthcare Diagnostics) Visfatin levels were determined by enzyme linked immunosorbent assay(ELISA) method(Phoenix-Pharmaceuticals Visfatin C-Terminal(Human) Enzyme Immunoassay(Katalog No:EK-003-80) Sensitivity:2.42ng/mL, Linear-Range: 2.42-38.1ng/mL, Intra-assay C.V.(%):< %10, Inter-assay C.V.(%):<%15. All analyses were performed using SPSS program (Version 16.0 for Windows).

RESULTS

Serum visfatin levels were markedly higher in the hypothyroidism group (8.96±4.27ng/mL) as compared with the hyperthyroidism(5.8±3.78ng/mL) and control (3.57±2.24ng/mL) groups(p<0.0001).Groups of hyper- and hypothyroidism demonstrated a significant difference(p=0.005),hyperthyroidism and control groups showed no significant difference(p=0.0167),hypothyroidism and control groups exhibited a significant difference(p<0.001). According to this result, hypothyroidism group was found to show statistically significant difference from other groups in visfatin levels. Visfatin, positively correlated with TSH, Total Cholesterol, LDL-C and negatively correlated with FT3 and FT4.

CONCLUSION

Visfatin is thought as a partly mediator to the effect of hyper/hypothyroidism on several metabolic parameters. Thyroid dysfunction may affect the visfatin clearance and may trigger visfatin secretion from visceral adipose tissue. The decrease in visfatin level due to thyroid hormones can be an additional result of the influence of thyroid hormones on the whole body metabolism.
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TESTOSTERONE VALUES AND CARDIOMETABOLIC RISK IN MALES

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BACKGROUND-AIM

The influence of testosterone levels on cardiometabolic risk is a topic increasingly more topical mainly due to the aging population. The aim of this observational study was to investigate the relationship between serum levels of total (TT) and free testosterone (TL) with markers of cardiometabolic risk in a Spanish male population of middle and advanced age.

METHODS

We studied 206 consecutive men with metabolic syndrome (ATP III criteria), between 40 and 70 years old. Anthropometric parameters (body weight, waist circumference and blood pressure) and biochemical (glucose, insulin, lipid profile, TT, TL (formula Vermeulen) and SHBG) were determined.

RESULTS

We studied our population by tertiles of TT and TL, with cutoff: 354 and 463 ng/dL for TT and 7.07 and 8.8 ng/dL for TL. Inversely find significant differences between the highest and lowest tertile of TT to body weight (p = 0.0093) and waist circumference (p = 0.0243). Furthermore between the highest and lowest tertiles of TL the inverse significant differences were found in age (p <0.0001), body mass index (p = 0.084), waist circumference (p = 0.0452), total cholesterol (p = 0.0513), LDL (p = 0.0295) and glucose (p = 0.0056).

CONCLUSION

From our findings could be seen that the values of TT and TL would risk markers of Diabetes Mellitus and Cardiovascular Disease.
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THE ROLE OF DETERMINING ALDOSTERONE, RENIN AND ALDOSTERONE/RENNR RATIO IN THE DIAGNOSIS OF PRIMARY ALDOSTERONISM

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BACKGROUND–AIM

Primary aldosteronism is one of the commonest forms of secondary hypertension. The aim of this retrospective study is to evaluate the role of the aldosterone/renin ratio (ARR) in the diagnosis of primary aldosteronism.

METHODS

A total of 362 hypertensive patients were included in this study. From all hospitalized patients blood samples were taken in order to measure concentration of total renin, aldosterone, potassium, sodium, chloride, total calcium, ionized calcium, bicarbonates and pH. Concentrations of total renin and aldosterone were determined using ELISA kit (IBL International). All the results were statistically processed by statistical package Data Analysis. According to the data collected from the medical history and the obtained values of aldosterone, renin and ARR, patients were divided into three groups: patients with primary aldosteronism (n=37), patients with secondary aldosteronism (n=76) and a group of hypertensive patients with a normal value of renin and aldosterone (n=249).

RESULTS

Concentration of renin was significantly lower in patients with primary aldosteronism compared to hypertensive patients (4.08±0.49 vs. 7.83±8.19; p<0.01) and patients with secondary aldosteronism (4.08±0.49 vs. 54.87±40.90; p<0.01). Concentration of aldosterone was significantly higher in patients with primary aldosteronism than in hypertensive patients (231.28±138.91 vs. 104.46±39.15; p<0.01), and significantly lower values of aldosterone were detected in hypertensive patients than in patients with secondary aldosteronism (104.46±39.15 vs. 233.05±179.81; p<0.01). Aldosterone/renin ratio was significantly higher in patients with primary aldosteronism compared to hypertensive patients (56.79±34.55 vs. 20.11±11.30; p<0.01) and patients with secondary aldosteronism (56.79±34.55 vs. 8.13±8.25; p<0.01). According to the ROC analysis, the cut-off value of 35 for ARR gives a 94% sensitivity, 90% specificity and accomplishes 91% accuracy in the evaluation of primary aldosteronism.

CONCLUSION

In our examined group of hypertensive patients, the aldosterone/renin ratio proved to be a good screening test in the detection of primary aldosteronism and for defining the level of functional renin/aldosterone axis.
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HOW SHOULD CORTISOL ASSAYS BE UTILIZED WHEN EVALUATING THE CORTISOL STATUS OF A WOMAN USING CONTRACEPTION CONTAINING ESTROGEN? - REFERENCE INTERVALS FOR SERUM CBG, AND SERUM AND SALIVARY CORTISOL IN HEALTHY FEMALES USING ESTROGEN CONTRACEPTION.


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BACKGROUND-AIM

Measuring the concentration of cortisol in serum (s-cortisol) plays a crucial role in diagnosing certain endocrine pathology. The metabolic syndrome epidemic has increased the number of patients screened for suspected hypercortisolism; hence the need for reliable cortisol assays and reference intervals for specific populations. During estrogen treatment, the concentration of serum corticosteroid binding globulin (s-CBG) increases. It is critical to understand how today’s doses of estrogen in contraception affect the s-CBG and s-cortisol. The aim was to establish reference intervals for s-cortisol, s-CBG and salivary cortisol (sa-cortisol) in a healthy female population using ethinylestradiol (EE) contraception, and to calculate a free cortisol index (FCI).

METHODS

s-cortisol and sa-cortisol were measured using Elecsys Cortisol (Roche Diagnostics, Mannheim, Germany). s-CBG was measured using a manual method (DIAsource ImmunoAssays, Louvain-La-Neuve, Belgium). The reference intervals of morning s-cortisol, morning s-CBG, and morning and evening sa-cortisol were calculated, given as the 2.5 and 97.5 percentiles of the reference values in a population of volunteer females (blood donors, students and laboratory workers), aged 18-45 years. 158 were not using estrogen contraception and 121 were using contraceptives containing low doses of EE (20-35 μg/day). FCI was calculated as s-cortisol/s-CBG.

RESULTS

Median and reference intervals for women not using and using EE contraception are respectively:

s-cortisol (8-10.30 am): 408 nmol/L, 197-737 nmol/L (n=158) and 867 nmol/L, 362-1297 nmol/L (n=121), p<0.001

s-CBG (8-10.30 am): 1101 nmol/L, 860-2425 nmol/L (n=157) and 2153 nmol/L, 865-3363 nmol/L (n=115), p<0.001

sa-cortisol (7-9 am): 15 nmol/L, 6-25 nmol/L (n=122) and 13 nmol/L, 6-28 nmol/L (n=102), p=0.057

sa-cortisol (9 pm-12 am): 5 nmol/L, 3-10 nmol/L (n=125) and 5 nmol/L, 3-9 nmol/L (n=103), p=1.000

FCI: 0.36, 0.15-0.65 (n=157) and 0.38, 0.20-1.41 (n=115), p=0.107

CONCLUSION

Due to increased s-CBG, s-cortisol is significantly higher in women using EE contraception, and the analytical results have to be compared against appropriate reference limits. There is no statistically significant difference in sa-cortisol or FCI between the groups.

sa-cortisol is the preferred measurand for cortisol status in healthy women using contraceptives containing EE, since it is not influenced by estrogens effect on CBG.
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MULTICENTER EVALUATION OF A THIRD GENERATION ESTRADIOL IMMUNOASSAY ON ROCHE DIAGNOSTICS COBAS SYSTEMS
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BACKGROUND-AIM
Objective of the Multicenter study was to evaluate the analytical performance of a new Estradiol immunoassay (E2 III) developed by Roche Diagnostics on cobas systems.
METHODS
The Roche electrochemiluminescence immunoassay for E2 III is a quantitative, two step, competitive assay using two monoclonal rabbit antibodies and 25 µL of sample. The Estradiol concentration is determined automatically from a 2-point calibration and a master curve which is traceable to CRM 6400a via ID-GC/MS. The result is generated within a total assay time of 18 minutes. To characterize QC data commercially available PreciControl Universal was used.
RESULTS
Functional sensitivity, defined as the lowest Estradiol concentration that can be reproducibly measured with an inter-assay CV of ≤ 20 %, was found between 13.2 – 18.3 pg/mL on cobas e 601 and cobas e 411 analyzers. For the within-lab precision profile 5 different native serum pools and 2 QC pools were assayed on 21 days, 2 runs per day based on CLSI guidelines. Standard deviations (SD) for imprecision were found ≤ 3.6 pg/mL (22 – 45 pg/mL) and CV ≤ 4.6 % (85 – 2406 pg/mL). Statistical Passing/Bablok analysis yielded the following results: Elecsys Estradiol III (y) versus Siemens Advia Centaur enh. Estradiol y= 1.07x+1.8, r= 0.987, N= 376; vs. Siemens Immulite y= 1.27x−7.1, r= 0.984, N=371; vs. Abbott y= 1.20x−9.1, r= 0.999, N= 506; vs DiaSorin y= 1.16x−3.3, r= 0.988, N= 530 and Roche Estradiol II on cobas e 602 analyzer y= 0.90x+7.1, r= 0.997, N= 530.
CONCLUSION
The new cobas Estradiol III assay shows improved comparability to competing non – Roche Estradiol assays and good precision over the entire measuring range. Reliability, convenience and robustness of the new application make it well suited for routine use in clinical laboratories.
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ALTERATIONS OF THYROTROPIN HORMONE IN WOMEN WITH SPONTANEOUS PREGNANCY LOSS
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BACKGROUND-AIM
Hypothyroidism has proven to be related with ovulatory problems, implantation and infertility, as well as miscarriages and pregnancy complications. Hypothyroidism is defined as a hyposecretion of the thyroid hormones from the thyroid gland. Standards for the diagnosis and treatment of subclinical hypothyroidism have changed according to some studies that demonstrate that there is an alteration of the thyroid gland in women with values of thyroid stimulating hormone (TSH) between 2.5-4.2 mIU/L, and that probably the only noticeable symptom is unexplained infertility/sterility.

Thyroid function is highly related to human reproduction and the screening of every woman is necessary, mostly in those who desire pregnancy or those who are in their first trimester of pregnancy

METHODS
The present is a retrospective study of women who asked for spontaneous pregnancy loss in our hospital from 2012 to 2014. They were screened for serum TSH using an immunochemical assay in Cobas 8000 analyzer (Roche)

RESULTS
A total of a hundred and twenty women with spontaneous pregnancy loss were included in the study. Of the 120 subjects, sixty-one (50.8 %) showed an elevated TSH levels, up to 2.5 mIU/L. Of them, the 54 % (33) had a TSH between 2.5 and 4.2 mIU/L in two or more analytical controls and were diagnosed of subclinical hypothyroidism. The 23.3 % of the studied women had a TSH levels above of upper limit of normal. Ultimately, in our study, a 50.8 % of women were diagnosed of subclinical hypothyroidism and hypothyroid women

CONCLUSION
In our study, we found a strong increased evidence of pregnancy loss in pregnant women with TSH levels between 2.5 and 4.2 mIU/L. More of 50 % of the women presented TSH values considered pathological to be upper than 2.5 mIU/L in recent studies.

The increased incidence of pregnancy loss in pregnant women with TSH levels between 2.5 and 4.2 mIU/L provides strong physiological evidence to support redefining the TSH upper limit of normal in women with spontaneous pregnancy loss and in the first trimester of pregnancy. Finally, we propose to make regular screening by TSH in desire pregnancy women to start thyroid treatment an early stage, when it was necessary
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COMPARISON OF NEW METHOD TO MEASURE SERUM ESTRADIOL LEVELS ON THE ROCHE COBAS E 602

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BACKGROUND-AIM
Estradiol, or more precisely, 17β-estradiol (17E), is the primary female sex hormone. One of its main clinical indications are the monitoring of fertility therapy and determining the time of ovulation within the framework of in vitro fertilization. The aim of the present study was to comparative the new Elecsys Estradiol III assay, which employs a competitive test principle using two monoclonal antibodies specifically directed against 17β-estradiol, with the actual Elecsys Estradiol II assay, which only uses one monoclonal antibody; both from Roche Diagnostics® (Mannheim, Germany).

METHODS
Serum samples from 70 women with a wide range of estradiol levels (5-2540 pg/mL) were analyzed with Elecsys Estradiol II (x) and Elecsys Estradiol III (y) at the same time. Data were statistically analyzed using MethVal® statistical package by Spearman correlation and Passing–Bablok regression to estimate the relationship between the two analytical techniques. Significance was set at p<0.05.

RESULTS
Regression analysis showed that the 2 methods were highly correlated (r = 0.99, P < 0.001, n = 70). Median 17E concentrations for method 1(x) was 215.5 (range: 5-2540) and by method 2(y) was 178 pg/mL (range: 5-273). However, Passing–Bablok analysis gave a regression equation of y= 0.869x-7. The 95% confidence interval (CI) for the slope did not include 1 [95% CI: 0.855-0.883; P < 0.001] and the 95% CI for the intercept did not include 0 [95% CI: -7 - (-3.8); P < 0.001].

CONCLUSION
The findings made in the present study showed a very good correlation between both methods. However, there is both a proportional error as a constant error which reported an underestimation of approximately 16% (10.04-22.84) of serum estradiol levels with Elecsys Estradiol III assay. Therefore we conclude that both methods are not transferable.
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REVIEWING THE MEASURE OF INTRAERYTHROCYTIC FOLIC ACID AND ITS POSSIBLE SUBSTITUTION FOR SERUM FOLIC ACID DETERMINATION

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BACKGROUND-AIM
Folic acid is one important component in erythropoiesis and its deficit can cause anaemia or dementia. For this reason clinical laboratory professionals have always tried to improve the methodology of measuring this component. Actually there are two principal ways for determining the amount of folic acid in the patients: serum folic acid (SFA) and intraerythrocytic folic acid (IFA). The first one is better for this low cost and simpler technology, and the second one is not affected by oral intake but is more expensive, to the point that there are numerous studies trying to review if it is possible to replace the intraerythrocytic measure. We have analysed the results of our organization in 2013 to contributing to assess the efficiency of these two determinations and establish appropriate recommendations.

METHODS
We recovered the results of all the determinations of SFA and IFA performed simultaneously in 2013.

RESULTS
We found 2582 determinations in which measurement were performed about both SFA and IFA. There was no sample with both deficit for SFA (<2.8 ng/mL) and IFA (<176 ng/mL). Thirty seven samples (1.4%) had normal levels of IFA and deficit for SFA. Six samples (0.2%) had deficit for IFA and normal levels for SFA. The rest (2539, 98.4% of the total) of the samples had both normal levels of IFA and SFA. Therefore in our population there were only 0.2% of IFA justifiable determinations. Any patient which SFA was less than 2 ng/mL had IFA deficiency. On the other hand when the determination of SFA was greater than 4 ng/mL there was no IFA deficiency.

CONCLUSION
Too many routine laboratory measurements of IFA are not performed justifiably, as we found in our results review. Only in 6 samples (0.2%) we found that the patient had normal levels of SFA while having deficit in IFA. To avoid these cases, our organization made a rule: to perform IFA determination if the SFA is between 2 to 4 ng/mL. If SFA is greater than 4 ng/mL, IFA deficiency can be ruled out. On the other hand, if SFA is less than 2 ng/mL the deficiency can be assumed. But there are some special situations that can evade this recommendation:
- Deficit in vitamin B12: this vitamin is necessary for the folic acid intake during the erythropoiesis. Therefore in a deficit of this component it can be found IFA falsely decreased.
- Haemodialysis: this technic can falsely decrease SFA, so after this process is more accurate to measure IFA.