Endocrinology

Cod: 0557

EFFECT OF EXERCISE ON BIOCHEMICAL PARAMETERS IN THE HEART TISSUE OF HYPERTHYROID RATS

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BACKGROUND: It is known that oxidative stress is increased in hyperthyroidism due to increments in the rate of oxygen consumption in target tissues. In addition, there is some information in the literature that regular exercise may improve oxidative stress in trained specimens. The purpose of this study was to investigate whether any effect of a regular endurance exercise on oxidative stress parameters in heart tissue of hyperthyroid rats.

METHODS: Twenty-three male Sprague Dawley rats were divided into four groups: control, exercise, hypothyroidism and hyperthyroidism + exercise. The rats in exercise groups were submitted to run on a treadmill at a speed of 23 m/min for 45 minutes, 5 day/week for 8 weeks. Hyperthyroidism was induced by L-thyroxine (0,25 mg/kg/day s.c. for 20 days), and was confirmed by the measurements of TSH, FT3 and FT4 in serum. The extent of oxidative stress was assessed by measuring the levels of the malondialdehyde (MDA), protein carbonyl content (PCO), the total antioxidant status (TAS), total oxidant status (TOS) and 8-hydroxydeoxyguanosine (8-OHdG) in heart tissues.

RESULTS: As compared with control, hyperthyroidism and exercise groups, TOS levels in hyperthyroidism+exercise group were significantly high (6.24±0.74, 6.21±0.76, 6.97±0.81, 8.72±1.19 μmol H2O2 eq/L, respectively, $p<0.05$). MDA levels of exercise (37.95±7.22 μM) and hyperthyroidism+exercise (35.05±7.34 μM) groups were statistically higher than those of control (13.34±5.32 μM) and hyperthyroidism (24.01±3.54 μM) groups ($p<0.05$). While PCO levels in hyperthyroidism group were not different from the control group, the values (15.23±4.93 nmol/mL) in hyperthyroidism+exercise group were significantly different from the values (7.99±0.65 nmol/mL) in control group ($p=0.012$). 8-OHdG levels in exercise and hyperthyroidism+exercise groups were the highest of all groups and these values were about twice of the values in control and hyperthyroidism groups.

CONCLUSIONS: The results show that regular endurance exercise does not has a positive effect in terms of reducing oxidative stress in heart tissues of hyperthyroid rats.
Key words: Endurance training, experimental hyperthyroidism, oxidative stress

Endocrinology

Cod: 0558

SERUM VITAMIN D STATUS AND ITS ASSOCIATION WITH HBA1C LEVELS

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BACKGROUND: Recent studies suggest that Vitamin D deficiency may play a role in the development of diabetes and the metabolic syndrome. It may be due to its potential anti-inflammatory and immune-modulating properties. The aim of this study was to investigate the relation of Vitamin D status with HbA1c, insulin and fasting glucose levels.

METHODS: We studied on 156 patients who were admitted to internal medicine clinic in July and August 2013. Patients with 25-Hydroxy Vitamin D (25-OH Vitamin D) levels <30 ng/ml were considered as Group 1 and ≥30 ng/ml as Group 2. HbA1c, serum insulin and fasting glucose levels were measured. Insulin levels measured by Beckman Coulter DxI-800 with chemiluminescence method. 25-OH Vitamin D levels were measured by DiaSorin, LIAISON XL instrument with chemiluminescence method. Datas were expressed as means and standart deviations. For statistical analysis SPSS 21.0 software was used. p- values <0.05 was considered statistically significant.

RESULTS: According to serum 25-OH Vitamin D levels, there were 113 patient (72.4%) in Group 1 and 43 patient (27.6%) in Group 2. The mean age was 59.12±10.6 year. The mean levels of Group 1 were as follows; insulin: 12.88±14.22, fasting blood glucose:165.8±78.7, HbA1c: 7.67±2.04; The mean levels of Group 2 were; insulin: 13.81±19.7, fasting blood glucose:134.7±44.4, HbA1c: 7.03±1.29. There were no correlation between two groups insulin levels ($p>0.05$) but we found statistically significant negative correlation of HbA1c and fasting blood glucose levels among groups ($p<0.05$).

CONCLUSIONS: We found that Vitamin D status was not associated with insulin levels. It may be due to our retrospective population based study group. Our findings may suggest that raising vitamin D levels may be effective in controlling glucose metabolism.

Endocrinology

Cod: 0559

EFFECT OF SEPIAPTERIN REDUCTASE ON ADIPONECTIN IN PATIENTS WITH THYROID DYSFUNCTION

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The aim of this study is to estimate the concentration of Adiponectin hormone and Sepiapterin reductase in blood serum of Hyper and Hypothyroidism patients. This work is performed in Tikrit teaching Hospital by collecting 86 samples (31 Hyperthyroid patients and 25 Hypothyroid patients) and 28 samples of control group, Sepiapterin reductase level showed a high significant increase ($P<0.001$) in both Hyper and Hypothyroidism patients as compared with controls. Adiponectin decreased ($P<0.001$) in both Hyper and Hypothyroidism patients in male and female. The correlation coefficient (r) (-0.32) and (-0.35) between Sepiapterin reductase and Adiponectin calculated with regression plot showed a negative correlation in patients with Hyperthyroidism and Hypothyroidism.

Endocrinology

Cod: 0560

INTERCOMPARISON BETWEEN METHODS FOR THE DETERMINATION OF URINARY IODINE

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BACKGROUND: Iodine is an essential micronutrient that is part of thyroid hormones necessary for growth and development throughout life. Iodine deficiency (ID), during pregnancy and lactation, is the cause of alteration in the most common preventable mental development in the world. Many methods have been described for the measurement of urinary iodine, standard indicator of ID, being the reference method the ICP-MS. We ponder on all methods are equally valid and which one correlates better with the ICP- MS.

METHODS: 20 control samples (6 replicates each), from Centers for Disease Control and Prevention (CDC) program, were processed by the method used in our laboratory, HPLC (G), and the results were compared with ICP-MS (R) by Passing-Bablock and Spearman correlation coefficient. The results obtained with colorimetric methods (digestion with ammonium persulfate Sandell- Kolthoff (A), digestion with chloric acid Sandell- Kolthoff (B), digestion ammonium persulfate in microplates (C), digestion with chloric acid in microplates (D), automated system urine ions (F) and ICP-MS (E) were also compared with R by following the EQUIP program (Ensuring the Quality of Urinary Orine Procedures).

RESULTS: Comparing HPLC vs ICP-MS, the equation obtained with Passing Bablock was y=1.0075x+1.9197 with a 95% confidence interval of slope of 0.966-1.059 and Spearman correlation was 0.9963. When comparing colorimetric methods with ICP-MS, the following regression lines in Passing Bablock were obtained: R-A: y=0.9697x+0.2172, R-B: y=0.9728x-0.8282, R-C: y=0.9493x+0.24, R-D: y=0.9739x-0.4382, R-E: y=1.0266x-0.3924, R-F: y=1.0824x-0.7001 and Spearman correlation coefficients between 0.9397 and 0.9988 were shown.

CONCLUSIONS: The HPLC correlates appropriately with the reference method, resulting a valid method for measuring urinary iodine. Colorimetric methods correlate well with the reference method, with the exception of digestion with chloric acid in microplates, which in high concentrations has a large dispersion.

Endocrinology

Cod: 0561

INSULIN RESISTANCE IN OVERT AND SUBCLINICAL HYPOTHYROIDISM IS COMPARABLE AND DETERMINED BY ANTI-TPO LEVELS MAINLY : IMPLICATION IN RISK STRATIFICATION FOR CARDIOVASCULAR DISEASE

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BACKGROUND: Thyroid hormones are involved in metabolic regulations which are altered in thyroid hypo-function. and the most frequent cause of hypothyroidism is the autoimmune thyroid disease manifested by elevated thyroid antibodies The present study was designed (1) to find out occurrence of insulin resistance in hypothyroid patients and (2) to compare insulin resistance in sub- clinical and overt thyroid hypo-function and association with ATPO levels.

METHODS: One hundred eighteen patients with the diagnosis of hypothyroidism based on their clinical and thyroid function test profile were included in this cross sectional hospital based descriptive study with their informed consent. HOMA-IR as an index of insulin resistance was calculated for each subject from their fasting plasma glucose and serum insulin levels. Autoimmunity against thyroid was evaluated by estimating anti TPO antibodies. Lipid profile and urine microalbumin was also assessed.

RESULTS: HOMA-IR as an index of insulin resistance was comparable in overt (5.8±3.24) and subclinical hypothyroidism (6.27±3.87) but was above the reference range for this population. Hypothyroid anti TPO positive cases has high TSH and deranged lipid profile with markedly increased LDL levels, compared to negative cases in both overt hypothyroidism and subclinical hypothyroidism.

CONCLUSIONS: Hypothyroidism induces insulin resistance, which is an index of cardiovascular health and the degree of insulin resistance is not dependent on severity of thyroid hypo-function (TSH value) however is associated with autoimmunity (ATPO levels) against thyroid. Therefore all the newly diagnosed cases and suspected cases of hypothyroidism must be screened for ATPO antibodies so that appropriate management can be started.

Endocrinology

Cod: 0562

NEUROPEPTIDE Y COULD REPLACE PLASMA NORMETANEPHRINES AS A PHEOCHROMOCYTOMA SCREENING PARAMETER

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BACKGROUND: Expert opinion favors free plasma normetanephrines (NMN) and metanphrines (MNp) as markers with highest sensitivity for the detection of pheochromocytoma.A number of studies precise that although only a subset of chromaffin cells express neuropeptide Y (NPY), this peptide was present in a lot of pheochromocytomas and neuroblastomas.We considered an opportunity to test plasma NPY besides plasma NMNp/MNp in a selected pheochromocytoma group and to underline the relevance of these parameters for screening studies.

METHODS: This prospective case-control study included a group of 15 hypertensive patients confirmed with pheochromocytoma and an age-matched control group of 15 normotensive subjects. The pheochromocytoma diagnosis was suspected on clinical ground and confirmed by imaging studies and by plasma neuroendocrine markers. Differentially free plasma NMNp/MNp and plasma NPY were assayed using enzyme-immunoassay for all three markers. The pheochromocytoma group was also evaluated post-surgery for all three endocrine markers.

RESULTS: In tumor cases all three parameters were increased and normalized after surgery. Values for all 3 parameters markers (mean±SEM) differed significantly in all tumor cases pre-surgery versus the same markers post-surgery.In tumor patients, plasma values post-surgery are not significantly different versus the same parameters in control group. In tumor group:pre-surgery free plasma NMNp was: 1106.06±301.23pg/mL vs. (versus) post-surgery NMNp: 58.25±6.64pg/mL and vs. control group NMNp: 46.45±6.32pg/mL; pre-surgery free plasma MNp was: 513.46±237.50 pg/mL vs. post-surgery MNp: 34.58±2.52pg/mL and vs. control group MNp: 22.62±2.35pg/mL; pre-surgery NPY was: 1088.02±185.57ng/mL vs.post-surgery NPY: 58.25±7.24ng/mL and vs. control group: NPY: 43.77±6.46ng/mL. Pairwise comparison of receiver operating characteristic curve (ROC) analysis showed no significant differences between NMNp and NPY areas, so both markers showed identical maximum sensitivity and specificity.

CONCLUSIONS: In our selected pheochromocytoma group, plasma NPY could be used instead of free plasma NMNp with the same sensitivity and specificity. Future studies could confirm the utility of NPY as a screening marker of pheochromocytoma.

Endocrinology

Cod: 0563

BASELINE AND ACTH STIMULATED SERUM 17-HYDROXYPROGESTERONE VALUES IN MACEDONIAN AND SERBIAN 21-HYDROXYLASE DEFICIENCY PATIENTS

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BACKGROUND: Steroid 21-hydroxylase deficiency is present in 90-95% of all cases with congenital adrenal hyperplasia (CAH), an autosomal recessive disorder. It can present as a severe classical salt wasting (SW) and simple virilizing form (SV), and a mild nonclassic late onset form (LO). The diagnostic biochemical marker for CAH is increased serum level of 17-hydroxyprogesterone (17-OHP). In the mild form it is increased only on ACTH stimulation test (0.25 mg i.v. cosyntropine). The analysis of this steroid hormone, therefore, plays a significant role in the differential diagnosis of CAH as well as for determination of the severity of the disease. Untreated patients with 21-hydroxylase deficiency present levels between 2 to 1000 times higher than healthy people.

METHODS: Enzyme immunoassay for the in-vitro quantitative diagnostic was used to determine serum 17-OHP (ng/ml) in the 56 patients from R. Macedonia and 22 patients from R. Serbia, with different clinical presentation of CAH. Baseline 17-OHP levels >4.95 ng/ml and ACTH-stimulated 17-OHP values >9.9 ng/ml are the cutoffs established for the diagnosis of the disease.

RESULTS: Baseline serum 17-OHP levels were significantly higher in patients with SW (>24.8 ng/ml) and SV (21.7±4.6 ng/ml) clinical forms of 21-hydroxylase deficiency than in LO patients (10.9±4.7 ng/ml), $p<0.05$. Also, there was significant difference between baseline (10.9±4.7 ng/ml) and ACTH-stimulated 17-OHP levels (17.4±6.4 ng/ml) in LO patients ($p<0.05$).

CONCLUSIONS: Enzyme immunoassay for the in-vitro quantitative diagnosis of serum 17-OHP with analytical sensitivity of 0.03 ng/ml and analytical specificity of 1.7% allows detection of different forms of CAH and provides basis for treatment and follow up.

Endocrinology

Cod: 0564

ALDOSTERONE-TO-RENIN RATIO IN HYPERTENSIVE PATIENTS FROM A ROMANIAN TERTIARY CENTER

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BACKGROUND: Primary aldosteronism (PA) increases the risk of cardiovascular events in hypertensive patients. The screening for PA is based on the aldosteron-to-renin ratio (ARR). This is a retrospective study aiming to evaluate the prevalence of positive screening tests for PA in hypertensive patients referred to a Romanian tertiary center in 2013.

METHODS: We investigated the medical records of 498 hypertensive patients, 343 females and 155 males. Patients were referred to our center for different endocrine disorders. We assessed the results for plasma aldosteron concentration (PAC), direct plasma renin concentration (DRC) and ARR at the admission. Confirmation tests for PA were not taken into account in this study. PAC was measured with ELISA (LDN GmBH, Germany, sensitivity 10 pg/ml), DRC with an automated immunochemiluminiscent method (Liaison, DiaSorin, sensitivity 0,3 pg/ml). Statistical analysis was performed by SPSS 17.0. Data were expressed as mean ± standard deviation, median, frequencies. t-test was used for comparison, $p<0,05$ was considered statistically significant.

RESULTS: Mean age of the study group was 48,41 (±16,42) years, 50,92 (±15,34) years for women, 41,9 (±18,08) years for men, respectively. Median values in all patients for PAC, DRC and ARR were 113,35 pg/ml, 11,35 pg/ml and 9,62, respectively. No statistical differences were found between sexes in respect of PAC ($p=0,12$) and ARR ($p=0,08$). We found higher values for ARR in women (10,55 versus 7,77 in men). We considered a cutoff for ARR of 57 (The Endocrine Society's Clinical Guidelines). When applying the definitions of positive screening results for PA, 70 patients showed increased ARR values (14%), 27 patients (5,4%) had increased ARR with increased PAC and low DRC values and 57 patients (11,4%) had high ARR with low DRC values.

CONCLUSIONS: Our results show a prevalence of positive screening test results for PA in hypertension patients of maximum 14%. As ARR was designed as a screening test and not a diagnostic one, the results and therapeutic approaches should be interpreted with caution.

Endocrinology

Cod: 0565

USE OF URINARY STEROID PROFILING FOR DIAGNOSING DIFFERENT VIRILIZING DISEASES

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BACKGROUND: An increased androgen production and virilization may be caused by a number of different diseases, e.g. adrenocortical carcinoma, a highly malignant tumor, deficiencies of certain steroid converting enzymes, particularly 21-hydroxylase deficiency (congenital adrenal hyperplasia), Cushing's syndrome and polycystic ovary syndrome. Since some of these diseases require immediate treatment, it is important that they can be readily diagnosed by methods in clinical routine use. In our laboratory we use urinary steroid profiling for diagnosing such diseases and the potential of the method is demonstrated.

METHODS: Urine samples (24-hour collections) were from patients of various age with symptoms of virilization and/or hirsutism. Metabolic steroid profiles in urine were analyzed by gas-liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC/MS) following extraction, hydrolysis/solvolysis of steroid conjugates, purification and derivatization (MO-TMS). Twenty-one urinary steroids are routinely quantified by GLC and new steroids are identified by GC/MS.

RESULTS: The results show that the urinary steroid profiles of patients with adrenocortical carcinoma and enzyme deficiencies, 21-hydroxylase deficiency being most frequent, are usually very characteristic and easy to recognize (diagnose) and so is also the case with Cushing's syndrome. Steroid profiles of patients with polycystic ovary syndrome (PCOS) and some other virilizing disorders demonstrate an increased production of testosterone. The method can be used for diagnosing children with precocious puberty. Since the former diseases generally need immediate treatment, it is important that they are readily diagnosed.

CONCLUSIONS: Urinary steroid profiling is a highly specific and sensitive analytical tool for diagnosing malignant adrenocortical tumors and various enzyme deficiencies, diseases that usually cause virilization and require immediate treatment. For patients with other virilizing disorders steroid profiling provides information about normal and increased production rates of testosterone. Steroid profiling is also useful for following up treatment of such diseases.

Endocrinology

Cod: 0566

SERUM SIALIC ACID LEVELS IN PATIENTS WITH DIABETIC FOOT AND ITS RELATIONSHIP TO C-REACTIVE PROTEIN

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BACKGROUND: Sialic asid (SA) is an independent risk factor for cardiovascular disease. SA is known to increase in type 2 diabetes mellitus. However, there is no study concerning the SA levels in patients with diabetic foot. The aim of this study was to determine serum SA levels in patients with diabetic foot, investigate the relationship between SA and C-reactive protein (CRP) and evaluate the importance of nitric oxide (NO) in development of diabetic foot.

METHODS: 30 patients with diabetes and 30 patients with diabetic foot and also 30 healthy volunteers as a control group were included in the study. The patients with diabetic foot were divided into two groups who had or had not undergone extremity amputation. Fasting blood samples were taken twice from patients with diabetes undergoing amputation due to diabetic foot as once before and once after the amputation. Serum SA, CRP and NO levels were measured. Serum SA level was measured by the method described by Svennerholm, NO level was detected by using the Griess reaction and CRP level was analysed by using immuno nephelometry. Study groups with a normal distribution were compared with one-way analysis of variance (ANOVA) and those without a normal distribution were compared with the Kruskal-Wallis analysis.

RESULTS: Serum SA and NO levels were higher in diabetes and diabetic foot groups than the control group ($p<0.05$). The comparison of the subgroups of the diabetic foot group as amputated and not amputated showed no significant difference in terms of NO and SA ($p>0.05$). Serum SA was significantly correlated with CRP in diabetic foot group ($p<0.001$). In addition, there was a statistically significant difference between pre-amputation SA values and post-amputation SA values ($p<0.023$).

CONCLUSIONS: We can say that the levels of high NO play an important role in development of diabetic foot and elevated serum SA levels may be an indicator extensive vascular damage and increased acute phase response in patients with diabetic foot.
Keywords: C-reactive protein, diabetic foot, nitric oxide, sialic asid

Endocrinology

Cod: 0568

FREQUENCY OF THYROID PATHOLOGY IN WOMEN OF REPRODUCTIVE AGE

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BACKGROUND: Thyroid disease are the most common endocrine pathology. The role of thyroid dysfunction is undeniable, both in terms of the reproductive capacity of women, and in terms of successful completion of the pregnancy and birth of a healthy child. According to the American Thyroid Association (2011), the incidence of hypothyroidism in women of childbearing age is 2-3%, hyperthyroidism is less common around 0.1-0.4%. The purpose of this work is a study on the incidence of thyroid pathology in women of reproductive age in Bulgarian population.

METHODS: 853 samples of women in reproductive age were examined. The analysis of TSH and fT4 is performed with ECLIA of Cobas 6000 with reagents and calibrators of Roche and BioRad controls.

RESULTS: Of the 853 analyzed samples in 88.04% of cases, the results of TSH were in the reference area (0.27-4.2 mIU/L). At 8.79% of TSH results was above 4.2 mIU/L, and 3.17% was below 0.27 mIU/L. It would be interesting redistribution of the results using the upper limit of 3.0 mIU/L (according to the literature above 95% of healthy euthyroid subjects have a serum TSH values below 2.5 mIU/L). When so inserted reference limit of TSH, the group with normal TSH values will decrease by 12.66% and will become 75.38%, against 24.62% with abnormal values.

CONCLUSIONS: The frequency of thyroid pathology in the studied women of fertile age is higher than expected according to the literature data. Implementation of an lower upper reference limit of TSH, would contribute to early detection and treatment of thyroid dysfunction in women of reproductive age.

Endocrinology

Cod: 0569

OVERTREATMENT DUE TO SPURIOUS HYPERCALCITONINEMIA: A CASE REPORT

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BACKGROUND: Serum calcitonin (CT) has important diagnostic and prognostic value in medullary thyroid carcinoma (MTC). Although measurement of CT is a highly sensitive method for the detection of MTC, it presents a low specificity for this tumor. It has been shown that only 10% to 40% of all patients with high levels of CT associated with a thyroid nodule actually have MTC. Moreover, several Authors have attributed high CT levels to heterophilic antibodies (HA) presence in patients'sera. In the overall clinical picture, elevated serum CT levels should be interpreted in each individual case and carefully validated before therapeutic decisions, considering that any method is not completely free from possible interferences.

METHODS: We present the case of a 38 years woman with thyroid nodules and hypercalcitoninemia, underwent total thyroidectomy. At clinical diagnosis, serum CT level was performed on IMMULITE 2000 (Siemens) in two different laboratories. After surgery, CT was analyzed in the same previous laboratories and also in our hospital on Liaison XL (DiaSorin).

RESULTS: Before surgery, on IMMULITE 2000 instrument, CT was 843 and 860 pg/ml. After surgery, the final pathology was: "Thyroid with focal and isolated aspects of nodular hyperplasia: there aren't aspects associated with MTC and C-Cell hyperplasia". Despite thyroidectomy, high CT values were confirmed in two consecutive measurements within one month (910 and 882 pg/ml) in the same laboratories. On the other hand, CT obtained in our laboratory, was undetectable in two consecutive measurements within one month (< 3 pg/ml).

CONCLUSIONS: Spurious positive CT results due to immunoassay interferences have led to incorrect indications of preventive total thyroidectomy because of erroneous MTC diagnosis. In order to avoid misdiagnosis and unnecessary thyroid surgery, it's imperative a collaboration between clinicians and laboratorians. In absence of familiarity and cytological confirmation for MTC, it may be useful to confirm hypercalcitoninemia retesting the sample by a different manufacturer assays.

Endocrinology

Cod: 0570

SYNACTHEN TESTING: DO WE NEED A 30- OR 60-MINUTE SAMPLE OR BOTH?

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BACKGROUND: For over 50 years, the gold standard to assess abnormal glucocorticoid production has been the insulin tolerance test. However, for reasons of risk, patient convenience and overall practicality the short Synacthen test (SST) is now the almost universal first line procedure to rapidly assess adrenal function. Some centres measure cortisol at baseline and 30 minutes while several recent studies have argued the necessity and sufficiency of the 60-minute cortisol and that the 60-minute is equally effective as combined 30- and 60-minute sampling to identify adrenal insufficiency.

METHODS: We have reviewed over 1100 SSTs over past 15 years. Over this period we have changed our protocol from sampling at 0, 30 and 60 minutes to 0 and 60 minutes. Cortisol was measured by Beckman Access. There is no universal consensus for either protocol but we will present justification for the latter protocol based on our data and other recent publications.

RESULTS:
• Cortisol response at 60 minutes invariably exceeded the 30 min response.
• Even in this well controlled study phlebotomy can be delayed due to venous access difficulty by up to 5 minutes. As cortisol level is rising much less rapidly at 60 minutes compared to 30 minutes it follows that any given delay in phlebotomy will have less impact at 60 minutes than at 30 minutes.
• If a normal cortisol peak level is taken as 550 nmol/L then a 30 min sample only, will misclassify 38% of people who show a normal response at 60 minutes.
• Deleting the 30 minute sample will also significantly reduce cost and patient stress.
• The 60 minute cortisol response in women is approximately 6% greater than in men.

CONCLUSIONS:
• We recommend, in harmony with other recent reports, that Synacthen testing be harmonised to a baseline ACTH and cortisol plus a single cortisol measurement 60 minutes after Synacthen injection.
Further harmonisation of Synacthen testing requires
• Harmonisation of different cortisol assays.
• Consensus on whether 550 is the optimal decision point for adrenal insufficiency.
• Consensus as to whether, well documented gender differences in cortisol response whereby women show higher cortisol response than men, should be translated into different cut-off limits for men and women.

Endocrinology

Cod: 0571

SCREENING FOR PRIMARY ALDOSTERONISM ALDOSTERONE TO RENIN RATIO USING TWO AUTOMATED CHEMILUMINESCENT IMMUNOASSAYS

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BACKGROUND: Primary Aldosteronism (PA) is the most common form of secondary hypertension. Prevalence in patients with Resistant Hypertension (uncontrolled blood pressure despite ≥ 3 antihypertensive drugs) is estimated in prospective studies to be 14-23%. The Aldosterone to Renin Ratio (ARR) can be used in the screening and is recommended by clinical practice guidelines in patients at risk. Use of ARR depends on the availability of reliable and sensitive methods to measure concentration of Aldosterone (PAC) and Renin.

METHODS: We investigated the performance of a new automated Chemiluminescent Immunoassay (CLIA) for measurement of PAC in comparison to our standard Aldosterone assay (RIA) with clinical samples. PAC was measured using CLIA on the LIAISON Analyzer (DiaSorin, Saluggia, Italy) and the Siemens coat-a-count RIA. Direct Renin (DRC) was measured on the LIAISON Analyzer. EDTA plasma samples were taken from 200 normotensive patients (N), 50 essential hypertensive (EH) patients and 50 PA patients. The latter groups were defined by post-saline Aldosterone >50pg/mL (RIA). 50 matched serum/plasma samples were measured with the Aldosterone CLIA to analyze matrix commutability.

RESULTS: Median PAC by RIA were 58pg/mL in N; 60 pg/mL in EH and 221pg/mL in PA. Median PAC by CLIA was 70pg/mL in N; 81 pg/mL in EH and 234pg/mL in PA. DRC was 20µU/mL in N; 50µU/mL in EH and 7µU/mL in PA. ROC analysis of the data identified a range of ARR values between 9.5 to 12.0 (PAC pg/mL; DRC µU/L) that provide acceptable balance between sensitivity and specificity (100%/80% to 98%/86%). Passing Bablok comparison of PAC from CLIA versus RIA revealed good correlation and agreement (y=1.04x+4.28). Preliminary data from salt load suppression tests and the high correlation to aldosterone concentrations measured by RIA indicate that the cut-off value (50pg/mL) can be used also for aldosteron measured by CLIA.

CONCLUSIONS: Our data indicate that the new aldosterone CLIA in conjunction with the established automated CLIA for renin can be used as a reliable and sensitive method for definition of the ARR.

Endocrinology

Cod: 0572

MEASUREMENT OF ANTI-MULLERIAN HORMONE (AMH): PERFORMANCES OF A NEW ULTRASENSITIVE IMMUNOASSAY

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BACKGROUND: Anti-müllerian hormone (AMH), a member of the transforming growth factors-β family, is produced by the granulosa cells of the growing ovarian follicles. Measurement of circulating levels of AMH is relevant for the evaluation of primary ovarian insufficiency, success of assisted reproductive therapies, and also to support the diagnosis of polycystic ovary syndrome. AMH testing is based on immunoassay format but most of the laboratories are using the same assay. The aim of our study was to evaluate the performances of a new enzyme linked immunosorbent assay (ELISA) in two different European laboratories.

METHODS: The performances of a new ultrasensitive AMH ELISA method (Ansh Laboratories, Inc, Webster, TX, USA) were evaluated in Maribor (Slovenia) and Brussels (Belgium). The limit of detection was determined with a 10g/L bovine serum albumin solution and the method imprecision was determined with pools of serum samples. Method comparison was performed with the AMH Gen II® (Beckman Coulter) ELISA assay in 213 patient's samples.

RESULTS: The limit of detection of the Ansh AMH assay was 0.10 ng/mL (n=10). The within-run coefficients of variation (CV) were 5.8% at 0.21 ng/mL (n=5), 3.0% at 0.54 ng/mL (n=5) and 4.1% at 0.88 ng/mL (n=10) with the Ansh AMH assay. In the 213 patients, the median AMH levels were 2.23 ng/mL (range: 0.10 – 23 ng/mL) with Ansh assay and 2.71 ng/mL (0.14 – 24) with the AMH Gen II®. A positive and significant correlation was observed between the two methods ($r = 0.92$, $p<0.0001$). Passing and Bablock regression analysis provides a slope of 0.99 and an intercept of -0.102, without significant deviation of linearity. The mean difference between the two AMH assays and observed on Bland an Altman plot was -03 ng/mL.

CONCLUSIONS: Our study demonstrated excellent analytical performances for the Ansh Laboratories AMH immunoassay and results comparable to those obtained with the AMH Gen II assay. This new AMH assay might therefore be relevant for the infertility workup and management of ovarian disorders.

Endocrinology

Cod: 0573

HYPERPROLACTINEMIA: CHALLENGES IN FINDING THE RIGHT ETIOLOGY

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BACKGROUND: Prolactin is a polypeptide hormone of the anterior pituitary. Several factors such as dopamine, estrogens, thyrotropin releasing hormone determine the secretion of prolactin. Hyperprolactinemia may cause galactorrhea, menstrual irregularity, infertility, hypogonadism and sexual dysfunction. We present three patients, being referred to our hospital for complaints of secondary amenorrhea, galactorrhea, infertility and chronic headache.

METHODS: Three patients were subject to a systematic clinical and biochemical evaluation.

RESULTS: Case 1 was a 19 years old woman with a prolactin level of 3.21 IU/l (reference value <0.60 IU/l), caused by medication (domperidone). Case 2 was a 28 years old woman with a prolactin level of 1.16 IU/l, caused by chronic nipple stimulation. Case 3 was a 20 years old man with a prolactin level of 6.0IU/l and a testosterone level of 2.4 nmol/l (reference range 7.6-31.4 nmol/l). His elevation was caused by a pituitary adenoma and macroprolactin. The first two cases illustrated the importance of a carefully taken medical history in the diagnosis of hyperprolactinemia. Cessation of medication and physical stimulation revealed normal levels of prolactin and restored clinical symptoms. In addition complementary imaging studies were spared. The third case illustrated the importance of additional laboratory testing in symptomatic patients. Besides the macroprolactin, the patient had elevated prolactin levels, which was treated by medication. Prolactin and testosterone levels returned to normal and complaints were restored.

CONCLUSIONS: A complete anamnesis and additional laboratory tests are the most important steps in the evaluation of a patient with hyperprolactinemia. In this perspective a close collaboration between clinician and laboratory specialist is a necessity for a fast and a correct diagnosis.

Endocrinology

Cod: 0574

MOLECULAR BIOLOGY INVESTIGATION OF SOMATOSTATIN AND ESTROGEN RECEPTORS IN CLINICALLY NON-FUNCTIONING PITUITARY TUMORS

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BACKGROUND: Somatostatin analogs (SA) are effectively used in the treatment of acromegaly, neuroendocrine tumors and Cushing's disease. In contrast, pharmacological treatment of non-functioning pituitary adenomas (NFA) has not been successful in most cases. A low SA effectiveness could associate with variable expression of target receptors on the adenomas. The aim of the study was to determine the somatostatin (SSTR subtypes 1–5) and estrogen receptor 1 (ER1) expression profile and describe biological variability of the receptors in 184 NFA specimens.

METHODS: The group of patients was made up of 96 men (22–82 years old; median 59) and 88 women (15–87 years old; median 60). Adenoma tissue samples were obtained from patients during transsphenoidal surgery. RNA was isolated by Trizol Reagent (Invitrogen) and transcribed to cDNA by SuperScript III (Invitrogen). The receptor expression was determined by quantitative real time PCR and obtained results were normalized to the beta-glucuronidase housekeeping gene.

RESULTS: SSTR1, 2 and ER1 receptors were expressed in all the 184 examined adenomas. SSTR3, 4 and SSTR5 were detectable in 97.2%, 79.4% and 64.9% of them, respectively. Absolute median values of mRNA expression were: 187 copies/ul for SSTR1, 1671 copies/ul for SSTR2, 1147 copies/ul for SSTR3, 3.4 copies/ul for SSTR4, 6.1 copies/ul for SSTR5, and 2649 copies/ul for ER1. Also, normalized data on SSTR4 and SSTR5 were lower compared with all other SSTRs. The median relative quantification values were: 2.88% (for SSTR1), 20.60% (SSTR2), 16.49% (SSTR3), 0.06% (SSTR4), 0.68% (SSTR5), and 42.03% (ER1).

CONCLUSIONS: Our molecular biology data on SSTR and ER1 revealed individual expression profiles in NFA. It demonstrates a large biological variability of the receptors in human, with the SSTR1, SSTR2, and ER1 expression dominance. This fact makes the SA treatment of NFA individual and very difficult. Determination of the receptors expression profile could serve to predict the SA dosage and increase effectiveness of NFA treatment.

This study was supported by Charles University Grant Agency, no. 723912.

Endocrinology

Cod: 0575

RELATION OF SELENIUM AND THYROID HORMONE LEVELS

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BACKGROUND: Selenium is an important essential element with proven anti-carcinogenic activity. As a member of selenoprotein family, it has structural and enzymatic activities. Its antioxidant and catalytic role in active thyroid hormone production is one of the best known activity of selenium. In this study, our aim is to determine the effect of different levels of selenium on thyroid hormone synthesis.

METHODS: 145 male participiants aged between 32 and 40 years were included in this study and divided into three groups according serum selenium levels (Group 1: 50-75 ug/L; Group 2: 75-100 ug/L; Group 3: 100-125 ug/L). Serum selenium levels were determined by Ankara Occupational Diseases Hospital toxicology laboratory using Agilent ICP-MS. Serum free triiodothyronine, free thyroxine and thyroid stimulating analyses were performed in Architect i200 SR autoanalyzer.

RESULTS: There was no significant difference for serum thyroid stimulating hormone and free triiodothyronine levels between three groups ($p = 0.92$ and $p = 0.16$ respectively). Serum free thyroxine levels were significantly higher in Group 1 compared to other groups ($p < 0.005$).

CONCLUSIONS: According to this study's result low selenium levels lead to increase free thyroxine levels. Further studies must be performed to establish the link.

Endocrinology

Cod: 0576

CARDIOPROTECTIVE EFFECT OF SEX HORMONES IN PRE AND POST MENOPAUSAL WOMEN WITH METABOLIC SYNDROME

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BACKGROUND: Metabolic Syndrome(MS) which affects 33.1% of Africans, predisposing them to cardiovascular disease(CVD) risk has been associated with the female gender. The cardioprotective effect of oestradiol(E2) against CVD is now controversial and was investigated in premenopausal(PM) and postmenopausal(POM) women with MS.

METHODS: A subset of a cohort study involving 191 women[44 PM,126 POM and 21 apparently healthy premenopausal women(controls)] aged 41-60 years was carried out in Ibadan. The IDF criteria(2005) was used for the diagnosis of MS. Reproductive history, adiposity measures[weight(BW), height, body mass index (BMI), waist circumference (WC), hip circumference(HC), waist hip ratio(WHR), percentage body fat(PBF)] and blood pressure [systolic(SBP and diastolic(DBP)] were obtained by standard methods. 10ml of fasting blood sample was collected from each participant. Hormones were analysed by EIA(Immunometrics(UK)Ltd) while oestradiol(E2)/testosterone(T) ratio was calculated. Glucose and lipids-total cholesterol(TC), triglycerides(TG) and high density lipoprotein cholesterol(HDLC) were analysed by enzymatic methods while low density lipoprotein cholesterol(LDLC) was calculated using the Friedwald's formula. Data obtained were statistically significant at $P<0.05$.

RESULTS: Parity, adiposity measures, glucose, leptin, E2/T and follicle stimulating hormone (FSH) were higher while HDLC and T were lower in PM compared with controls. Age, parity, WHR, SBP, TG, luteinizing hormone (LH), and FSH were higher while BW, HC, leptin and prolactin (PRL) were lower in POM compared with PM. E2 correlated positively with DBP, T, FSH, E2/T but inversely with PRL in PM. E2 and T correlated inversely with BW, BMI, WC, PBF and TG but positively with FSH in POM. E2 only correlated inversely with PRL but positively with T and E2 /T ratio. T only correlated inversely with HC, TC, LDLC and leptin but positively with SBP and DBP while E2/T correlated positively with age,TC and LDLC in POM. P was ≤ 0.049.

CONCLUSIONS: Elevated oestradiol in premenopausal or reduced oestradiol in postmenopausal women with metabolic syndrome may not be protective against cardiovascular risk. Oestradiol/testosterone may be critical and important in cardiovascular risk assessment in women with metabolic syndrome.

Endocrinology

Cod: 0577

STATE OF THE ART OF ALDOSTERONE IMMUNOASSAYS. A MULTICENTER COLLABORATIVE STUDY ON THE BEHALF OF THE CARDIOVASCULAR BIOMARKERS STUDY GROUP OF THE ITALIAN SECTION OF EUROPEAN SOCIETY OF LIGAND ASSAY (ELAS) AND SOCIETà ITALIANA DI BIOCHIMICA CLINICA

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BACKGROUND: aldosterone, the primary member of the class of mineralocorticoids, is exclusively produced by the zona glomerulosa of the adrenal cortex and mainly acts on the distal renal tubule by increasing sodium reabsorption and potassium excretion, thus controlling sodium and potassium metabolism. The knowledge of aldosterone levels is very usefull in screening and diagnosis of primary aldosteronism. Immunoassays normally used in routine laboratory for aldosterone measurements are usually made by manual. The reference techniques require very expensive equipment, skilled staff and are more time consuming. Recently fully automated methods for aldosterone assay are available and needs to be clarified if the use of these platforms could improve some flaws of commonly used manual assays.

METHODS: in this study analytical performances of two automated aldosterone assays, IDS iSYS and DiaSorin LIAISON, were evaluated. Results obtained with the two platforms for clinical samples (healthy subjects and patients with cardiovascular disease and/or hyperaldosteronism) were compared with those obtained by immunoassays (RIA and EIA) or separative methods (GC-MS and LC-MS). Aldosterone was also measured with the two automated methods in the control specimens distribute during the last two annual cycles of a national External Quality Assessment scheme named Immunocheck study.

RESULTS: the two automated methods showed similar analytical performances: LoD 30.2 vs 33.2 pg/mL, LoQ 37.6 vs 40.0 pg/mL respectively; the within-run and total imprecision evaluation showed CV% between 8.1 and 14.1 for samples with 65.1 and 139.4 pg/mL concentration for both methods. There was a close linear regression between methods, however we found a significant bias (mean 27.5%, SD 59.5%) between Liaison and iSYS. In the evaluation of the EQA samples results obtained with these two methods were highly correlated to the consensus mean values.

CONCLUSIONS: our data indicate that aldosterone values measured with the two automated methods actually show better analytical performances, shorter TAT and require less "hands on labor" compared to other RIA and EIA immunoassays. Furthermore, the analytical performance of the two automated aldosterone immunoassays are quite similar.

Endocrinology

Cod: 0578

THYROID LABORATORY TESTS REQUESTING PATTERNS IN PRIMARY CARE SETTING: A PILOT STUDY IN SPAIN

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BACKGROUND: To compare the inter-practice and inter-regional variability in thyroid tests requested by General Practitioners (GPs) in Spain, according geographic characteristics, using the measurement of appropriateness indicators, to try to ascertain the degree of requesting appropriateness.

METHODS: 76 Spanish laboratories from diverse regions across Spain covering a population of 17679195 inhabitants, filled out the number of tests requested by GPs for the year 2012. Every patient seen in any primary care center, regardless of the reason for consultation, was included in the study. Five thyroid laboratory tests were examined in a cross-sectional study: thyrotropin (TSH), free thyroxine (FT4), free triiodothironine (FT3), antitiroglobulin antibody (TgAb) and antiperoxidase antibody (TPOAb). Two types of appropriateness indicators were calculated: every test requests per 1000 inhabitants or ratios of related tests requests: FT4/TSH, FT3/TSH and TgAb/TPOAb. The indicators results obtained in different geographical areas were compared.

RESULTS: The rate of request of TSH ranged from 90.8 to 327.3 per 1000 inhabitants, from 15.7 to 233 in the case of FT4 and from 0 to 32.1 for FT3. The rate of antithyroid antibodies requesting was from 0 to 50.4 in the case of TPOAb and from 0 to 26.8 for TGAb. FT4/TSH indicator results showed that FT4 was generated depending on TSH results in the majority of laboratories, being 1 the indicator result only in 3. 24 laboratories achieved the FT4/TSH indicator target (0.25). FT3/TSH target (0.1) was achieved in 74 laboratories and only 8 of them achieved the TGAb/TPOAb indicator goal (0.1). TSH/1000 inhabitants was significantly inferior in urban-rural hospitals when compared to urban and rural areas.

CONCLUSIONS: The high variability in thyroid tests requesting in Spain emphasizes the need to accomplish interventions to improve appropriate laboratory use. Inter-departmental and inter-regional cooperation would be crucial to develop protocols and guidelines.

Endocrinology

Cod: 0579

OXIDATIVE STRESS PARAMETERS AND INTERACTION OF THESE PARAMETERS WITH THE TREATMENT IN PATIENTS WITH HYPOGONADISM

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BACKGROUND: This study has been conducted in order to reveal the presence of oxidative stress in patients with hypogonadotropic hypogonadism over the antioxidant parameter thiol with protein, lipid and nucleic acid oxidation products, and further to evaluate the probable effects of current patient treatments on oxidative stress.

METHODS: 30 male patients with a diagnosis of hypogonadism and age-and sex-matched control group of 20 healthy volunteers were included in this study. In order to assess oxidative stress; advanced oxidation protein products (AOPP), thiol, malondialdehyde (MDA), nitric oxide (NO) and 8-hydroxydeoxyguanosine (8-OHdG) levels of the subjects were measured.

RESULTS: When the pre-treatment and post-treatment evaluations were made to the patient group, it was observed that thiol level was statistically higher, AOPP, MDA and NO levels were statistically lower and 8-OHdG level showed no statistically significant difference after treatment. The view that oxidative stress is a significant factor in hypogonadism pathogenesis is supported with high AOPP, MDA, NO levels and low thiol level measured in the study. Our study is the first to evaluate AOPP, MDA, NO and thiol levels of patients with hypogonadism in plasma/serum.

CONCLUSIONS: We are in the opinion that this study will make significant contributions to the literature for the explanation of the pathogenesis of the disease and will be useful in treatment protocols.
Key Words: Hypogonadism, AOPP, thiol, MDA, NO, 8-OHdG.

Endocrinology

Cod: 0580

ASSOCIATIONS OF SERUM VITAMIN D STATUS WITH THYROID HORMONE LEVELS

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BACKGROUND: The relationship between Vitamin D and autoimmune disorders has long been investigated and it may affect autoimmune thyroid diseases. Vitamin D also has been shown to influence thyrocytes directly by reducing Thyroid Stimulating Hormone (TSH)- stimulated iodide uptake and cell growth. However, it is unclear how Vitamin D status is related to TSH, free triiodothyronine (FT3) and free thyroxine (FT4) levels at the population. The aim of this present study was to investigate the relation between Vitamin D status and TSH-FT3- FT4 levels.

METHODS: We studied on 162 patients who were admitted to internal medicine clinic in July and August 2013. Patients with 25-Hydroxy Vitamin D (25-OH Vitamin D) levels <30 ng/ml were considered as Group 1 and ≥30 ng/ml as Group 2. 25-OH Vitamin D were measured by DiaSorin, LIAISON XL instrument with chemiluminescence method . TSH, FT3 and FT4 levels were measured by Beckman Coulter DxI-800 with chemiluminescence method, too. Datas were expressed as means and standart deviations. For statistical analysis SPSS 21.0 software was used. p- values <0.05 was considered statistically significant.

RESULTS: According to serum 25-OH Vitamin D levels, there were 118 patient (%72.8) in Group 1 and 44 patient (%27.2) in Group 2. The mean age was 58.9 ± 10.5 year. The mean levels of Group 1 were as follows ; TSH:1.59 ± 0.8, FT3:2.69 ± 0.5, FT4: 0.89±0.1; The mean levels of Group 2 were TSH:1.53 ± 0.8; FT3:3.08 ± 0.4; FT4: 0.91±0.2. There were no statistically significant correlation between Vitamin D status and TSH-FT3-FT4 levels (p>0.05).

CONCLUSIONS: We found that, Vitamin D status was not associated with FT3,FT4 or TSH levels. It may be due to our retrospective population based study group. Prospective studies are needed to confirm the relationship between thyroid and Vitamin D status.

Endocrinology

Cod: 0581

RELATIONSHIP BETWEEN PLASMA CORTIZOL LEVELS AND SCORE OF DEPRESSION IN HEROIN ADDICTS OF METHADONE TREATMENT

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BACKGROUND: Although endocrine abnormalities are recognized in opiates users and methadone maintenance patients, very little is known about the range of hormones affected, their pathophysiology and their clinical relevance. Various endocrine abnormalities have been reported in these patients with included increased levels thyroxin (T4), Triiodthyronine (T3), insulin and glucose metabolism abnormalities similar for those seen on type 2 diabetes, increased prolactin levels and abnormalities in sexual hormone. Adrenal insufficiency decrease noradrenaline levels and increase plasma cortisol levels. Pathophysiological mechanism postulated does explain these findings included a direct action of heroin or methadone at the hypothalamic or pituitary level. The aim of this study was to determine the relationship between serum cortisol levels and score of depression in methadone maintenance treatment (MMT) patients. The study was cross sectional. In this clinical study were assessed morning serum concentrations of cortisol in 60 patients of MMT in Day hospital on methadone treatment of heroin addicts.

METHODS: Serum cortisol levels were determinated with chemiluminescent enzyme immunoassay (CLIA) methods of Immulite 2000 analyzers, Beck Depression Inventory (BDI) was used to determinate the depressive condition.

RESULTS: The correlation between serum cortisol levels and score of BDI depressive symptoms in all patients in MMT was statistically significant ($p<0.05$).

CONCLUSIONS: Higher serum levels of cortisol are in direct dependence with high scores that conditions striking depressing symptomology. The results are of great importance for diagnosing depressive state of the patients, and therefore the application of certain antidepressant therapy

Endocrinology

Cod: 0582

LONGITUDINAL CHANGES IN TSH AND FT4 DURING NORMAL PREGNANCY AND POST-PARTUM

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BACKGROUND: Thyroid physiology changes during pregnancy. Therefore the use of trimester-specific reference intervals (RI) for TSH and free T4 (fT4) are recommended. Each laboratory should verify RI for their population due to influences of ethnicity, iodine status and differences between assays. No trimester-specific RI for TSH and fT4 are available for The Netherlands. Current Dutch guidelines recommend using non-pregnant RI to diagnose thyroid dysfunction during pregnancy.

METHODS: 172 apparently healthy pregnant Dutch women were recruited via primary midwife practices in the southern part of The Netherlands between 2012 and 2014. Exclusion criteria included multiple pregnancy and past history of thyroid disease. We longitudinally assessed TSH and fT4 at 10-12 weeks, 27-29 weeks and 36-40 weeks of gestation (trimesters (Tr) 1, 2 and 3 respectively) and at 4-11 weeks postpartum in a subgroup of 69 women who had a full set of samples available. Antibodies to thyroid peroxidase (TPOAb) and to TSH-receptor (TSH-RAb) were analysed at T3. All analyses were performed on a Cobas 6000 analyser (Roche Diagnostics). Women who were TPOAb positive (n=5; 7%) or subsequently developed significant disease (pregnancy-induced hypertension n=4, diabetes n=1) were excluded from statistical analyses, leaving a total of 59 women. None of the women was TSH-RAb positive. Mean±SD age of the longitudinal subgroup at Tr1 was 30±4 years.

RESULTS: Mean (95% confidence interval, CI) TSH increased during pregnancy: 1.47 (1.23-1.72) mIU/L in Tr1, 1.73 (1.50-1.95) mIU/L in Tr2, 2.04 (1.79-2.28) mIU/L in Tr3 (ΔTr1-Tr2 P=0.01, ΔTr2-Tr3 P<0.001). Mean postpartum TSH was 1.62 (1.43-1.82) mIU/L. Mean fT4 decreased during pregnancy from 14.9 (14.5-15.4) pmol/L in Tr1 to 11.7 (11.4-12.1) pmol/L in Tr2 and 11.2 (10.8-11.6) pmol/L in Tr3 (ΔTr1-Tr2 and ΔTr2-Tr3 P<0.0001). Mean postpartum fT4 was 13.9 (13.4-14.3) pmol/L.

CONCLUSIONS: Non-pregnant RI of TSH (0.4-4.0 mIU/L) are too liberal to identify pathological deviations during pregnancy. FT4 concentrations are at the lower end of the non-pregnant RI (9-23 pmol/L), which is seen with most immunoassays. Consequently, they may more easily be interpreted as hypothyroid while actually reflecting normal physiology.

Endocrinology

Cod: 0583

CALCULATION OF FREE TESTOSTERONE: A COMPARISON OF 3 PUBLISHED EQUATIONS

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BACKGROUND: Testosterone is mainly transported in blood by sex hormone-binding globulin (SHBG). The clinical situation is more accurately reflected by free testosterone (FT) levels, which correspond to the small unbound fraction. The available laboratory methods for the measurement of FT are laborious and cannot be automated. Alternatively, there are several methods published for the calculation of FT basing on the most frequently cited algorithm and relying on mass action equilibrium formulas described by Vermeulen et al. (1999). Algorithms for the calculation of FT are based on the measurement of total testosterone (TT) and SHBG. In this study we compared the results obtained using 3 different equations.

METHODS: The 3 different calculation formulas published by de Ronde et al. (2006), Berding & Krämer (2004) and the online calculator ISSAM have been used to estimate the FT concentrations. In serum samples of 30 patients (15 men and 15 women) TT and SHBG concentrations have been measured with commercial immunometric methods on the Modular E170 and the Immulite 2000, respectively. According to Vermeulen et al. (1999), a fixed albumin concentration of 43 g/l was used.

RESULTS: The Passing & Bablok correlation showed a very good agreement between the 3 different calculations. The Pearson correlation coefficients in men were always 1.00, the slopes varied between 0.99 and 1.00 using the different formulas. The intercepts always were less than 1% of the corresponding average of the calculated values. In women the Pearson correlation coefficients were 1.00, the slopes always 1.00 and the intercepts were all 0. We demonstrated the validity of Roche FT reference values which were originally determined by measurements of Elecsys TT and SHBG assays. The analysis of method-dependent median values from the Elecsys and the Immulite 2000 SHBG tests derived from external quality controls showed a relative difference of only -2.02 %.

CONCLUSIONS: The calculation formulas published to calculate FT must be revalidated in the local setting, in order to avoid an overestimation or underestimation of FT. After validation, we have decided for reasons of practicability to use the ISSAM calculator to estimate the FT levels.

Endocrinology

Cod: 0584

CAN NEUTROPHIL/LYMPHOCYTE RATIO AND MEAN PLATELET VOLUME BE USED FOR DIFFERENTIAL DIAGNOSIS OF LYMPHOCYTIC THYROIDITIS AND BENIGN COLLOIDAL GOITER?

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BACKGROUND: Thyroid nodules are a very common finding, especially present in iodine-insufficient population. Lymphocytic thyroiditis (LT), which is one of the common thyroid pathologies and resulting from the autoimmune process, is characterized by widespread lymphocyte infiltration, fibrosis, and parenchymal atrophy of thyroid tissue. Radiological and histopathological evaluations are essential in differentiating true nodules from pseudonodules, which are an expression of the inflammatory infiltrate.
Neutrophil/Lymphocyte ratio (NLR) and mean platelet volume (MPV) are indicators of the subclinical inflammatory status of the body. An alteration in NLR and MPV levels may be found in LT patients those have thyroid nodules. The aim of this study was to investigate the utility of NLR and MPV as simple and readily available predictors for distinguishing LT and benign colloidal goiter (BCG) with thyroid nodules.

METHODS: We retrospectively evaluated 167 patients with thyroid pathologies, those of 97 were histologically confirmed as LT, and 70 were confirmed as benign colloidal goiter (BCG). Patients with LT were divided into two groups according to their thyroid-stimulating hormone (TSH) levels [TSH> 4 IU/mL (n:30) and TSH< 4 IU/mL (n:67)] to assign the effect of thyroid hormones on white blood cell (WBC) counts and MPV.

RESULTS: NLR and MPV levels were significantly higher in both LT subgroups than patients with BCG ($p<0.05$). However NLR and MPV levels did not differ among LT subgroups in respect to TSH levels ($p>0.05$). WBC, neutrophil, platelet counts and free triiodothyronine (fT3) levels were not significantly different between groups ($p>0.05$).

CONCLUSIONS: According to the results of this study, both NLR and MPV can be used as potential biomarkers in discriminating BCG and LT patients independently from serum TSH concentrations, before pathological and radiological assessments.

Endocrinology

Cod: 0585

DOES LOW SERUM 25-HYDROXYVITAMIN D LEVEL PREDICT PROGRESSION PREDIABET TO TYPE 2 DIABETES?

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BACKGROUND: Vitamin D deficiency appears to be related to the development of type 2 diabetes mellitus (T2DM). Mild to moderate vitamin D insufficiency has been proposed as a risk factor for T2DM. We hypothesized that vitamin D deficiency may be prevalent in a population of T2DM patients and that vitamin D may be related to glucose control in this group of patients.

METHODS: A total of 89 impaired fasting glycaemia group (IFG) (45 female, 44 male, age: 48,94±6,08 years) and 78 T2DM (38 female, 40 male, age: 50,12± 6,85 years) were enrolled to this study.Serum levels of glucose, was measured with enzymatic method Insulin, vitamin D were measured by chemiluminesant method, HOMA-IR was calculated by Formula HbA1c levels were measured with high performance liquid chromotography (HPLC).

RESULTS: There were no statistical differences between groups for glucose, (p>0,05). T2DM group had significantly lower serum levels of (p=0,0001), insulin (p=0,013), HOMA (p=0,014) and vitamin D (p=0,0001) than IFG. HbA1c levels were higher in the group of T2DM than in the IFG group(p=0,0001), There were negative correlations in between vitamin D and HbA1c (r=-0,327, p=0,006). There were positive corelations in between vitamin D and insulin, HOMA-IR (respectively, r=0,215,p=0,006, r=0,236, p=0,002). In the group of T2DM 25(OH) D3 levels were lower when compared to IFG, 25 (OH) D3 levels were detected as 20,98±10,39 ng/ml and 13,65±7,11 ng/ml in the patient and IFG (p<0,0001). 25(OH)D3 levels were found to be inversely associated with HbA1c levels in the group of T2DM (p=0,0001, r=0,215, linear regression analysis) Linear regression analysis was converted for HbA1c, insulin, HOMA-IR variants which were found to be related with vitamine D by univariate tests; R2: 0,203, corrected R2: 0,197, HbA1c (p=0,001), Insulin (p=0,013), HOMA-IR (p=0,006) were found to be in relation with vitamine D.

CONCLUSIONS: Further studies are required especially in subjects that are affected by a high risk of developing diabetes (impaired fasting glucose and/ or glucose tolerance). Based on the hypothesized mechanism of action of vitamin D, these subjects may be the main beneficiaries of the effects of vitamin D on the prevention of T2DM.

Endocrinology

Cod: 0586

A COMPARISON OF SIX 25-HYDROXY VITAMIN D ASSAYS IN THE ASSESSMENT OF VITAMIN D DEFICIENCY AND RESPONSE TO TREATMENT

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BACKGROUND: Accurate measurements of 25-hydroxy vitamin D (25(OH)D) are important for vitamin D status assessment and treatment control. Previous data suggest that commercially available assays vary considerably. We therefore investigated the performance of 4 commercially available 25(OH)D assays against two liquid chromatography - tandem mass spectrometry (LC MS/MS) methods with respect to assessment of serum 25(OH)D before and after vitamin D supplementation over a period of 4 months.

METHODS: Serum samples (n=490) were obtained from 35 healthy postmenopausal women who were participating in a randomised, double-blind 7-arm clinical trial evaluating different vitamin D supplementation strategies. For each participant, serum 25(OH)D was measured on 14 days over a period of 4 months. Samples from all treatment arms were collected and assessed by a radioimmunoassay (RIA), three automated immunoassays (Roche, Abbott, and Liaison), and two LC MS/MS methods, one of which was calibrated using standards traceable to NIST SRM972 standards and therefore defined as a reference method in this study.

RESULTS: The 25(OH)D values obtained with automated immunoassays were in average lower than those obtained by the reference method. The mean difference ranged between -2.47 ng/mL for Abbott to -4.92 ng/mL for Roche. RIA demonstrated a positive mean difference of 2.24 ng/mL. The biases between results measured by RIA, Liaison, Abbott and Roche and results determined by ÌCC LC/MS/MS as reference method, exceeded the recommended thresholds (±25%) for the total allowable Error (TEa) of a 25(OH)D test in 12%, 32%, 15% and 28% respectively, of the participant samples tested in this study. All assays showed significant (P<0.001) increases in 25(OH)D concentrations following vitamin D supplementation. Compared to the LC MS/MS, sensitivity of different methods in detecting vitamin D deficiency (<20 ng/mL) varied from 82.32% for RIA to 98.99% for Abbott, and specificity ranged from 67.02% for Liasion to 97.17% for RIA.

CONCLUSIONS: In conclusion, the accuracy of commercially available 25(OH)D assays varies in a clinically meaningful manner by under- or over-diagnosing vitamin D deficiency

Endocrinology

Cod: 0587

CALCITONIN MEASUREMENT ON FINE-NEEDLE ASPIRATE WASHOUTS IN MEDULLARY THYROID CARCINOMA

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BACKGROUND: Ultrasound-guided fine-needle aspiration biopsy cytology (FNAB-C) is the most common procedure for confirming the diagnosis of primary or metastatic medullary thyroid carcinoma (MTC). In the needle wash-out (FNAB) the measurement of calcitonin (CT), as biochemical marker, combining to citology has been proposed to provide the diagnostic accuracy.

METHODS: FNAB-C was performed on 62 thyroid lumps or lymph nodes in 38 patients with suspicious MTC before initial surgery or during post-surgery follow up. Samples for serum CT (sCT) measurement were obtained in all the patients before performing FNAB-C. After obtaining a FNAB-C specimen, the needle was washed with 0.5 ml of saline solution. Receiver operating characteristic (ROC) analysis was performed to determine the absolute cut-off levels of CT in the washing fluid and the cut-off ratio between FNAB-CT and sCT with the highest sensitivity and accuracy. Diagnostic accuracies of the established cut-offs were compared with that of FNAB-C.

RESULTS: Overall, primary or metastatic MTC were found at final histological examination in 18 cases (29.0%). sCT and CT-FNAB values were 217.5 pg/ml ± 599.1 (range 3.0-3110.0 pg/ml) and 317.9 pg/ml ± 687.4 pg/ml (range 1.6-2000.0 pg/ml), respectively. ROC analysis indicated absolute levels of FNAB-CT >10.4 pg/ml and FNAB-CT/sCT ratio >1.39 as the more accurate cut-offs. Sensitivity, specificity, overall accuracy, positive (PPV) and negative predictive values (NPV) were 50%, 100%, 85%, 100 and 83%, respectively, for FNAB-C, 89%, 100%, 97%, 100% and 96% for FNAB-CT and 83%, 93%, 90%, 83% and 93% for FNAB-CT/sCT ratio. One out 2 patients with false negative FNAB-CT result and one out 3 patients with false negative FNAB CT/sCT ratio were correctly diagnosed by FNAB-C. Eight out 9 non-diagnostic FNAB-C were correctly classified by FNAB-CT and seven by FNAB CT/sCT ratio.

CONCLUSIONS: FNAB-CT should integrate but not substitute FNAB-C to detect primary or metastatic MTC. FNAB-CT is particularly useful in presence of non diagnostic FNAB-C because the biochimical variation is ahead of time than cytological diagnosis.

Endocrinology

Cod: 0588

PITUITARY MAMMOTROPES IN LONG-TERM OVARIECTOMIZED ADULT RATS CHRONICALLY TREATED WITH ESTRADIOL

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BACKGROUND: Bilateral ovariectomy in women causes estrogen deficiency, which leads to many profound changes, such as hot flushes, bone mass loss, decreased libido, depression, increased risk of cardiovascular diseases and general acceleration of the aging process. Estradiol modulates the activity of hypothalamic-pituitary system and performs a beneficial effect in osteoporosis and some other ageing symptoms. The present study investigate the effects of chronic estradiol treatment on the immunohistochemical and hormone secreting characteristics of pituitary mammotrope (PRL) cells in ovariectomized (Ovx) and estradiol treated ovariectomized (Ovx+E) rats.

METHODS: Adult Wistar female rats were divided into three experimental groups (n=7), while two of them were bilaterally Ovx. After recovery period (1 month), one Ovx group was i.p. treated with 0,625 mg/kg b.w. of estradiol-dipropionate (Ovx+E), for four weeks. The sham controls (C) and the other Ovx group received the adeqate volume of dissolvent (sterile olive oil) during the same period. All females were sacrificed 24h after the last injection. PRL cells were studied using the peroxidase-antiperoxidase (PAP) immunohistochemical procedure. Serum PRL concentrations was measured by the hPRL- Delfia kit.

RESULTS: Body weights in Ovx+E group were significantly ($p<0.05$) decreased by 27.7% and 25.1% in comparison to the C and Ovx animals, respectively. Absolute pituitary weights in Ovx+E group were 3.3 and 2.5 higher ($p<0.05$) compared to the C and Ovx groups, respectively. Relative pituitary weights in Ovx+E group were 4.2 and 3.2 higher ($p<0.05$) then in C and Ovx groups, respectively. Immunohistochemically labeled PRL cells in the control rat pituitaries were polygonal, elongated in shape, with a spherical, centrally located nucleus. In Ovx+E rats PRL cells were longer, irregularly shaped, with more intense imunopositive signal. The PRL serum levels in Ovx+E group were significantly ($p<0.05$) increased by 42.4% and 51.8% compared to the C and Ovx, respectively.

CONCLUSIONS: It can be concluded that estradiol treatment in Ovx female rats have the stimulatory effect on the PRL secretion.

Endocrinology

Cod: 0589

FUNCTIONAL CHARACTERISTICS OF THYROID TISSUE CAN BE PRESERVED IN LONG TERM CULTURE

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BACKGROUND: Two-dimensional cell cultures lack the metabolic and proliferative gradients. Human thyroid cell culture models do no entirely mimic the pathophysiologic behavior of the thyroid tissue in vivo. Setting-up the experimental conditions and investigate the functional characteristics of the human thyroid tissue in a long term culture.

METHODS: Thyroid tissue was collected from patients that underwent thyroid surgery. Samples were harvested in DMEM+10% FBS+antibiotic/antimycotic both from nodular and perinodular tissue. Tissue fragments have been cultured in thyroid specific culture medium, supplemented with TSH and Insulin, for 3-6 months. Samples from cell culture medium were harvested at three different time points. Thyroglobulin T4, fT4, T3 and ATPO in cell culture medium were assayed. The study is approved by the Ethical Commission of the Institute. Patients were enrolled after they signed the Informed Consent.

RESULTS: Thyroid tissue fragments showed long term preserved functional characteristics in culture. In papillary carcinoma tissue culture, Tgl levels in nodular tissue was significantly higher than in perinodular tissue (594, 08 vs. 74.9 ng/ml) The hormonal secretion showed a constant increase in time. In chronic thyroiditis Tgl, T4, fT4, have lower vales in the nodular tissue than in the perinodular one (46, 24 vs. 787,55 ng/ml for Tgl, 1.28 vs. 8.22 ug/dl for T4 and 5.57 vs. 38.42 pmol/l for fT4).

CONCLUSIONS: Thyroid tissue fragments can be cultured over long time periods maintaining their phenotypic characteristics. Our model can be useful to test new therapeutic agents in vitro in similar conditions as in vivo.
Acknowledgements: This work was done under PCCA2 no.135/2012 grant.

Endocrinology

Cod: 0590

CONNECTION BETWEEN POSTOPERATIVE THYPOCALCAEMIA AND PTH MEASURED IN THE FIRST POSTOPERATIVE HOURS AFTER THE TOTAL THYROIDECTOMY

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BACKGROUND: Frequency of thyroid disease in Croatia corresponds the world average (7%). Because of the intimate contact between parathyroid and thyroid glands the total thyroidectomy might cause postoperative hypocalcaemia. In this study we want to establish the total number of postoperative hypocalcaemia during one year period and estimate quality of PTH activity as predictive hypocalcaemia markers in the early postoperative period.

METHODS: In this study we included 93 patients: 80(85%) women and 13(15%) men, average age 52±14 undergoing to total thyroidectomy during 2013 in our hospital. We had been measured total calcium concentration (Arsenazo III method, Abbott c4000) and PTH(ECLIA, Elecsys 2010, Roche).

RESULTS: Categorizing patients on the basis of the cut-off PTH activity (15 pg/mL) in the first postoperative hours we were obtained two groups. Group with PTH values below the cut-off include 27 patients with a median(range) PTH 8.24(2.76-14.2)pg/ml, while in the group with values above 15 pg/ml counted the remaining 66 patients with PTH 30.35(15.7-78.3)pg/mL. There was a significant difference between PTH those two groups, $p < 0.001$. All patients postoperative manifest hypocalcaemia had a PTH activity in the early postoperative hours below the cut-off. In 18 of 27 patients with PTH under 15 pg/mL(67%) exhibited the hypocalcaemia. 19.3%(18/93) patients developed hypocalcaemia which intensity is higher if it is manifested late. Patients with reduced PTH were divided into two groups. In the group of PTH 0 -7.5 pg/mL, 10/11 (91%) and in group PTH 7.5-15pg/mL, 8/17 (47%) exhibited hypocalcaemia. The average serum Ca is lower if it is manifested later (1st 1.98, 2nd 1.89, and 3rd day 1.84mmol/L ($p < 0.05$). Patients which had developed postoperative hypocalcaemia, were establish eucalcaemia average after 5.1 days.

CONCLUSIONS: Our results confirm the necessity of PTH measuring in the early postoperative period after the total thyroidectomy. Delayed noticed changes in the level of Ca can result unrecognized hypoparathyroidism, fast and more intensive hypocalcaemia and highly risked postponed supplementary therapy intervention. It would be worth exploring further extended, in which the same would be repeated frequent sampling at shorter intervals in the early postoperative period.

Endocrinology

Cod: 0591

ANALYTICAL AND CLINICAL EVALUATION OF THE NEW VIDAS® FT4 METHOD FOR FREE THYROXIN MEASUREMENT

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BACKGROUND: The measurement of FT4 is an important tool for the diagnosis and monitoring of thyroid diseases. Recently, bioMerieux (Marcy l'Etoile, France) has redeveloped its FT4 test on the VIDAS instrument. The new test (VIDAS FT4-ref 30459) has been evaluated in our laboratory. The method is based on the technique Enzyme Linked Fluorescent Assay (ELFA). All of the assay steps are performed automatically by the instrument.

METHODS: We evaluated 71 routine serum samples : 11 normal subjects, 9 hyperthyroid, 9 hypothyroid, 7 low-T3 syndrome, 4 subclinical hyperthyroid, 5 renal failure and 4 liver cirrhosis patients, plus 12 pregnant women and 10 paediatric patients undergoing cardiac surgery. Moreover, control samples from an External Quality Assessment (EQA) scheme (QualiMedLab/CNR, Pisa Italy) were measured. For comparison, in addition to VIDAS FT4, all samples were tested on Cobas-Roche FT4 and AIA-Tosoh FT4 immunoassay techniques.

RESULTS: Regression analysis between VIDAS FT4 and the two compared methods (VIDAS FT4 = 1.24 Cobas – 0.62, n = 70, r = 0.974;VIDAS = 1.27AIA – 0.44, n = 71, r = 0.986) indicates that results produced by VIDAS are slightly higher but well correlated with those of the other two techniques. In addition, the results of the control samples obtained by the EQA in laboratories using VIDAS, Cobas and AIA (including our lab) confirms the trend obtained on patients' samples. Regarding precision, the CVs of three control samples measured in 10 different runs are: sample A, 5.54 pg/mL, 13.5%; sample B, 8.31 pg / mL; 8.7%; for sample C, 21.8 pg / mL 6.6%; this level of precision is comparable to that observed with the two other compared methods.

CONCLUSIONS: These preliminary data confirm the good analytical and clinical performance of redeveloped VIDAS-FT4 assay in various pathological thyroid conditions and in the presence of physiological variations of thyroid hormone binding proteins.

Endocrinology

Cod: 0592

LABORATORY DETERMINATION OF MACROPROLACTINAEMIA USING PEG METHODOLOGY

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BACKGROUND: According to literature, macroprolactinaemia is mainly cause of hyperprolactinemia due to two different circulating forms of prolactin, big (50 kDa) and big-big (>150 kDa) molecules. The relatively low frequency of the symptoms in patients with macroprolactinaemia, created the need for a relatively simple, inexpensive and at the same time sufficiently reliable method which can detect large, biologically inactive prolactin molecules such as polyethylene glycol precipitation method (PEG). The aim of this study was to evaluate aforementioned methodology and its applicability in everyday laboratory practice.

METHODS: This study included 82 patients who had come to the Center for Laboratory Medicine, Clinical Center of Vojvodina in order to determine the serum prolactin levels. Obtained serum samples were poured into two aliquots. The first aliquot was frozen and stored at -20˚C and the other was immediately analyzed. Both kinds of aliquots were treated with the respective, aqueous solution or phosphate buffered saline of Merch 6000 (PEG). All serum and supernatant samples were analyzed on the automated system Abbott Architect i2000sr. Macroprolactinaemia was assessed using calculated cut off Recovery value, less than 40%. All the results were statistically processed by Data Analysis package.

RESULTS: Supernatant prolactin concentrations and calculated Recovery values obtained from unfrozen serum samples using phosphate buffered saline are the lowest (15.64±18.98 ng / ml). Statistical difference was not determined between Recovery values obtained from fresh and frozen serum samples after precipitation with PEG dissolved in phosphate buffer (p=0.89). The results of linear regression showed statistically insignificant relationship between the calculated values of the coefficient of variation and the initial values of prolactin (R^2=0.01; p=0.262).

CONCLUSIONS: Based on the results, today is in use a reproducible method for laboratory determination of macroprolactinaemia based on PEG precipitation (dissolved in phosphate buffer) measuring low, elevated and high prolactin levels with equal reliability using fresh and previously frozen serum samples. It provides the opportunity of laboratory assessment of macroprolactinaemia once a week.

Endocrinology

Cod: 0593

ANALYTICAL EVALUATION OF FREND TSH ASSAY

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BACKGROUND: The FREND™ TSH test is a newly developed- rapid quantitative sandwich immunoassay, utilizing antibody-conjugated fluorescent nanoparticles and capture antibody in microfluidic flow system. We investigated the analytical performance of FREND™ TSH test on the FREND™ system according to the CLSI guidelines.

METHODS: The analytical sensitivity, precision, linearity and method comparison were performed. General analytical requirements (interference, crossreactivity) were also evaluated.

RESULTS: Analytical sensitivity was determined using blank sample and sera with lowest TSH concentration. Limit of blank(LoB) and limit of detection (LoD) was 0.033 and 0.030 mIU/L, respectively. In precision test, using three levels pooled human sera with a concentration of 0.68 - 19.4 mIU/L, the inter-assay CVs and lot-to lot variation ranged from 3.5 to 8.2%. We confirmed the linearity covering the measuring range (0.06-25.0 mIU/L), which manufacturer declared (r=0.9965). In method comparison, Deming regression analysis demonstrated close agreement between FREND TSH and ADVIA centaur TSH assay, showing a slope 0.3988 (95% CI: -0.554 to 1.351) and an intercept of 7.2356 (95% CI:-2.103 to 16.575). The mean percentage difference between two assays was +6.36%. The interference test by endogenous substance or drugs, and cross-reactivity test by hCG, LH or FSH showed no significant affect on the TSH measurement. The heterophilic antibodies (anti-mouse; HAMA) up to 52.5 ng/mL did not interfere with the FREND™ TSH assay.

CONCLUSIONS: The FREND™ TSH test demonstrated reliable analytical performance from our evaluation. It can be used for thyroid function test in clinical laboratories.

Endocrinology

Cod: 0594

EVALUATION OF THE AGE-SPECIFIC AMH VALUES IN A LARGE POLISH WOMEN POPULATION

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BACKGROUND: The aim of the study was to determine the age-specific serum anti-Mullerian hormone (AMH) values from a large population of the women presenting in DIAGNOSTYKA Company, Poland.

METHODS: Tests were performed on a group of 4157 women in age 18-45 (mean 33±4,91) years between 2011-2014 at Diagnostyka Company. Serum AMH was determined using the Beckman Coulter Gen II AMH ELISA assay. Serum follicle-stimulating hormone (FSH) were performed using FSH (Roche Diagnostics, Elecsys Cobas e 100 test).

RESULTS: Mean AMH level was 3,12 (SD±3,63) ng/ml. Women were divided into seven group depending on age: (1)18-21; (2) 22-25; (3) 26-29; (4) 30-33; (5) 34-37; (6) 38-41; (7) 42-45. Medians (quartiles) of AMH level in each group were: 6,09 (3,09-8,36) ng/ml; 5,40 (2,36-7,12) ng/ml; 4,90 (1,89-6,16) ng/ml; 3,56 (1,28-4,53) ng/ml; 2,34 (0,69-3,04) ng/ml; 1,77 (0,42-2,13) ng/ml; 1,00 (0,16-1,20) ng/ml respectively. 1702 women were tested with both AMH and FSH. Mean FSH level in the studied population was 6,64 (SD±6,45) mIU/ml. Medians (quartiles) for FSH in each age group were: (1) 5,32 (3,26-62,84) mIU/ml ; (2) 5,03 (3,97-5,91) mIU/ml; (3) 5,27 (4,36-6,32) mIU/ml; (4) 5,43 (4,48-6,79) mIU/ml; (5) 5,55 (4,43-6,79) mIU/ml; (6) 5,74 (4,52-7,87) mIU/ml; (7) 6,52 (4,77-9,86) mIU/ml. Significant correlation between AMH and age was found in studied population (R=0,39; p<0,001), and in each group (1) R=0,07,p=0,002; (2) R=16;p<0,001; (3) R=0,22, p=0,001; (4) R=-0,11,p=0,005; (5) R=0,07,p=0,005; (6) R=-0,15,p<0,001; (7) R=-0,14;p<0,001. Significant correlations were found between: FSH and AMH (R=-0,14,p<0,001); and FSH and age (R=0,05,p=0,042). Odd ratio (OR) in logistic regression was performed to evaluate relation between AMH and age (OR=0,84, -95% CL=0,82; 95%CL=0,86; p<0,001). Receiver Operating Characteristic (ROC) curve analysis was performed to determine a cutoff value for age (37 years) using as the predictor of the fertility reduction AMH level <1,5 ng/ml.

CONCLUSIONS: The references value were established for age-specific AMH value. AMH level decreased dependently on age and correlated with the age and FSH level.

Endocrinology

Cod: 0595

MALE HYPOGONADISM OF A PATIENT WITH 45X0/46XX MOSAICISM AND WITHOUT SRY GENE. A CASE REPORT

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BACKGROUND: Technological advances in cytogenetics and molecular biology, allow the diagnosis of diseases in many occasions; other times the finding of chromosomal abnormalities is casual. Male hypogonadism, disorder causing a decrease in testicular function, is a common but underdiagnosed disease. Diagnosis is established by a low concentration of circulating testosterone but molecular biology helps in complicated diagnosis cases.

METHODS: 67-year-old man, known by the Service of Endocrinology since 2005, with right breast gynecomastia, decreased sex drive, erectile dysfunction with several years of evolution and loss of hair on chest and legs; without interesting family history. The biochemical study shows, at the baseline study, values of testosterone (TEST), luteinizing hormone (LH) and follicle stimulating hormone (FSH) of 0.36 ng / mL, 8.4 mIU / mL and 22.7 mIU / mL respectively and 10.3 ng / mL, 0.1 mIU / mL and 1.7 mIU / mL within a few days after administration of testosterone. In order to verify clinical suspicion of hypogonadism, biochemical study was started and decreased values of testosterone and increased values of luteinizing and follicle stimulating hormones were found. He began treatment with depo-testosterone intramuscular injection and the study was repeated before and after treatment administration. Bilateral scrotal radiology and a cytogenetic study by karyotyping with fluorescence in situ hybridization (FISH) were requested to rule out possible genetic cause.

RESULTS: Analytical and radiological results set up the diagnosis of hypergonadotropic hypogonadism with bilateral testicular atrophy. The cytogenetic study shows a kariotype in mosaicism with a cell line (84% of the studied metaphases) compatible with a translocation between X chromosome and Y chromosome and the other cell line compatible with Turner syndrome. In both lines, heterochromatin of Y chromosome is not observed. The final chromosome formula was 45,X[32]/46,X,der(X)t(X;Y)(p22.3;p11.3)[168].

CONCLUSIONS: The availability of cytogenetics and FISH technology is able to detect chromosomal abnormalities that occur with an exceptional frequency. Often, this information helps in understanding complex manifestations in diseases with a basically clinical diagnosis.

Endocrinology

Cod: 0597

A CORRELATION STUDY OF SERUM C – PEPTIDE TO APO-PROTEINS IN NEWLY DIAGNOSED HYPOTHYROID SUBJECTS: A NOVEL MARKER OF CVD RISK

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BACKGROUND: With an ever increasing incidence of Cardiovascular diseases (CVD) in hypothyroidism, there has been a demand for newer markers that could help in the early detection of the risk of this disease complex. The present study was planned to establish the correlation between C– peptide and metabolic parameters like apo – proteins in newly diagnosed hypothyroid subjects for an early evaluation of CVD risk of these patients.

METHODS: The present study has been conducted in 250 subjects (100 healthy controls and 150 hypothyroid subjects). The hypothyroid subjects were all newly diagnosed and were analyzed for anthropometric (Body mass Index BMI), clinical (Blood pressure) and biochemical (Fasting Blood Sugar (FBS), Lipid profile, apo – proteins) parameters. Statistical analysis was done using the students -'t' test and Pearson's coefficient of correlation.

RESULTS: The newly diagnosed hypothyroid subjects presented with obesity (male 28.7 ± 4.44 kg/m^2, female 28.73 ± 5.12 kg/m^2 v/s control males 20.38 ± 3.15 kg/m^2, control females 22.16 ± 3.33kg/m^2), hypertension (Systolic blood pressure (SBP) 132.60 ± 12.18 mm of Hg v/s 115.10 ± 3.87 mm of Hg) (Diastolic blood pressure (DBP) 88.79 ± 8.02 mm of Hg v/s 78.32 ± 3.64 mm of Hg), hyperglycaemia (142.49 ± 65.07 mg/dl v/s 83.53 ± 11.23 mg/dl), dyslipidaemia, raised Apo – B (175.58 ± 34.56 mg/dl v/s 82.9 ± 10.94 mg/dl) and C – peptide (3.45 ± 1.93 ng/ml v/s 1.46 ± 1.08 ng/ml) levels and reduced Apo –A1 (139.76 ± 17.40 mg/dl v/s 140.75 ± 10.20 mg/dl). Statistically significant association of C – peptide was observed with SBP (r=0.195, p=0.016), DBP (r=0.205, p=0.011), FBS(r=0.71 p<0.0001), total cholesterol (0.63 p<0.0001), TG (r=0.36 p<0.0001), low density lipoprotein (r=0.618 p<0.0001), Apo – B (r=0.47 p<0.0001) and CVD risk ratios TCH/HDL (r=0.50, p=0.001) and apo – B/ Apo – A1 (r=0.27 p=0.006).

CONCLUSIONS: Serum C – peptide levels in hypothyroid subjects at diagnosis can serve as a noval marker for an early evaluation of CVD risk of these patients owing to the strong association with SBP, DBP and CVD risk ratios.

Endocrinology

Cod: 0598

MULTICENTER EVALUATION OF A HIGHLY SENSITIVE THYROGLOBULIN IMMUNOASSAY ON ELECSYS SYSTEMS

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BACKGROUND: Objective of the Multicenter study was to evaluate the analytical performance of a highly sensitive thyroglobulin assay (Tg II) developed recently by Roche Diagnostics on Elecsys systems.

METHODS: The Roche Elecsys electrochemiluminescence immunoassay for Tg II is a quantitative, one step, double antigen sandwich assay using 35 µL of sample. The Tg concentration is determined automatically from a 2-point calibration and a master curve which is traceable to CRM 457. The result is generated within a total assay time of 18 minutes. To characterize QC data PreciControl Thyro Sensitive Elecsys (~ 1 ng/ml Tg) was used in addition to the commercial available QC material PreciControl Universal.

RESULTS: Functional sensitivity, defined as the lowest Tg concentration that can be reproducibly measured with an inter-assay CV of ≤20%, was found between 0.05-0.1 ng/ml on cobas e 601/411. For the within-lab precision profile 4 different serum pools were assayed on 21 days, 2 runs per day based on CLSI guidelines. Standard deviations (SD's) for imprecision were found ≤ 0.04 ng/mL (0.04 – 0.5 ng/mL) and CV's ≤ 5.2% (0.5 – 500 ng/mL). The test was found to be linear from 0.04 – 500 ng/mL (acceptance criteria: 0.04 – 0.5 ng/mL ± 0.05, 0.5 – 500 ng/mL ≤ 10%). Statistical Passing/Bablok analysis using anti-Tg negative samples (Elecsys anti-Tg ≤ 22 IU/mL) yielded the following results: Elecsys Tg II (x) versus Beckman-Coulter y= 0.843x+0.051, r= 0.9891, N= 115; vs. Siemens Immulite y=1.065x+0.007, r= 0.9878, N=115; vs. Brahms Kryptor hTg y= 0.453x+0.129, r= 0.9643, N= 73 and vs. Elecsys Tg on cobas e 601 y=1.109x +0.14, r= 0.9986, N= 112.

CONCLUSIONS: The new Elecsys Tg II assay shows excellent results for functional sensitivity and precision. Reliability, convenience and robustness of the new application make it well suited for routine use in clinical laboratories.

Endocrinology

Cod: 0599

ELECSYS® AMH IMMUNOASSAY: EVALUATION OF THE NOVEL HIGH-THROUGHPUT ASSAY'S PRECISION UNDER ROUTINE CONDITIONS

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BACKGROUND: Anti-Müllerian-Hormone (AMH) is a promising marker for assessing the ovarian reserve and optimization of in-vitro fertilization treatment. Current existing manual AMH assays exhibit limitations with respect to throughput and reliability of results. Recently a fully automated AMH assay has been developed on the Elecsys®/cobas® e electrochemiluminescence immunoassay platform. The objective of the technical performance study was to evaluate the precision of this high-throughput assay under routine conditions.

METHODS: The precision performance of Elecsys® AMH immunoassay was evaluated in five different routine laboratories across Europe. The evaluation of the immunoassay was performed on a total of two cobas® e601 instruments, three cobas® e411 instruments and one Modular Analytics E170 and compared to the respective routinely used manual method. Each laboratory evaluated the performance of the immunoassay independently. The study design encompassed a repeatability experiment and the evaluation of the within-run and intermediate precision, following the procedure recommended according to CLSI-EP5-A2. For conducting the experiments, each laboratory was provided with an identical set of spiked sample and control material covering major parts of the measuring range (sample concentration between 0.24ng/mL up to 18.5ng/mL).

RESULTS: For native and spiked sample material, the repeatability was demonstrated to be below 3.2% coefficient of variation (CV) in all five laboratories. The assay's precision performance following the CLSI-EP5-A2 protocol resulted in a maximum day-to-day imprecision CV of 3.7% and part to part imprecision CV of 2.1%. The within part variance did not exceed CV 1.8%. At all testing sites the within-lab-precision exhibited CVs below 4.4%. The automated Elecsys® AMH immunoassay exhibited a better precision performance than the routinely used manual methods.

CONCLUSIONS: The fully automated Elecsys® AMH immunoassay run on routine cobas® e instruments demonstrates a very good precision. As a consequence, the availability of the fully automated Elecsys® AMH assay will represent a fast and precise alternative to manual AMH assay testing.

Endocrinology

Cod: 0601

THE RELATIONSHIP BETWEEN LEPTIN AND PCOS

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BACKGROUND: The ob gene produces a leptin hormone that regulates energy balance and works as a satiety signal to the hypothalamus. Also, leptin has important effects on the reproductive system. Insulin resistant, hyperinsulinemia, and obesity are important features of polycystic ovary syndrome. Many studies suggested that leptin may have a role in polycystic ovary syndrome (PCOS) via its role on obesity and insulin resistance. This study was done to evaluate leptin levels among Qatar university female students who were presented with clinical and hormonal features suggestive of PCOS criteria, and to investigate its relationship with PCOS among overweight and obese subjects.

METHODS: A prospective, retrospectives cross- sectional study included 78 females student aged 17-25 years. The retrospective data included clinical, anthropometric and hormonal profile such as Progesterone, Insulin, Estradiol, SHBG, Testosterone, DHEAS and Prolactin. Leptin concentration was measured by enzyme absorbent enzyme-linked immune sorbent assay by BioVendor Human Leptin ELISA. The study subjects were divided into two main groups based the cut off value of body mass index (BMI=25 Kg/m^2) into overweight/obese group and non-overweight/non-obese group.

RESULTS: Overweight/Obese female subjects had significantly higher leptin level (22.85 ng/ml) than non-overweight/non-obese subjects (2.82 ng/ml) with (p-value <0.05). OW/Ob group showed higher frequency of family history of PCOS and diabetes (8.70%) and (78.30%) respectively. Also, leptin found to be significantly higher in those women with PCOS than in women without PCOS (0.037 significant p- value). Spearman's correlation analysis, revealed that leptin is significantly correlated with insulin, testosterone, BMI, DHEAS, progesterone, FAI and PCOS.

CONCLUSIONS: PCOS subjects exhibit high leptin level. Overweight and obese subjects exhibit higher levels of androgens and leptin, which could highlight the possible implication of leptin for the pathophysiology of PCOS.

Endocrinology

Cod: 0602

CORONARY RISK FACTORS-HOMA-IR, HS-CRP AND FIBRINOGEN IN PRE-MENOPAUSAL WOMEN WITH POLY CYSTIC OVARIAN SYNDROME (PCOS)

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BACKGROUND: Polycystic ovarian syndrome (PCOS) is one of the commonest endocrinopathies in women of reproductive age. We aimed to establish coronary risk factors in pre-menopausal Indian women with Poly-cystic ovary syndrome (PCOS).

METHODS: The study was conducted on 150 females (100 PCOS and 50 controls) of Indian origin from reproductive age (25-45 years). Both the study groups were examined and analysed for anthropometric, clinical and biochemical parameters. The observations were subjected to statistical analysis using the descriptive statistics (mean±SD), chi-square test, t-test etc. Pearson's coefficient helped assess the correlation of the various parameters. Multiple regression analysis was used to identify the strongest coronary risk factor.

RESULTS: Women with PCOS were obese (26.65±4.69 v/s 26.10±4.48, p=0.493 NS), hypertensive (130.89±7.63 v/s 123.3±8.02, p<0.001), had hyperinsulinemia (29.02±8.85 v/s 19.46±10.80, p<0.001), insulin resistance (6.99±3.10 v/s4.73±3.66 p<0.001), dyslipidemia, and had raised luteinizing hormone (LH) (7.52±2.39 v/s 6.18±1.29, p<0.001), fibrinogen (215.7±58.87 v/s 182.3±14.61, p<0.001) and high sensitivity C - reactive protein (hs-CRP) (2.27±0.95 v/s 2.17±0.3, p=0.74). Metabolic syndrome was observed in 66% of the women with PCOS. There was a strong association observed between central adiposity and serum insulin (r=0.22; p=0.007) and between coronary risk factor HOMA-IR and BMI, Waist to Hip ratio, hypertension and pro-thrombotic marker fibrinogen and inflammatory marker hs-CRP. Multiple regression analysis showed HOMA-IR (p<0.005) to be the strongest cardiovascular (CVD) risk factor in these women.

CONCLUSIONS: We conclude that obesity in PCOS enhances the CVD risk due to raised fibrinogen, hs CRP and insulin resistance and possibly lead to CVD complications in later life.

Endocrinology

Cod: 0603

DETECTION OF MACROPROLACTIN IN HYPERPROLACTEMIC SERA: STEP TOWARDS CLINICAL EFFICIENCY AND COST EFFECTIVENESS

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BACKGROUND: It has been shown that macroprolactin (MaPRL) is biologically inactive because the large molecular size of this complex prevents its crossing through the capillary blood barrier and reaching target cells. The aim of this study is to detect the frequency of MaPRL in hyperprolactinaemic patients by using PEG precipitation method and to report the post-PEG treated results in order to facilitate interpretation of the result, provide more clinically useful information, and enhance patient care. This will have overall clinical impact on diagnosis, management and cost effectiveness proving to be a beneficial quality improvement initiative.

METHODS: A prospective study was conducted at the Section of Chemical Pathology, Department of Pathology and Microbiology Aga Khan University (AKU) between 5th to 20th Oct 2013. During this period total 4375 patients were tested for serum prolactin levels. From this, subset of 100 randomly selected subjects with high prolactin levels i.e. from 25-150 ng/ml in females and 15-150 ng/ml in males were selected for PEG precipitation. PEG precipitation was performed by PEG 8000 and results were interpreted by using post PEG treated reference range of Males 3.6–12.4 ng/ml and Females 4–18.5 ng/ml. Data was analyzed by SPSS version 19.

RESULTS: Sera from 100 patients with elevated serum PRL concentrations were screened for macroprolactinaemia by PEG. Out of which 98 were females and 2 were males. Serum prolactin levels became normal after post PEG treatment in 52% individuals both males included suggestive of macroprolactin while it remained elevated in 48% individuals demonstrating true hyperprolactinemia.

CONCLUSIONS: A significant number of subjects are found to have high serum prolactin levels due to MaPRL, which is a significant cause of misdiagnosis, unnecessary investigation, and inappropriate treatment. We recommend treatment of high prolactin results with PEG for accurate results and enhancing clinical efficiency and cost effectiveness for providing quality care to the patients.

Endocrinology

Cod: 0604

HYPOTHYROIDISM ONE OF THE FACTORS WITCH MAY AFFECT FERTILITY

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BACKGROUND: Hypothyroidism as one of the factors that affect fertility in women act in a way that increases the level of serum prolactin. Reduced secretion of thyroid hormones leading to increased secretion of TSH from the thyrotrophs in pituitary gland. So it stimulates increased secretion of TRH from the hypothalamus, which is a strong prolactin - stimulating factor, which through their metabolic pathways via phospholipase C activates voltage -sensitive calcium channels that enhance exocytosis of prolactin from laktotrophs. Hyperprolactinemia inhibits the secretion of gonadotropin releasing hormopnot (CnRH) from the hypothalamus, which decreased excretion leads to decreased concentrations of FSH and LH in serum and insufficient creation of ovarian follicles, which secrete reduced amounts of estradiol and progesterone.

METHODS: Blood was taken from hypothyroid female patients before and after therapies for hypothyroidism. It was examined the concentration of serum TSH level, and prolactin levels, gonadotropins FSH and LH levels and ovarian steroids: estradiol and progesterone.

RESULTS: Patients before treatment has increased concentration of TSH and prolactin, while FSH, LH, estradiol, and progesterone were decreased. After treatment the serum TSH and prolactin levels has decreased TSH, while concentrations of FSH, LH, estradiol, and progesterone increased.

CONCLUSIONS: Thyroid replacement therapy removes and improves fertility in women with hypothyroidism.

Endocrinology

Cod: 0605

EFFECTS OF HYPERTHERMIC STRESS IN DIFERENT DEVELOPMENTAL STAGES IN WHITE RAT ON THE TABLE

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BACKGROUND: High environmental temperature of 40°C, applied in different developmental stages in White laboratory rat, is a stress on the body that load the adrenal glands. In terms of hypertermic stress adrenal glands work faster, producing increased amounts of adrenaline, noradrenaline and cortisol. In terms of long-term stress, and load of the glands are entering a phase of temporary hypofunction.

METHODS: We were examined the effect of hyperthermic stress in the White laboratory rat strain Wistar, the temperature of 40°C, applied for two hours daily. The animals were divided into five groups: control, which resides at room temperature, exposed during pregnancy, exposed during lactation in exposed after the period of lactation and exposed continuously from pregnancy until the 50th day of life. After the sacrifice of young animals examined were the mass, total protein, content of DNA, and RNA content of the adrenal glands .

RESULTS: Hyperthermic stress applied during pregnancy is not or has little effect on the parameters examined, because of the protective effect of the placenta. During lactation hyperthermic stress causes the adrenal gland hyperplasia, that is why it comes to increasing the value of all tested parameters. Hyperthermic stress applied after the period of lactation doesn't cause special effects on the examined parameters, because the body is recovering and the values are almost back to those values in the control group of animals. Continued hyperthermic stress (animals exposed from pregnancy until the 50th day of life) causes a significant increased values of all examined parameters, because of the increased levels of ACTH and cortisol in serum.

CONCLUSIONS: During pregnancy hyperthermic stress has a small effect, but it is much higher during lactation. This is due to hyperplasia and adrenal hyperfunction. Due to continuous hyperthermic stress applied during the entire experimental period comes to a significant increase in all parameters examined for irreversible impairment of the function of the adrenal glands (chronic hypofunction).

Endocrinology

Cod: 0606

IMPACT OF SHORT-TERM HYPOTHYROIDISM AFTER LEVOTHYROXINE-WITHDRAWAL ON N-TERMINAL PRO-A-TYPE AND N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDES IN PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA

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BACKGROUND: Acute hypothyroidism after levothyroxine-withdrawal in patients with DTC may induce untoward cardiovascular effects. This study investigates the effects of short-term levothyroxine-withdrawal after total-thyroidectomy on N-terminal pro-A-type natriuretic peptide (NT-proANP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels in patients with DTC, as compared with controls and with the same patients evaluated during thyrotropin (TSH) suppressive therapy with levothyroxine.

METHODS: Serum concentrations of TSH, NT-proANP and NT-proBNP were measured in 51 female patients with DTC (48.7±4.2 years) at three time points: 4 weeks after levothyroxine-withdrawal, before radioiodine (131I) therapy (T1-under acute hypothyroidism), 5 days after 131I (T2-under acute hypothyroidism) and 12 weeks after 131I (T3-under TSH suppression). Thirty healthy euthyroid women served as controls (42.8±5.6 years).

RESULTS: At T1/T2/T3, median (IQR) TSH levels were 81.1(64.1-99.6)/79.4(62.3-98.3)/0.2(0.1-0.2) mU/L in patients group vs. 2.1(1.8-2.5) mU/L in control group (P<0.001), median (IQR) NT-proBNP levels were 50.1(12.8-88.9)/36.5(2.8-68.4)/79.5(62.5-101.3) pmol/L in patients group vs. 64.5(59.6-87.4) pmol/L in control group (P<0.001) and median (IQR) NT-proANP levels were 5.2(2.1-32)/1.7(1.1-16)/487(133-691) pmol/L in patients group vs. 297.7(198.7-411.3) pmol/L in control group. At T1/T2/T3, the median (IQR) of NT-proBNP/NT-proANP ratios were 4.8(1.2-14.4)/5.5(0.7-31.2)/0.2(0.1-0.5) vs. 0.24(0.2-0.3) in control group (P<0.001).

CONCLUSIONS: Short-term hypothyroidism after levothyroxine-withdrawal and 131I therapy induced deleterious effects on natriuretic peptides profiles. NT-proANP and NT-proBNP reduced activity may increase the risk of developing arterial hypertension due to the sodium retention and vasoconstriction and can impair quality of life. The NT-proBNP/NT-proANP ratio during short-term acute hypothyroidism and 131I therapy was very high and corresponds to pathological states with cardiac dysfunction. Though these natriuretic peptides alterations are mostly reversible after restoration of euthyroidism by levothyroxine-therapy, over time these hormone fluctuations may lead to cardiovascular diseases.

Endocrinology

Cod: 0607

TESTOSTERONE DISTURB THYROID HOMEOSTASIS IN MIDDLE-AGED MALE RATS

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BACKGROUND: Testosterone replacement therapy has significant benefits in some aspects of lipid profile, prevention of atheromas deposition, coronary vasculature and in exercise performance in men with heart failure. Endogenous androgens and ageing are important, yet poorly defined factors in regulation of thyroid function. In this study we examined the effects of testosterone treatment on serum TSH levels in orchidectomized (Orx) middle-aged male rats, which are experimental model of andropause.

METHODS: Sixteen month old Wistar rats were subcutaneously administrated with 0.5 mg/kg of testosterone propionate (TP; n=6) every day for three weeks. The control sham-operated (n=6) and Orx (n=6) groups received vehicle (sterile olive oil) alone. Twenty four hours after the last treatment all animals were decapitated, their blood was collected from the trunk, and serum prepared according to standard procedures. Serum TSH was determined by rat RIA, and total T4 by ECLIA.

RESULTS: Orchidectomy of middle-aged males decreased body weight gain by 7% ($p<0.05$), while subsequent TP treatment reversed this change to the values obtained for SO group. Orx did not affect serum TSH and total T4 levels, while subsequent testosterone treatment increased serum TSH by 11%, $p<0.05$, and decreased serum T4 by 33%, $p<0.05$, in comparison with the Orx control value.

CONCLUSIONS: Our findings provide evidence that castration at middle age had no disturb thyroid homeostasis, while subsequent testosterone administration decreased serum T4 level. This decrease probably consequently increased serum TSH.

Endocrinology

Cod: 0608

THE RELATIONSHIP BETWEEN ANTI-MüLLERIAN HORMONE (AMH) CONCENTRATION AND OLIGO/AMENORRHOEA IN SUBFERTILE WOMEN WITH POLYCYSTIC OVARIAN MORPHOLOGY

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BACKGROUND: Serum anti-Müllerian hormone (AMH) concentration is related to hyperandrogenism (HA) and oligo/amenorrhoea (OA) of polycystic ovary syndrome (PCOS). Objectives of this study were firstly to determine whether AMH is predominantly associated with OA or HA in subfertile women diagnosed as having PCOS according to the Rotterdam criteria (RC) and secondly to determine whether AMH association with the number of follicles measuring 2-9 mm in diameter (AFC) is independent of other clinical and biochemical characteristics that define PCOS.

METHODS: A total of 233 subfertile women with polycystic ovarian morphology (PCOM) who met the RC criteria for PCOS were divided into phenotype groups as follows: PCOS A = PCOM+HA+OA, n=90; PCOS C = PCOM+HA, n=51; PCOS D = PCOM+OA, n=91. PCOM was defined as the presence of >11 AFC in at least one ovary. HA was defined as testosterone (T) concentration >2.8 nmol/L and/or modified Ferriman-Gallwey score (mFG) >7. OA was defined as menstrual cycle length (MCL) >35 days. Kruskal-Walis test with post-hoc analysis for pair wise comparison was used to determine the between-group difference in AMH and other patient characteristics. The relationships between AMH and patient characteristics that define RC were assessed by multiple regression analysis (MRA). P-value <0.05 was considered as statistically significant.

RESULTS: Significant difference in AMH was found when two hyperandrogenic PCOS phenotypes differing in MCL (PCOS A vs PCOS C) were compared to each other (P < 0.001). In contrast, the AMH values of the two oligo/anovulatory PCOS phenotypes (PCOS A vs PCOS D) did not differ despite the difference in the signs of HA (P = 0.174). AMH was found to be associated with AFC, T and MCL, but not with mFG. MRA revealed an independent association between AMH and AFC. AMH was included together with MCL, T and mFG in the AFC prediction model explaining 36.9% of the variance of AFC.

CONCLUSIONS: OA seems to be the PCOS feature predominantly associated with the AMH concentration in PCOS women having PCOM. The antral follicle accumulation in PCOS could be partially explained by an androgen-independent role of AMH.

Endocrinology

Cod: 0609

PROLACTIN AND REPRODUCTIVE HORMONE STATUS IN OLIGOMENORRHEIC AND INFERTILE FEMALES

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BACKGROUND: Oligomenorrhea is one of the significant problems of female 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 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. Hormonal assay were measured by ELISA method (Eliscan, India). Data were analyzed using SPSS ver. 20, p-value <0.05 was considered significant.

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.. The median IQR values of Prolactin, FSH and LH of 96 oligomenorrheic women were 697.94 (500.00;919.20) mIU/mL, 8.49(6.22;15.05) mIU/mL and 6.39(3.62;15.30) mIU/mL respectively. Likewise in 30 patients of infertilie women, median and interquartile range of Prolactin, FSH and LH were 783.51(515.57;988.60)mIU/mL,11.01(8.1;60.6)mIU/mL and 6.34 (3.5; 27.11) mIU/mL respectively. Prolactin and LH values were not significantly different among the oligomenorrhea and infertility group (p=0.043).

CONCLUSIONS: Our study showed that there was no remarkable difference of serum Prolactin and LH between oligomenorrheic and infertile women.
Keywords: Oligomenorrhea, infertility, Prolactin, FSH,LH

Endocrinology

Cod: 0610

PREVALENCE OF OVARIAN CYST WITH HYPERPROLACTINEMIA IN DYSMENORRHIC PATIENTS

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BACKGROUND: Dysmenorrhoea apart from being distressing may be associated with pathologies and hormone imbalance. These patients were examined for ovarian cyst, galactorrhoea, prolactin and TSH levels. Hormonal irregularity of FSH and LH levels causes formation of ovarian cyst, but its association with hyperprolactinemia and dysmenorrhoea have not been studied.

METHODS: 250 patients with primary dysmenorrhoea visiting the OPD of Obstetrics & Gynecology Dept. of tertiary care hospital were enrolled for the study. 250 age matched females without dysmenorrhoea and any gynecological complaints were taken as controls. Detailed history of dysmenorrhoea, menstrual cycle and breast discharge was taken. All the patients were assessed for pain on VAS scale and effect on daily activity and analgesic requirement was noted. TSH and prolactin levels were analyzed by chemiluminescence on cobas 2010. Sonography of pelvis was done for pelvic pathology like ovarian cyst. Patients with raised levels of PRL were put on Cabgoline 0.25mg biweekly along with medroxyprogesterone 10 mg /d for 10 days in luteal phase for 12 weeks and those with high TSH were put on eltroxin. After treatment repeat testing of PRL, TSH and ultrasound pelvis for resolution of cyst was done.

RESULTS: The mean age of presentation was 21.58 ±4.68 years. Study group patients had significantly higher incidence of galactorrhoea (30% vs 10%) and hyperprolactinemia (20% vs 5%) compared to control group. Incidence of hypothyroidism was also higher (10% vs 3%). Ovarian cyst of 5cm was noted in 22.5% patients. After treatment dysmenorrhoea and ovarian cyst resolved in 99% and 20% patients respectively.

CONCLUSIONS: Significant number of dysmenorrhoea patients presented with breast discharge, ovarian cyst and high levels of TSH and PRL, which resolved after treatment. So all dysmenorrhoea patients should be investigated for hormone imbalance and get appropriate treatment for it, as it might affect their fertility.

Endocrinology

Cod: 0611

COMPARISON OF BLOOD COLLECTION TUBES CONTAINING K3EDTA PLUS APROTININ FOR THE PRESERVATION OF ACTH LEVEL

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BACKGROUND: The susceptibility of plasma adrenocorticotropic hormone (ACTH) to enzymatic degradation has limited the stability of ACTH. This study examined if a proteinase inhibitor, aprotinin, mixed with blood at the time of collection, would change the stability of ACTH in plasma at various temperatures for several hours and days using immunoassay.

METHODS: Sequential human blood samples were collected from 22 patients in EDTA collection tubes and EDTA plus aprotinin tubes, respectively. Plasma obtained was stored for 4 days at 4°C and on the other hand a second aliquot at room temperature. ACTH was measured immediately and after storage times of 1, 2, 4, 24 h, and 96 h at 4°C and other aliquots at room temperature.

RESULTS: Aprotinin plus K3EDTA-plasma samples centrifuged and stored at 4°C showed the best stability of ACTH up to 96 h compared to the ACTH concentration collected into K3EDTA tubes. Also, aprotinin plasma stored at room temperature showed no significant change according to baseline values. ACTH in plasma samples without aprotinin centrifuged and stored at 4°C declined in 2 h.

CONCLUSIONS: The addition of aprotinin showed an improvement of the stability at 4°C/RT. These data indicate that aprotinin has a profound preservative effect upon plasma ACTH level and that it may be possible to submit unfrozen samples collected with this inhibitor to appropriate reference laboratories for analysis of ACTH immunoassay. From a practical standpoint, reference laboratories may wish to prepare collection tubes containing both EDTA and aprotinin for distribution to other laboratories.

Endocrinology

Cod: 0612

COMPARISON OF DETERMINATION OF ALDOSTERONE BY THE CHEMILUMINISCENCE LIAISON® ALDOSTERONE ASSAY WITH THE RADIOIMMUNOASSAY

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BACKGROUND: The fully automated DiaSorin LIAISON® platform was developed for reliable detection of aldosterone (serum, plasma, urine). We compared determination of aldosterone in serum, plasma, urine by the different methods, the DiaSorin LIAISON® (chemiluminescence immunoassay - CLIA) and RIA (radioimmunoassay).

METHODS: The comparison of results of aldosterone levels serum and urine by two analytical methods (RIA and CLIA) and the comparison of aldosterone concentration in serum vs in plasma using RIA and in serum vs in plasma using CLIA. The series included samples of aldosterone in serum and urine with concentration 26.4 – 678.0 ng/ml (for serum) from subjects > 20 years of age. These samples were routinely analyzed by the RIA. The samples were stored at -20oC until the measurements not later than one month. Aldosterone was determined using the ALDOSTERONE RIA kit, (IMMUNOTECH a Beckman Coulter, France), for CLIA we used the LIAISON®Aldosterone assay (DiaSorin Inc–Stillwater–USA). The statistical analysis was conducted using MedCalc, version 4.31.010. The required values of Pearson's correlation coefficient rP were > 0.90.

RESULTS: The results of statistical comparison of aldosterone concentration in serum using CLIA and RIA were n = 69, p = 0.05, rP=0.934, y (CLIA) = 0.573 (RIA) + 38.42. The results of statistical comparison of aldosterone concentration in urine using CLIA and RIA were rP = 0.962 n = 23, p = 0,05, y (CLIA) = 0.771 (RIA) + 215.6. Furthermore, we used both methods to determine the correlation between the serum and plasma levels of aldosterone: RIA (n = 45, p = 0.05, rp=0.979, y (plasma) = 1.08 (serum) + 13.1); CLIA (n = 39, p = 0.05, rP = 0.962, y (plasma) = 1.070 (serum) – 4.57).

CONCLUSIONS: Our data demonstrate that aldosterone concentrations determined using CLIA correlate with aldosterone concentrations established using RIA. The correlation Pearson's coefficients (rP) between these two methods were > 0.93. Fully automated LIAISON® Aldosterone assay is due to its reduced TAT (Turn around time) when compared to RIA meeting current laboratory needs.