Prenatal and postnatal testing

T185

ANALYTICAL PERFORMANCE OF NOVEL ASSAY FOR MEASURING SEX HORMONE-BINDING GLOBULIN (SHBG) USING FLUORESCENCE IMMUNOASSAY BY AIA-2000 ANALYSER AND COMPARISON WITH CHEMILUMINISCENCE IMMUNOASSAY PERFORMED BY ARCHITECT I1000 ANALYSER

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BACKGROUND-AIM

SHBG is a glycoprotein synthesized in the liver and released into the bloodstream. It has a high affinity for steroid hormones especially testosterone and estradiol. It is involved in transport of these sex steroids in plasma and to the target cells. Due to the high binding affinity of SHBG to steroids, it will limit the access of these steroids to their target cells. SHBG is widely measured together with testosterone for calculation of "Free androgen index" (FAI), which is used as indicator of abnormal androgen status.
In this study we report the results of a study evaluating the analytical performance of the new assay ST AIA-Pack SHBG for measuring SHBG in human serum on AIA-2000® (Tosoh Bioscience) and comparison with other commercial method SHBG assay on Architect i1000® (Abbott Diagnostics). The aim of our study was to evaluate analytical performance of this new method to introduce method on group of patients from in vitro fertilisation (IVF) clinic.

METHODS

We evaluated total imprecision, accuracy, reproducibility and uncertainty for each assay using patient serum pools, patient samples and controls. The method comparison study of SHBG assays was performed on group of patients (n=143), who underwent IVF procedures, with the AIA-2000 and ARCHITECT i1000 analysers.

RESULTS

For novel SHBG assay we determined levels for our patients and we divided them to the groups based on age and sex. Total CVs determined for intraday repeatability of the assay ranged from 2,7 to 3,6 % and interday reproducibility ranged from 2,5 to 2,6 %. The results of SHBG measured by the AIA-2000 has excellent correlation with Architect i1000 ($r = 0.988$) with a regression equation of $y_{(AIA)} = 1{,}03\ x_{(Architect)} - 0{,}62$.

CONCLUSION

The novel SHBG assay fulfil the spectrum of fertility tests for measuring in in vitro fertilisation centres. The method has very good analytical performance, excellent correlation with other established method for measuring SHBG and can be used for routine measurement and for calculating of FAI.

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T186

MEDIANS FOR MATERNAL SERUM UNCONJUGATED ESTRIOL DURING NORMAL PREGNANCY IN RUSSIAN POPULATION WITH COMPETITIVE ELISA

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BACKGROUND-AIM

Unconjugated estriol (uE3) is the major estrogen formed by the fetoplacental unit during pregnancy. Maternal serum uE3 levels have been recommended to monitor fetal status. Determination of the normal range limits plays an important role in this process.

METHODS

Serum concentrations of uE3 were measured using a quantitative competitive solid phase enzyme immunoassay (DS-EIA- free Estriol).

RESULTS

Maternal serum uE3 values from 323 unaffected, singleton white pregnancies (Central Russia and Volgo-Viatsky Region, mean age 26 years) were ranged according to the gestational week from 4 to 37. Medians and multiples of medians (MoM) were calculated for every gestational week. Normal range limits (0.5 MoM-2 MoM) for gestational weeks from 12 to 33 are represented:
12 gestational week - median 1.3ng/ml, normal range from 0.6 to 2.6 ng/ml;
13 gestational week - 2.4 ng/ml, from 1.2 to 4.8 ng/ml;
14 gestational week - 3.3 ng/ml, from 1.6 to 6.5 ng/ml;
15 gestational week - 4.2 ng/ml, from 2.1 to 8.4 ng/ml;
16 gestational week - 4.3 ng/ml, from 2.2 to 8.6 ng/ml;
17 gestational week - 4 ng/ml, from 2 to 8 ng/ml;
18 gestational week - 5 ng/ml, from 2.5 to 10 ng/ml;
19 gestational week - 6 ng/ml, from 3 to 12 ng/ml;
20 gestational week - 6.8 ng/ml, from 3.4 to 14 ng/ml;
21 gestational week - 6.1 ng/ml, from 3 to 12 ng/ml;
22-23 gestational week - 7.3 ng/ml, from 3.6 to 15 ng/ml;
24-25 gestational week - 7.9 ng/ml, from 4 to 16 ng/ml;
26-27 gestational week - 11.3 ng/ml, from 5.6 to 23 ng/ml;
28-29 gestational week - 10.9 ng/ml, from 5.4 to 22 ng/ml;
30-31 gestational week - 12.7 ng/ml normal range from 6.3 to 25 ng/ml;
32-33 gestational week -13.6 ng/ml, normal range from 6.8 to 27 ng/ml.

CONCLUSION

The normal limits of maternal serum uE3 for Central Russia population with DS-EIA-Estriol free were defined. uE3 levels increase gradually during pregnancy and most rapidly in the third trimester. As the normal ranges for uE3 are very wide, it is recommended to monitor each patient for establishment of individual trend.

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T187

INDUVIDUAL RISK RATIO EFFECT OF SAMPLE STORAGE OF AFP, β-HCG AND E3 VALUES WITH SIEMENS IMMULITE AND BECKMAN DX DEVICE

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BACKGROUND-AIM

Triple test; a screening test used in the prenatal diagnosis of chromosomal defects such as Trizomi18, 21 (Down syndrome) and neural tube defects diseases (NTD). Purpose of the study to examine the impact of individual risk results of sample storage conditions and different devices for triple test.

METHODS

Serum samples were divided 4 section to work AFP, β-hCG and E3 tests. The aliquoted samples were studied the same day (1st day), 2nd day waiting in the refrigerator (+4C) and the freezer section of the refrigerator waiting 7th day (-20 C). Each sample were studied beckmandx 800(device 1) and Siemens IMMULITE 2000(device 2) devices separately. MoM values of AFP, β- Hcg E3 tests and individual risk of the patient were calculated using computer programs (PRA; Prenatal Risk Calculation, Benetech Software, Toronto and Prisca 4.0; Prenatal Risk Calculation, TYPOLOG Software / GmbH, Hamburg, Germany).

RESULTS

No significant difference was between both the device of the MoM measurement of β-hCG and E3 in the 1st day and the 2nd day results (device 1 $p = 0.95$ and $p = 0.09$, device 2 $p = 0.32$, $p = 0.27$ respectively). But there was found significant difference in the MoM values of AFP ($p < 0.05$). There was a significant difference between the two devices of MoM values of E3, β-HCG and AFP tests ($p < 0.05$). Although there were significant differences in analytical variation, there were no differences between the two devices, ($p = 0.58$, $p = 0.59$, $p = 0.33$, $p = 0.65$),when the individual risk of the patient assessed.

CONCLUSION

Stand by time of samples influence preanalytical systems of triple test results. Down syndrome who are not close the individual risk limits was assessed to have no effect on the individual risk for patients of the different analytical performance.

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T188

SCREENING FOR CHROMOSOMAL ABNORMALITIES IN THE FIRST TRIMESTER OF PREGNANCY USING ULTRASOUND AND MATERNAL SERUM BIOCHEMISTRY. A REVIEW OF THREE YEARS' EXPERIENCE.

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BACKGROUND-AIM

The first-trimester prenatal screening is a combination of tests performed during the first trimester of pregnancy. This approach combines biochemical analysis of maternal serum and fetal ultrasonography. It allows calculating the risk of a fetus of presenting the most frequent chromosomal abnormalities (i.e. Down's syndrome and Edwards syndrome) through multivariant statistical analysis. The aim of this study is to evaluate the accuracy and reliability of the one-step multidisciplinary clinical screening for fetal chromosomal anomalies in the first trimester of pregnancy supplied by our hospital.

METHODS

During a three-year study 5798 serum samples from pregnant women have been studied. PAPP-A and beta-hCG have been determined by chemiluminescent immunoassay. Ultrasonography has been performed to all patients. PRISCA 4.0 program has been used to estimate syndromes risks using the biochemical and sonographic data. A cut-off of 1/250 for Down's syndrome and 1/100 for Edward's syndrome risk has been set up in this study. Statistical analysis was performed.

RESULTS

Patients studied: 5798 singleton pregnancies. Down's syndrome: detection rate of 84,21 % at a false-positive rate of 3,44%. Edwards syndrome: detection rate of 100 % at a false-positive rate of 0,62%. Aneuploidies (overall): detection rate of 89,66% at a false-positive rate of 3,61%.

CONCLUSION

The results obtained are comparable to those previously published in the literature. The first trimester combined screening test appears as an efficient tool that reduces the number of invasive diagnostic tests, due to its high sensitivity, specificity and negative predictive value.

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T189

ALPHA-FETOPROTEIN IN AMNIOTIC FLUID AS A BIOCHEMICAL MARKER OF NEURAL TUBE DEFECTS AND OTHER FETAL ABNORMALITIES.

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BACKGROUND-AIM

The role of Alpha-Fetoprotein (AFP) concentrations in amniotic fluid as a diagnostic test in neural tube defects (NTD) has been repeatedly questioned, and not widely assessed in the Spanish population. Our objective was to assess the value of AFP in amniotic fluid as a marker for NTD and other fetal abnormalities.

METHODS

A review of all the amniocentesis performed in our centre in 2011-2013 was conducted, registering for every case maternal characteristics, as well as AFP concentrations and pregnancy outcome. AFP concentrations in normal pregnancies were used to calculate the gestational-specific medians (15-22 weeks); no medians were calculated for gestational ages >22 weeks as there were few pregnancies with normal outcomes in our sample. Multiples of the median (MoMs) for AFP concentrations were calculated for all the cases. Elevated AFP cases were classified according to whether or not the elevated AFP was incidental or central to the fetal abnormality identification, considering if the ultrasonographic test had already revealed an abnormality.

RESULTS

755 pregnant women were included in the study, with a mean age of 34.2 years (16- 45 years) and a mean gestational age of 18.3 weeks (15-30 weeks). 513 of these had normal pregnancy outcomes. Only 26 cases (3,4%) had AFP concentrations >3 MoMs; 12 NTD cases, 5 cystic hygromas, 2 gastroschisis, 3 severe intrauterine growth restriction cases, 2 fetal demise cases, 1 Edward's syndrome case and 1 normal case. There were other 9 cases of NTD, all with significantly elevated AFP concentrations, but MoMs could not be calculated because the gestational age was >22 weeks. All the NTD, cystic hygromas and gastroschisis cases showed >3 MoMs AFP concentrations, and all had been also previously diagnosed by ultrasonographic examination. Neither elevated (>3 MoMs) nor diminished (<0.5 MoMs) AFP concentrations proved to be useful to detect any other fetal abnormalities included in our sample.

CONCLUSION

Significantly elevated AFP concentrations in amniotic fluid are observed in NTD and other fetal abnormalities, but its measurement to rule out or confirm a NTD case does not seem justified in centres with proved expertise in targeted ultrasonographic testing.

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T190

THE ANALYSIS OF AMNIOTIC FLUID USING FLUORESCENT METHOD

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BACKGROUND-AIM

Amniotic fluid represent a stagnant pool, approximately circulating with a turnover time of one day. Adequate amniotic fluid volume is maintained by a balance of fetal fluid production and resorption. The chemical composition of its substances varies with gestational age and it is connected with different biochemical functions and participate in several metabolic processes in the body. Fluorescent spectroscopy is shown to be very sensitive and effective method for study of molecular interaction. The fluorescent properties of the amniotic fluid are investigated to determine spectral parameters that can be used in diagnosis. Results of our research using fluorescence spectroscopy can be helpful for screening of fetal malformations.

METHODS

50 samples of amniotic fluid (17 – 24 gestation week) with no visible traces of blood were collected according clinical indications by amniocentesis. The samples were centrifuged for 5 min at 3000 rpm and stored at -80°C. Fluorescent fingerprints were measured (250-650 nm, $\Delta\lambda$ = 30 nm) using Perkin-Elmer, Model LS 55 Luminescence Spectrometer, and quartz cuvette (QS, 1cm) at ambient temperature. Individual measurements were graphically processed into a three-dimensional contour synchronous fluorescent fingerprints maps using software WinLab.

RESULTS

Amniotic fluid has intensive fluorescence. The region 280 nm is characteristic for fluorophores of proteins and aromatic amino acids. The fluorescent peak detected in the range λex=340-360 nm and λem=450 nm is specific for endogenous cofactor NADH+H+. Compounds with the fluorescent maxima at λex=450 nm; λem=520 - 560 nm are connected with the presence of some pigments (bilirubin connected with protein) in amniotic fluid. Both emission spectra and excitation spectra of long-wave ($\lambda > 450$ nm) fluorescence of amniotic fluid which are connected with prenatal abnormal developments of a fetus (anencephaly and spina bifida) were not observed in our samples due to the fact that samples were collected from the women with no confirmed fetus defect.

CONCLUSION

Fluorescence is non-invasive, very fast and simple method, which can be useful in prenatal diagnosis. Fluorescence properties of the amniotic fluid are investigated to determine spectral parameters that can be used to reveal pregnant women with a high risk of congenital malformations of their offspring's.

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LUNG MATURITY ASSESSMENT IN NEONATAL GASTRIC ASPIRATE BY BIOCHEMICAL AND BIOPHYSICAL INVESTIGATION

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BACKGROUND-AIM

The optimal approach to detection of alveolar surfactant deficiency in prematurely born infants at birth remains unclear and the decision to apply exogenous surfactant is based mainly on the clinical and radiological signs of neonatal respiratory distress syndrome (NRDS).
The aim of the present study was to estimate the lung surfactant maturity by analyzing biochemical and biophysical properties of gastric aspirates (GA) from infants with NRDS and healthy full term infants, and to find an approachable method for assessment of surfactant maturity at birth.

METHODS

The study included forty-seven infants divided into two groups: 34 full-term healthy and 13 prematurely born infants developing clinical signs of NRDS and treated by assisted ventilation and exogenous surfactant. A biochemical analysis of the protein and lipid content of GA collected at birth was performed. The surface characteristics (equilibrium, maximal and minimal surface tension) were measured by the pending drop method.

RESULTS

The mean phospholipids' concentration in GA of the premature infants was lower (295.7 vs.374.5 μg/ml) than in the term infants.The mean protein content was less in GA of the premature babies than the term newborns (574.5 vs.641.5 μg/ml). The dynamic surface characteristics showed significantly higher mean values of the minimal surface tension in the premature infants, 20.5m/Nm compared to the term babies, 12.3 mN/m ($p<0,01$). There was no significant difference between the equilibrium and maximal surface tensions values of both groups.

CONCLUSION

Our findings revealed lower phospholipid and protein concentrations in GA from premature infants as compared to the healthy term infants. The dynamic surface characteristics of GA differed in the two groups, the minimal surface tension being the most important parameter for evaluation of surfactant maturity. Our results could find application into the clinical practice for fast surfactant maturity diagnostics in prematurely born children regarding lifesaving therapy with exogenous surfactants administration.

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T192

CENTER-SPECIFIC MEDIANS FOR FREE ß-HUMAN CHORIONIC GONADOTROPIN AND PREGNANCY-ASSOCIATED PLASMA PROTEIN-A IN FIRST TRIMESTER RISK CALCULATION FOR TRISOMY 21

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BACKGROUND-AIM

Reliable risk calculation for trisomy 21 in first-trimester screening depends on good estimates of the medians for fetal nuchal translucency thickness (NT), freeß-subunit of human chorionic gonadotropin (fß-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in maternal plasma from unaffected pregnancies. The concentrations of fß-hCG and PAPP-A greatly depend on gestational age and are therefore expressed in gestational age–adjusted multiples of the median (MoM) in unaffected pregnancies for risk calculation. Ideally, each center should establish its own medians. The purpose of our study was to establish our center-specific medians and to compare with the commercial software medians.

METHODS

Data from 819 normal singleton pregnancies between 10 and 14 gestational weeks were retrieved. Median MoMs of NT, fß-hCG and PAPP-A by gestational age (corrected by maternal weight, race, and tobacco) were evaluated by using commercial software medians. After calculating local medians, median MoMs of NT, fß-hCG and PAPP-A by gestational age and percentage of positive cases were determined with a cut-off of 1:250 at term.

RESULTS

Mean and standard deviation of the maternal age at expected date of delivery was 30.77 ± 5.25. By using commercial software medians, median MoMs of NT, fß-hCG and PAPP-A with 95% confidence interval (CI) were 0.91 (0.90-0.93), 1.30 (1.24-1.34) and 1.05 (0.99-1.09), respectively. Percentage of positive cases was 3.79%. After establishing our local medians, the recalculated median MoMs of fß-hCG and PAPP-A with 95% CI were 1.04 (1.0-1.08) and 1.03 (0.98-1.09), respectively and percentage of positive cases was 2.08%.

CONCLUSION

By using the default medians in the commercial software, we found that the median MoM of fß-hCG and PAPP-A were outside the acceptable limits (1MoM±10%) for most gestational weeks. After calculating our local medians, the median MoM of fß-hCG and PAPP-A were within the acceptable limits. As a result, we decided to use our center-specific medians in first trimester risk calculation for Trisomy 21

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T193

DO ASSISTED REPRODUCTIVE TREATMENTS ON PRENATAL SCREENING DURING THE FIRST TRIMESTER INFLUENCE?

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BACKGROUND-AIM

Background: The prenatal screening is a set of noninvasive tests. It allows the identification of pregnant women at risk of delivering a fetus suffering of aneuploidy, mainly Down syndrome, Patau syndrome and Edwards syndrome. To calculate this risk the following indicators are taken into account: the expectant mother´s age, the nuchal scan results and the concentration of maternal serum biochemical markers: pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin free (fb-HCG). To assess whether there is a difference in the values of PAPP-A and b-HCG during the prenatal screening from Group 0 (no fertility treatment) and Group 1 (fertility treatment), and its influence on the prenatal screening.

METHODS

Material and Methods: A retrospective study which included pregnant women that attended the San Carlos Clinical Hospital during 2012, 2013 and 2014 was conducted. The multiples of the median values (MoM) were analyzed for the the PAPP-A and fb-HCG serum concentrations of the pregnant women from Group 0 (n=1119) and Group 1 (n=170). Patients with twin pregnancies were excluded. The drug and doses to be used in the assisted reproductive treatments will vary according to the patient´s age, reproductive pathology, type of treatment (in vitro fertilization, artificial insemination, embryo transfer) and response to previous cycles.
The data statistical treatment was performed by comparing medians with the chi-square and Student´s t. SPSS18 statistical software was used.

RESULTS

Results: In determining the values of PAPP-A in maternal blood, a lower value was observed in Group 1 (median = 1.07; IQR = 0.71-1.57) compared to Group 0 (median = 1.21; IQR = 0.81-1.71). When determining fb-hCG in maternal blood a higher value was observed in group 1 (median = 1.24; IQR = 0.89-1.75) compared to group 0 (median = 1.05; IQR = 0.73-1.54). The MoM median of PAPP-A is higher in Group 1 ($p = 0.030$) and the MoM median of fb-HCG is higher in Group 0 ($p = 0.000$).

CONCLUSION

Conclusion: The differences found were statistically significant although we cannot conclude that this is clinically significant. Therefore, further studies are necessary, increasing the number of pregnant women undergoing fertility treatments to be able to accurately define the clinical relevance of such differences.

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DE NOVO X-TO-AUTOSOME TRANSLOCATION - A CASE REPORT

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BACKGROUND-AIM

X-to-autosome translocations are rare structural chromosome abnormalities, with an estimated incidence of 1-3/10000 live births. The phenotype of these rearrangements is variable, since not only depends on the breakpoints of both chromosomes, but also by the special circumstances of the phenomenon of X-chromosome inactivation.

METHODS

We described a case of a 18-year-old girl who consulted Endocrinology Service for primary amenorrhea and delayed puberty. On physical examination she shows a normal phenotype, weight 41 kg, height 159 centimeters and a BMI of 17 kg/m2 (classified as underweight). Tanner stage is categorized as P2 and S3 relating to pubic hair and breast development respectively. Referral consultation to Gynecology Service and high-resolution karyotype in peripheral blood were requested.

RESULTS

Gynecological ultrasound and hormonal study were performed, both with normal results. Chromosome analysis showed 46 chromosomes and the presence of an apparently balanced reciprocal translocation between the long arms of one X chromosome and one chromosome 12, with apparent breakpoints in Xq13 and 12q24.1 respectively. Since parental karyotypes were normal, this anomaly was considered de novo: 46,X,t(X;12)(q13;q24.1)dn.

CONCLUSION

Prenatal genetic counseling is specially complicated in this type of translocations, and it varies depending on several factors: the nature of inheritance, chromosomal breakpoints, X-chromosome inactivation and/or the presence of ultrasound abnormalities. In most X-to-autosome balanced translocations the X chromosome not involved in the translocation is preferentially inactivated, while the translocated X chromosome remains active to avoid the autosomal monosomy. Carriers of X-to-autosome balanced translocations have increased risk of infertility and ovarian dysfunction because of the alteration of genes in critical regions of X chromosome. It can also lead to recurrent miscarriages or children with abnormalities due to structural or functional changes. Gynecological examination and assessment of ovarian reserve is also recommended.

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T195

EVALUATION OF CYTOKINES LEVEL AT PREGNANCY COMPLICATED BY PRENATAL HYPOXIA

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BACKGROUND-AIM

Important predetermine role in the future development of the fetus and placenta play many immune factors, including violation of the immune barrier function of the placenta, increase the permeability for the maternal immune lymphocytes that attack fetus, disruption of the interaction between antigen-recognizing structures and the system of regulatory cytokines, growth factors. We had studied the level of pro-inflammatory and anti-inflammatory cytokines in the serum of pregnant women to investigate the influence of prenatal hypoxia on the changes of the immunological status.

METHODS

Patient blood serum samples (n=90) were selected from pregnant women (gestational week 34-37) complicated by prenatal hypoxia of 1-low severity, 2–medium severity, 3–severe hypoxia. The level of IL-1β, IL-4, IL-6, TNF-α, IGF-1 was tested by ELISA (Vector-Best, Russia).

RESULTS

The results indicate a progressive increase the content of pro-inflammatory cytokines at severe hypoxia versus control group: TNF-α (p<0,0001) and IL-1β (p<0,001). Women with prenatal hypoxia had progressively lower levels of serum IL-4 (p<0,001) and IGF-1 (p<0,005). The concentration of IL-6 was different from other cytokines: reduced at low hypoxia (p<0,01) with further increase at severe hypoxia to normal ranges of the control group. The negative correlation was found between TNF-α and IL-4 in the serum of pregnant women (r=-0,82), which made it possible to use the ratio of these factors as a biomarker for the prenatal diagnosis of hypoxia.

CONCLUSION

Our studies of the immune status of pregnant women with prenatal hypoxia showed a violation of the balance of pro-inflammatory and anti-inflammatory cytokines. The ratio of TNF-α and IL-4 can be suggested as a biomarker for the prenatal diagnosis of hypoxia.

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T196

A DISINTEGRIN AND METALLOPROTEINASE DOMAIN-CONTAINING PROTEIN 12 LEVELS IN FIRST TRIMESTER PREGNANTS

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BACKGROUND-AIM

The human disintegrin and metalloproteinase domain-containing protein 12 (ADAM12) is an enzyme that cleaves IGF-BP to release IGFs, thus increasing the effectiveness of IGFs, on fetal growth during pregnancy. In this study, we aimed to compare ADAM12 levels, Plasenta Associated Plasma Protein-A (PAPP-A) and Free-beta HCG (fβ-HCG) MoM values and birth weights of babies in two pregnant groups whose trizomy 21 risk found above and under threshold level in the first trimester screening test results, and to investigate the correlations of these parameters with each other.

METHODS

Fourty pregnants who were assessed as risky and as the control group 39 pregnants who were assessed as low risky, based on the first trimester screening test results, at the total 79 pregnants were included in this study. Maternal serum ADAM12 levels were determined by ELISA; PAPP-A and fβ-hCG levels were measured by chemiluminescence method. MoM values were calculated by Prisca program.Statistical analysis of data was performed with SPSS package program.

RESULTS

ADAM12 (ng/mL), PAPP-A MoM and infant birth weight in risky pregnants were significantly lower than control group (p<0,001; p<0,001; p=0,029, respectively), fβ-hCG MoM level was significantly higher than control group (p<0,001). ADAM12 levels in group with low birth weight (LBW) babies were significantly lower than group with normal birth weight (NBW) babies (p<0,033) and fβ-hCG MoM values in group with LBW was found significantly higher than group with NBW (p<0,029). Positive significant correlation between ADAM12 concentrations and PAPP-A MoM values (r = 0,630) was found.

CONCLUSION

It was concluded that maternal serum ADAM12 levels are useful as a biomarker to predict trizomy 21 risk besides PAPP-A and fβ-hCG MoM values. In addition, serum ADAM12 levels can help to predict birth weight of babies.

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T197

BIOCHEMICAL MARKERS PREGNANCY-ASSOCIATED PLASMA PROTEIN-A (PAPP-A) AND FREE BETA-HUMAN CHORIONIC GONADOTROPIN (FREE ß-HCG) IN THE FIRST TRIMESTER OF PREGNANCY AND PREECLAMPSIA

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BACKGROUND-AIM

Background: The first-trimester combined screening test and determining biochemical markers of pregnancy-associated plasma protein-A (PAPP-A) and the free ß -human chorionic gonadotropin (free ß-hCG) is used for the risk evaluation of the development hypertensive disorders during pregnancy after 20 weeks of gestation. Preeclampsia (PE) is one of the most serious pregnancy complications and the major cause of maternal and perinatal morbidity and mortality.
Objective: The aim of this study was to present the results of serum levels of the biochemical markers (PAPP-A and free ß-hCG) in the first trimester of pregnancy and identify correlations between these biochemical markers, maternal age, BMI with PE.

METHODS

Materials and Methods: In this study were included 70 pregnant women in the two groups:
(1) preeclampsia group (N = 40), (2) control group (N = 30). Biochemical markers (free ß-hCG and PAPP-A) have been measured by ECLIA on Roche COBAS E601 analyzer. The results of serum levels PAPP-A, free ß-hCG, BMI and maternal age were compared and statistically analysed by using Excel and SPSS version 22.0. (non-parametric method of Mann-Whitney U test).

RESULTS

Results: The screening test for Down syndrome is being done between 11 and 14 weeks of gestation. In the preeclampsia group the mean values of free ß-hCG (IU/L) and PAPP-A (mIU/L) were 37,18 ± 20,64 and 2711,15 ± 1788,60 with p=0,007. The mean values of BMI were 26,18 ± 4,93 kg/m² and maternal age 31,53 ± 5,00. In the control group the mean values of free-ß hCG (IU/L) and PAPP-A (mIU/L) were 29,97 ± 10,39 and 3411,30 ± 1227,59. The mean values of BMI were 23,47 ± 4,09 kg/m² and maternal age 28,10 ± 4,57.

CONCLUSION

Conclusion: There is a significant association between low levels of serum PAPP-A and a risk for PE. Free ß-hCG wasn't significant marker for PE. Maternal age and increased BMI have confirmed risk for development PE.

Prenatal and postnatal testing

T198

STUDY OF MEDIATORS OF APOPTOSIS AT EXPERIMENTAL PRENATAL HYPOXIA

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BACKGROUND-AIM

Background: Molecular features of induction and progress of apoptosis little studied during the pregnancy. There is insufficient attention to the study of serum factors and mediators of apoptosis. The purpose of the study - a comparative analysis of the concentration of soluble forms of sFas-R, sFas-L, Bcl-2 and caspase-1 in the blood serum of rats to elucidate the role of factors of apoptosis at prenatal hypoxia.

METHODS

Methods: Blood samples were collected from 30 healthy rats and 60 rats with experimental hypoxia at 16th, 18th and 20th days of gestation. The serum Fas-receptor, Fas-ligand, Bcl-2, caspase-1 were measured by ELISA using monoclonal antibodies.

RESULTS

Results: At prenatal hypoxia the level of sFas-R increased (1565,2±24,3 vs 1506±26,76 pg/ml at 16th day, 2046,8±55,3 vs 1564,3±25,1 pg/ml at 18th day, p<0,05; 2419,6±32,6 vs 1591,6±12,62 pg/ml at 20th day of gestation, p<0,001). The content of sFas-L rised (1,48±0,01 vs 1,46±0,01 ng/ml at 16th day, 2,2±0,11 vs 1,48±0,01 ng/ml at 18th day, p<0,001; 3,0±0,07 vs 1,54±12,6 ng/ml at 20th day, p<0,001). The concentration of Bcl-2 was higher at prenatal hypoxia (122±2,47 vs 119,2±0,8 U/ml at 16th day, 133,8±3,01 vs 120,6±1,78 U/ml at 18th day, p<0,05; 153,6±1,47 vs 126,4±1,44 U/ml at 18th day , p<0,001). The level of caspase-1 also increased (122±2,47 vs 110,4±2,79 U/ml at 16th day, 133,8±3,01 vs 115,2±1,02 U/ml at 18th day, p<0,05; 153,6±1,47 vs 121,2±1,16 U/ml at 20th day, p<0,001). There was a significant positive correlation between sFas-L and stage of hypoxia.

CONCLUSION

Conclusions: The activation of apoptosis factors synthesis at prenatal hypoxia indicates the dysfunctional changes at pregnant rats with prenatal hypoxia and may play a significant role in prenatal hypoxia pathogenesis.

Prenatal and postnatal testing

T199

THE ADDITION OF ENDOGLIN AND HCG DOES NOT IMPROVE PERFORMANCE OF AN A PRIORI MULTIVARIABLE EARLY PREDICTION RISK ALGORITHM COMBINING CLINICAL CHARACTERISTICS WITH PAPP-A AND ANGIOGENIC MARKERS

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BACKGROUND-AIM

We aimed to determine the impact of Endoglin (Eng) and hCG on the performance of a multivariable model combining a priori clinical characteristics and biomarkers to detect, early in pregnancy, women at higher risk of developing preeclampsia (PE).

METHODS

This is a nested case-control study from a cohort of 7,929 pregnant women recruited between 10 and 18 weeks of gestation. 350 developed hypertensive disorders of pregnancy (HDP) of which 139 had PE, comprising 68 with severe PE and 47 with preterm PE were matched with two women with a normal pregnancy. We first selected a priori clinical characteristics and promising markers to create multivariable logistic regression models: body-mass index (BMI), mean arterial pressure (MAP), placental growth factor, soluble Fms-like tyrosine kinase-1, pregnancy-associated plasma protein A and inhibin A. We then determined if the addition of potential markers Eng and hCG improved the predictive model.

RESULTS

Main Outcome Measures: PE, severe PE, preterm PE, HDP.
At false-positive rates of 5 and 10%, the estimated detection rates of the a priori risk-model were 31 and 42%, 15 and 54%, 26 and 39%, and 32 and 43%, while they were 26 and 44%, 22 and 34%, 25 and 42%, and 26 and 44% after the addition of Eng and hCG in the model. There were no significant improvement of positive predictive values and area under the ROC curves after the addition of Eng and hCG.

CONCLUSION

The addition of Eng and hCG did not significantly improve the a priori multivariable risk algorithm combining clinical and biochemical markers. Overall, the weak performance does not justify the clinical implementation of this approach as screening test early in pregnancy in a population having similar characteristics.
Keywords: Preeclampsia, biomarkers, model, early pregnancy

Prenatal and postnatal testing

T200

HORMONE MODELLING IN PRETERM NEONATES: ESTABLISHMENT OF PITUITARY AND STEROID HORMONE REFERENCE INTERVALS.

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BACKGROUND-AIM

Immaturity of the endocrine system and its potential impact on morbidity is the subject of numerous studies. Reports suggest significant differences in serum levels of hormones in extremely preterm compared to late preterm and full term infants. The aim of this study was to develop reference intervals for three pituitary hormones and five steroid hormones in serum collected from very and extremely preterm infants.

METHODS

Blood was collected from 248 (128 male, 120 female) preterm neonates born between 24 and 32 weeks' gestation. Participants were recruited from three neonatal intensive care wards in Melbourne Australia. No infant in this cohort had ambiguous genitalia or other endocrine abnormality. All infants included in the reference interval determination survived beyond the equivalent of term. Serum was analysed for prolactin, FSH and LH by automated electrochemiluminescence immunoassay (Roche Cobas 8000-E602). LC-MS/MS was employed for analysis of 17 hydroxy-progesterone, androstenedione, cortisol, cortisone and testosterone. The robust method was applied to define the central 95% reference interval, after each hormone measure was transformed using a Box-Cox transformation to correct for asymmetry.

RESULTS

Reference intervals were established for eight hormones. Gender specific intervals were developed for FSH, LH and testosterone. Cortisone and 17-OHP required division based on gestational age, with neonates born <30 weeks' gestation demonstrating higher levels than their older counterparts. Androstenedione, cortisol and prolactin did not require any division within this cohort for reference interval assignment.

CONCLUSION

This report provides the first characterisation of serum steroids measured by mass spectrometry in preterm neonates, with the additional characterisation of three pituitary hormones in infants born ≤32 weeks' gestation. Utilisation of this data allows for correct interpretation of results for very preterm neonates and reduces the risk of incorrect diagnosis due to misinterpretation of data.

Prenatal and postnatal testing

T201

LACTOFERRIN ACCUMULATION IN FETAL INTESTINE

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BACKGROUND-AIM

Lactoferrin (LF) is a component of secondary granules of mature neutrophils. Increased faecal LF concentrations (> 7.25 μg/ g faeces) have been associated with intestinal inflammation in adults and older children. Meconium is a specific type of faeces formed by the fetus and excreted in the first 48 hours after birth. It is not a homogenous material but a series of layers formed in the intestine starting from 12 weeks of gestation.
Aim of the study was assessment for possible inflammatory condition in fetal intestine in utero by measurements of LF concentrations in all consecutive meconium portions passed by healthy neonates. The total LF content of all serial meconium portions passed by a neonate was considered to equal the amount of LF accumulated in utero.

METHODS

LF concentrations were measured using AssayMax Human Lactoferrin ELISA Kit, Assaypro LLC in homogenized portions of meconium (n=81) collected from 20 neonates. One to nine meconium portions were obtained from one neonate. The weight of a single meconium portion [g]: range (0.18–18.93), mean±SD=5.52±4.02, median=3.29. The weight of meconium filling the fetal intestine [g]: range=4.72–36.95, mean±SD=18.29±8.64, median =18.97.

RESULTS

- LF concentration [μg/g]: range=1.69–511.43, mean ± SD = 45.07±78.53, median =18.98.
- LF content of the fetal intestine [μg]: range=20.48–2749.55, mean ± SD=757.23±745.41, median=514.73.
- LF concentrations were increased in the last meconium portions passed compared to first meconium portions passed after birth (p=0.017).
- Total LF content of meconium correlated with the birth weight (r=0.47, p<0.05).
- Total LF content did not correlate with the gestational age (r=0.39, p>0.05).

CONCLUSION

- LF concentrations in 80% meconium samples exceeded the upper limit of normal for adults.
- Increased LF concentrations in meconium of healthy neonates are evidence of ‘physiological’ inflammation in the period when the intestinal barrier of the fetus is immature.
- The factors responsible for differences in LF concentrations and LF accumulation between neonates (300-fold and 50-fold respectively) remain unclear and need further studies.

Prenatal and postnatal testing

T202

DETERMINANTS OF VITAMIN D STATUS IN PREGNANT WOMEN AND NEONATES: EFFECT OF SEASON AND LIFESTYLE FACTORS

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BACKGROUND-AIM

Evidence suggests a beneficial effect of vitamin D on perinatal health but low vitamin D status is prevalent in pregnant women and neonates.
The objective was to determine the sociodemographic and lifestyle characteristics that are associated with vitamin D status of mothers in early pregnancy and neonates.

METHODS

Included were 1635 pregnant women from Quebec City and Halifax, Canada, 2002-2010. Vitamin D status was based on the concentration of 25-hydroxy-vitamin D [25(OH)D] determined with a chemiluminescence immunoassay in maternal sera collected at a median of 15 weeks' gestation and in cord sera at delivery. A questionnaire that included information on potential determinants was completed in midpregnancy. Backward stepwise logistic regression was used to identify independent predictors of [25(OH)D] <50 nmol/L and odds ratios (OR) with 95% confidence intervals (CI) were estimated. Backward stepwise linear regression was used to identify independent predictors of [25(OH)D] on a continuous scale and adjusted mean [25(OH)D] by category of the predictors in the final model was estimated.

RESULTS

Of the mothers, 732 (44.8%) had 25(OH)D concentrations below 50 nmol/L. Independent determinants of maternal [25(OH)D] <50 nmol/L included season, education, income, parity, pre-pregnancy body mass index (BMI), physical activity, and dairy product consumption. Adjusted mean maternal 25(OH)D levels were higher in summer than winter by 16.1 nmol/L (CI: 13.6, 18.7), in the highest versus the lowest category of education by 6.1 nmol/L (CI: 0.5, 11.8), in BMI <25 kg/m^2 versus BMI ≥35 kg/m^2 by 8.2 nmol/L (CI: 4.0, 12.3), and in the highest versus the lowest physical activity category by up to 9.5 nmol/L (CI: 2.9, 16.1). Supplement use was not significantly associated with maternal 25(OH)D. Cord [25(OH)D] <50 nmol/L, observed in 24.4% of neonates, had similar determinants but with the inclusion of maternal age, supplement use, and maternal [25(OH)D].

CONCLUSION

This study suggests that vitamin D status of pregnant women and/or neonates can be improved through supplementation, adequate dairy intake, moving towards a healthy pre-pregnancy body weight, and physical activity, but controlled studies are needed to determine the effectiveness of interventions aimed at these factors.
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T203

SERUM MIR-155 AS A POTENTIAL BIOMARKER OF MALE FERTILITY

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BACKGROUND-AIM

Male subfertility has been associated with low grade systemic inflammation (LGSI) as well as with androgen deficiency. miR-155 and miR-146a are central regulators of inflammation and their level in cells and in the serum has been associated with several inflammatory conditions. Aim of this study is to determine whether serum levels of micro RNAs miR-155 and miR-146a associated with male fertility, LGSI and androgens.

METHODS

In this case-control study, two independent groups of 60 subjects each (exploratory and confirmatory cohort) were randomly selected from an ongoing study on subfertile men (in total: hypogonadal; n=40, eugonadal; n=40 and control group n=39) at a University Hospital Reproductive Medicine Centre. Total RNA was isolated from cell-free serum. As internal control a synthetic miRNA, UniSp6, was added to each sample prior to extraction. miRNA expression levels were measured by real-time RT-PCR and presented as fold difference (arbitrary units, U) from control. Sera from these individuals were analyzed for hormone and cytokine levels.

RESULTS

Serum levels of miR-155 were associated with levels of miR-146a ($p<0.0001$), but only miR-146a was associated with inflammatory markers. miR-155 was strongly associated with subfertility ($p=0.0001$). Receiver operating characteristic curve (ROC) analysis indicated that miR-155 but not miR-146a can be used as a marker of sub fertility ($p<0.001$). MiR-155 with a cutoff value of 1.77 had 47% sensitivity and 95% specificity for identifying subfertility and a positive predictive value (PPV) and negative predictive value (NPV) of 95% and 47%, respectively. When used in combination with FSH, sensitivity and specificity were 80% and 100%, respectively, while PPV and NPV were 100% and 71%, respectively, those values being higher than for the FSH alone.

CONCLUSION

In conclusion, circulating miRNAs are potential biomarkers of subfertility. Our results indicate that miR-155 may be biomarker of fertility, which is independent from androgens, estrogens or LGSI markers and can potentially be used in combination with FSH.

Prenatal and postnatal testing

T204

NEW LABORATORY BIOMARKERS IN PREECLAMPSIA(PE),OUR EXPERIENCE

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BACKGROUND-AIM

There is growing evidence that angiogenic growth factors such as placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) play a signficant role in the development of preeclampsia. The aim of the study was to examine whether increased serum sFlt1 levels are related to development of PE

METHODS

Eighty five preeclamptic patients and 85 normotensive, healthy pregnant women in women in 24-28 week of singleton pregnancies were involved in this case-control study. Serum was analysed for PlGF and sFlt-1 using the Roshe-Elecsys assay to obtain a sFlt-1/PlGF ratio, according to the manufacturer's instructions

RESULTS

The sFlt-1 levels were significantly higher in the preeclamptic women, median-interquartile range 4011.2 (157.7pg/ml) than in normal controls 679.2 (140.1 pg/ml) ($p<0.001$), while the PlGF levels were significantly lower, 402 (14 pg/ml) as compared to controls 1128.6 (62.5 pg/ml) ($p<0.001$). In preeclamptic women, sFlt-1 levels were negatively correlated with the PlGF levels ($r=-0.25$, $p=0.04$). In women with preeclampsia, the median sFlt-1/PlGF ratio was significantly higher, 9.9 (0.5) compared to the control group 0.6 (0.1), ($p<0.001$). The predictive accuracy of preeclampsia was higher as denoted by greater AUC (0.901).

CONCLUSION

The sFlt-1/PlGF ratio is a better predictor than either of these parameters alone This was the first attempt to establish periodic values for preeclampsia biomarkers sFlt-1 and PIGF levels in Albanian laboratory medicine. In future these biomarkers will be the first signals for preeclampsia development and help prevent its severe forms for a better outcome for the mother and baby health.