Distribution of Biochemical and Ultrasound Markers Values in the First Trimester Screening Program in Timisoara

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The aim of our study was to analyze the distribution of biochemical markers (free- \hat{a} hCG and PAPP-A) and nuchal translucency (NT) values and their deviation from expected median in our screening program. NT, free- \hat{a} hCG and PAPP-A values were measured in first trimester sera of 1313 singleton pregnant women. The age (mean ±standard deviation) of pregnant women at the time of screening was 28.94±4.95 years and the gestational age was 87.66±4.74 days. The value of NT (median±standard error of mean) was 0.87±0.01 multiple of median corrected (MoMc), of free- β hCG was 1.07±0.02 MoMc and of PAPP-A was 1.06±0.01 MoMc. Since the huge majority of pregnant women carried a healthy fetus it was assumed that the median value of measurements was equal to 1 MoMc and the deviation from median was equal to null. Our results showed that the greatest deviation from the expected median (-0.13 MoMc) was recorded for the NT measurement while the deviation for the free- \hat{a} hCG and PAPP-A values was 0.07 respectively 0.06. In conclusion the main disturbing factor in calculating the aneuploidy risk was the measurement of NT. A systematic audit of laboratory and ultrasound measurement is necessary.

Keywords: first trimester screening, aneuploidies, deviation from median, audit

Aneuploidies screening is one of the main challenges in materno-fetal medicine [1] and is performed at the same age of pregnancy as the screening for other pregnancy complications: preeclampsia [1], preterm birth [1,2,3], endocrine dysfunctions [4] or risk of materno-fetal infection transmission [5,6]. According to medical authorities first trimester screening implies ultrasonographic evaluation of crown-rump length, nuchal translucency, nasal bone, ductus venous velocity and tricuspid valve regurgitation [1]. Also first trimester screening involves measurement of biochemical markers: free fraction of beta chain of chorionic gonadotropin hormone (free-beta hCG), and pregnancy associated protein A (PAPP-A). The optimal period for first trimester ultrasound measurements is between 11 weeks of pregnancy (wp) and 4 days and 13 wp and 6 days, while the measurement of biochemical markers is recommended to be performed between 9 wp and 13 wp and 6 days.

Pregnancy-associated plasma protein A or PAPP-A is a protein with metalloproteinase features that cleaves insulin-like growth factor binding protein [7]. PAPP-A structure is well known [8] and is encoded by the PAPPA gene [9]. Low plasma levels are found in pregnancies with fetuses with aneuploidies or are predictive for pregnancy complications such as: intrauterine growth restriction, preeclampsia, preterm birth or intrauterine fetal demise [10].

Free beta-hCG is a subunit of hCG a hormone produced by trophoblastic vili during pregnancy [11]. Elevated freebeta-hCG were found in pregnancies with fetuses with Down syndrome and preeclampsia [12].

Screening of an euploidies allows the selection from the entire population of pregnant women of those with a high risk, who will further benefit from diagnostic invasive methods [1].

The aim of our study was to analyze the distribution of biochemical markers and nuchal translucency values in our screening program.

Experimental part

Patients and sera

Our first trimester screening program was applied to 1313 pregnant women with single spontaneously conceived pregnancies, without diabetes, who came to our hospital from 2010 to 2014. Sera were collected between 9 wp and 13 wp and 6 days from singleton pregnancies. Pregnant women were interrogated about the date of the last menstrual period, mode of conceiving, smoking behavior, diabetes and weight at the time of biochemical screening. In all pregnancies the gestational

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age was determined based on first trimester crown-rump length measurement.

Measurement of first trimester biochemical markers

First trimester biochemical markers (free Beta-hCG and PAPP-A) were measured by chemiluminescence method, using an ImmuliteOne Machine (DPC, Diagnostic Products Corporation, Los Angeles, USA) and commercially available kits (Siemens Healthcare Diagnostics Products Ltd., Llanberis, Gwynedd, LL55 4EL, UK). Values were expressed in corrected multiple of medians (MoM), calculated according to PRISCA software, Version 4 (Typolog Software, Tomesch, Germany). Data from pregnant women and biochemical markers were stored using ASTRAIA software, the materno-fetal module (Astraia GmbH, Munich, Germany) [13].

First trimester ultrasound markers measurement

Crown-rump length and nuchal translucency (NT) were measured according the fetal medicine foundation (FMF) guidelines although only a few of ultra-sonographers were accredited by FMF. Values for NT are expressed in MoM.

Ethical issues

The research meets the conditions of the ethical guidelines and legal requirements and was approved by the Committee of the University of Medicine and Pharmacy Timisoara. Informed consent was obtained from every patient.

Statistical analysis

Data are expressed in median+/- Standard error of mean (SEM). GraphPad InStat software, San Diego, California, USA and SPSS, IBM Inc. were used for statistical analysis.

Results and discussions

Evaluation of individual risk for an uploidies relies on an algorithm that cumulates the risks calculated on basis of independent biochemical and ultrasonographic parameters such as: age of the pregnant women, gestational age, NT thickness, presence of nasal bone or free-beta-hCĞ and PAPP-A concentrations [1]. The risk evaluation is adjusted (corrected) depending on mode of conception, demographical and behavioral data of the pregnant women (racial origin, smoker status, diabetes, etc.). An accurate evaluation of the risk implies both an accurate evaluation of the gestational age and of the biochemical and ultrasonographic markers [14]. Errors in ultrasonographic evaluation (crown-rump length, NT) or of measurement of biochemical markers could determine a false evaluation of the individual aneuploidy risk [15]. In order to assure a good quality of the screening program it is necessary to run audit activities [15]. We present herein the particularities of the screening program implemented in our center.

Demographic features of pregnant women

The mean age of pregnant women at the time of screening was 28.94 years, the gestational age was 87.66 days and the weight was 63.18 kg (table 1).

Deviation from median of nuchal translucency values

Statistical evaluation of nuchal translucency measurement values (expresed in Multiple of medians – MoM) is represented in table 2 and the deviation of NT values from the expected median in figure 1.

| | Age (years) | Gestational age (days) | Weight (kg) |
|--------------------------|----------------|---------------------------|----------------|
| Mean | 28.94 | 87.99 | 63.18 |
| Standard deviation | 4.95 | 4.74 | 12.79 |
| Number of pregnant women | 1313 | 1313 | 1313 |

Table 1DEMOGRAPHIC FEATURES OF PREGNANTWOMEN THAT UNDERWENT FIRST TRIMESTERSCREENING

Table 2

STATISTICAL EVALUATION OF NT MEASUREMENT VALUES EXPRESSED IN MULTIPLE OF MEDIANS (MoM)

| | | | | Median | SEM | lower 95% conf | upper 95% conf |
|----------------|-----------------|----------------|--------------------------|--------|------|----------------|----------------|
| Value measu | of rement (i | TN median 1 | thickness nultuiples) | 0.87 | 0.01 | 0.91 | 0.94 |



Fig. 1. Distribution of deviation from median of NT measurements (expressed in log 10 from multiples of median - MoM). The number of pregnant women with the same value of deviation of NT values from median (absicssa) are represented on ordinate

| | Median | SEM | Lower 95% conf | upper 95% conf | Table 3 STATISTICA |
|---|--------|------|----------------|----------------|---|
| Value of free-β hCG measurements (expressed in multiples of median corrected – MoMc) | 1.07 | 0.02 | 1.23 | 1.33 | EVALUATION FREE-β hC MEASUREME (EXPRESSED IN |

STICAL TION OF -β hCG REMENTS ED IN MoM)



Fig. 2. Distribution of deviation from median of free- β hCG measurements (expressed in log 10 from multiples of median corrected - MoMc). The number of pregnant women with the same value of deviation of free- β hCG value from expected corrected median (absicssa) is represented on ordinate

Table 4 STATISTICAL EVALUATION OF PAPP-A MEASUREMENTS (EXPRESSED IN MoM)

| | Median | SEM | lower 95% conf | upper 95% conf |
|--|--------|------|----------------|----------------|
| Value of PAPP-A measurements (expressed in multiples of median corrected – MoMc) | 1.06 | 0.01 | 1.17 | 1.24 |



Fig. 3. Distribution of deviation from median of PAPP-A measurements (expressed in log 10 from multiples of median corrected - MoMc). The number of pregnant women with the same value of deviation of PAPP-A value from expected corrected median (absicssa) is represented on ordinate

Deviation from median of free- β hCG values Statistical evaluation of free- β hCG values (expressed in Multiple of median corrected – MoMc) is represented in table 3 and the deviation of free- β hCG measurements values from corrected median in figure 2.

Deviation from median of PAPP-A values

Statistical evaluation of PAPP-A values (expressed in Multiple of medians corrected - MoMc) is represented in table 4 and the deviation of PAPP-A measurements values from expected corrected median in figure 3. Conclusions

Our research brings new data about the particularities of the first trimester aneuploidies screening in Timisoara and shows that:

- the age of the pregnant women at the time of the first trimester screening was $28,7\pm0.13$ years (median \pm SEM) with 5 to 6 years greater compared to the year 1995 (unpublished data).

- the weight of the pregnant women at this gestational age was 61 ± 0.35 kg (median \pm SEM).

- the nuchal translucency values were lower than expected (0.85 MoM). The reason is probably a nonadequate measurement tehnique.

- the free- β hCG values are with 0.07 MoMc increased compared to the expected values.

- the PAPP-A values are with 0.06 MoMc increased compared to the expected values.

- the measurement of nuchal translucency is the main disturbing factor in ensuring a high quality of the screening program.

- in order to improve the accuracy of the screening it is mandatory to roll a regular audit of the screening program.

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