Weight of Pregnant Women and their Influence on Second Trimester Biochemical Markers

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Fetal aneuploidies screening was based for a long time on ultrasonographic and biochemical markers measurement. The risk calculated in accordance with second trimester biochemical markers (STBM) values relies on calculation of corrected MoM values. MoM (multiple of Medians) signify the deviation of a measured value from the expected value (Median). The Median is measured at the same gestational age in pregnancies which involve healthy fetuses. The correction of MoM includes an adjustment for certain parameters that influence the STBM value: demographical (ethnicity), behavioral (smoking status, weight), and others (mode of conceiving, etc.). In our article we aim to analyze: (1) the accuracy of software to calculate STBM corrected MoM values, (2) the effect of weight of pregnant women on STBM and (3) the capability of software to counterbalance this influence. Pregnant women (n=1242) were screened for aneuploidies based on an integrated test: first trimester ultrasound and STBM (AFP, hCG and uE3). The absolute value, multiple of median (MoM) and corrected multiple of median (MoMc) values were 33.94±0.45, 1.04±0.12 and 0.98±0.01 for AFP, 22530±477, 0.87±0.01 and 0.85±0.01 for hCG, respectively 0.97±0.03, 0.99±0.01 and 0.98±0.01 for uE3. The weight of pregnant women inversely correlates with absolute and MoM AFP, hCG and uE3 values. No correlation was found with AFP and hCG MoMc values. A very weak inverse correlation was found between weight and uE3 corrected MoM values. Our study confirms that there is a difference between provider and own calculated hCG MoMc values. The weight of pregnant women inversely correlates with STBM values. The software used for aneuploidies risk evaluation corrects the influence of weight of pregnant women, but a minimal influence on uE3 corrected MoM values is still present.

Keywords: AFP, hCG, uE3, influence of weight, integrated screening, aneuploidies, software correction

Fetal aneuploidies screening in pregnancy was based on ultrasonographic and biochemical markers measurement for a long time [1-3]. Recent developments made it possible to appreciate the risk of aneuploidies based on free fetal DNA in maternal blood [4]. Because the access to the test mentioned above is not widespread enough, the methods based on biochemical and ultrasonographic measurements are still in use [4]. Nowadays the first trimester combined test is the most recommended test worldwide [1,2]. However, integrated test including first trimester ultrasound markers and second trimester biochemical markers (STBM) is still recommended by many physicians [3].

The STBMs included in integrated test are alpha fetoprotein (AFP), human chorionic gonadotropin hormone (hCG), and free Estriol (uE3) [3,5,6].

Human Alpha-fetoprotein (AFP) is together with albumin, and vitamin D-binding protein a member of the *albumin gene family* [7]. The gene that encodes synthesis of AFP is located on the long arm of chromosome 4 [8-10]. The AFP has a molecular mass of around 70kD. [7]. The concentration of AFP increases in fetal plasma at a maximum value at the end of the first trimester and slowly decreases at the end of the pregnancy [11]. It is produced by cells from the yolk sac and the fetal liver. Elevated concentrations were found in pregnancies which imply fetuses with open neuronal defect or abdominal wall defect. A decrease in concentration is found in sera of pregnant women who carry fetuses with Down syndrome. Beyond the pregnancy period AFF could be synthesized in different tumors and diseases [7].

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Human chorionic gonadotropin (hCG) synthesis takes place in syncytiotrophoblast after implantation[12-15]. hCG has a key role in maintaining pregnancy because it sustains corpus luteus formation and the production of progesterone and estradiol [16]. hCG also modulates the immune system and the angiogenesis processes having a role in placentation and placental growing [17]. The two subunits of hCG are: α (alpha) subunit which is identical to luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and â (beta) subunit that is specific to hCG. It was well described before the association between a high hCG concentration and Down syndrome pregnancies. Cancerous cells could produce hCG too [18].

Unconjugated Estriol (uE3) is the main estrogen in pregnancy [5]. uE3 is produced in fetal tissue and in placenta. This is why the estriol concentration reflects both the fetal wellbeing and the placenta function. Pregnant women who carry fetuses with chromosomal anomalies such as Down syndrome or Edward's syndrome show modified uE3 values [5]. As effect uE3 could be used in screening of fetal aneuploidies. Outside pregnancy estriol exerts immunonodulatory effects on certain diseases (autoimmune, inflammatory, osteoporosis, vascular diseases, etc.). It was recommended as a treatment in menopause [19].

Since in pregnancies with Down syndrome STBM serum concentrations show an alteration compared to pregnancies with healthy fetuses, STBM could be used to screen for fetal aneuploidies [20]. The complete risk evaluation formula relies on the calculation of an individualized risk which includes a likelihood ratio obtained from evaluation of following parameters: native risk (age related risk), age of pregnancy, ultrasound parameters (nuchal translucency thickness, presence of nasal bone) and the risk calculated based on STBM values [21]. The risk calculated on basis of biochemical markers values rely on calculation of corrected MoM values. MoM (multiple of Medians) values quantifies the deviation of a measured value from the expected value (Median). The medians are obtained from healthy fetus pregnancies at the same gestational age [20,21]. The correction of MoM includes an adjustment for certain parameters that influence the concentration of STBM: demographical (ethnicity), behavioral (smoking status, weight), and others (mode of conceiving, etc.) [20,21].

In recent studies we analyzed the capability of the risk calculation software to counterbalance the effect of smoking on first and second trimester biochemical markers values [3,21,22].

In our article we aim to analyze (1) the accuracy of software to calculate second trimester biochemical markers (STBM) corrected MoM values, (2) the effect of weight of pregnant women on STBM and (3) the capability of software to counterbalance this influence.

Experimental part

Patients and sera

Pregnant women (n=1242) were screened for aneuploidies based on an integrated test including first trimester ultrasound markers (crown-rump length and nuchal translucency) and second biochemical markers (AFP, hCG, uE3). First trimester ultrasound markers were measured between 11+4 and 13+6 weeks of pregnancy (wop) in all pregnant women. Second trimester biochemical markers were measured between 15 and 22 wop. Only pregnant women of Caucasian ethnicity who conceived spontaneously, without diabetes, and with singleton pregnancies were included in our study. We collected data about last menstrual period, mode of conceiving, smoking behavior, presence of diabetes, and weight at the time of biochemical screening using an protocol presented before [23,24]. Research results by Kalish, Chervenak et al. were used to establish pregnancy age on basis of CRL values [25].

Measurement of second trimester biochemical markers concentration

STBM (AFP, hCG and uE3) were measured by the 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). STBM concenrations were expressed in absolute values, multiple of medians (MoM), corrected multiple of medians (MoMc), and calculated according to PRISCA software, Version 4 (Typolog Software, Tomesch, Germany).

Gestational age determination

The gestational age was established based on first trimester crown-rump length measurement [25].

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

We used GraphPad InStat software, San Diego, California for statistical analysis. Data were expressed in median +/- Standard error of mean (SEM). Spearman non-parametric correlation test was used to calculate correlations.

Results and Discussions

It is well known that among other factors (smoking, ethnicity, method of conception or presence of diabetes) the weight of pregnant women influences the STBM concentration [9,10,22]. The accuracy of risk evaluation depends on the capacity of risk calculation software to counterbalance the influence of these parameters on STBM.

Demographic and serological features of pregnant women in the study

The first trimester ultrasound evaluation was performed at a crown-rump length (CRL) of 59.37±0.30 mm. The age of pregnant women was 28.71±0.13 years and the weight 61.8±0.34 kg at the time of STBM measurement. (table 1) The absolute concentration, multiple of median (MoM), and corrected multiple of median (MoMc) values were 33.94±0.45, 1.04±0.12, and 0.98±0.01 for AFP, 22530±477, 0.87±0.01, and 0.85±0.01 for hCG, respectively 0.97±0.03, 0.99±0.01 and 0.98±0.01 for uE3 (table 2).

Correlation between the weight of pregnant women and second trimester AFP values

The weight of pregnant women inverse correlate with absolute AFP values (rho = -0.29, p< 0.0001) and MoM AFP values (rho = -0.32, p<0.0001). No correlation was found with AFP corrected MoM values (table 3).

Demographic features	Median±SEM
Age (years)	28.71±0.13
Gestational age (days)	117.28±0.21
Weight (kg)	61,8±0.34
CRL (mm)	59.37±0.30
Number of pregnant women	1242

Data are expressed in median ± SEM

Table 1
DEMOGRAPHIC FEATURES OF PREGNANT WOMEN

Serological parameter	AFP	hCG	uE3
Absolute value	33.94±0.45	22530±477	0.97±0.03
MoM	1.04±0.12	0.87±0.01	0.99±0.01
MoMc	0.98±0.01	0.85±0.01	0.98±0.01
Number of pregnant women	1242	1242	1242

Data are expressed in median ± SEM

Table 2
SEROLOGICAL FEATURES OF PREGNANT WOMEN

weight vs. AFP	absolute value	Multiple of Median (MoM)	Multiple of Median corrected (MoMc)
Rho	- 0.2927	- 0.3211	0.0262
p- value	< 0.0001	< 0.0001	0.35 (NS)

Table 3
CORRELATION BETWEEN SECOND TRIMESTER SERA AFP VALUES AND WEIGHT OF PREGNANT WOMEN

Correlation between the weight of pregnant women and second trimester hCG values

The weight of pregnant women inversely correlates with absolute hCG values (rho = -0.21, p< 0.0001) and MoM AFP values (rho = -0.21, p<0.0001). No correlation was found with hCG corrected MoM values (table 4).

Correlation between the weight of pregnant women and second trimester uE3 values

The weight of pregnant women showed a very weak but significant inverse correlation with second trimester uE3 absolute values (rho = -0.07, p< 0.011) respectively an intense inverse correlation with uE3 MoM values (rho = -0.17, p< 0.001). A very weak inverse correlation was still found between the weight and uE3 corrected MoM values. (Table 5)

We analyze herein for the first time if the software used for fetal aneuploidies risk calculation fits the particular features of the pregnant women in our country [1,3,21,26]. Our results are very relevant because a huge number of pregnant women undergo fetal aneuploidies risk evaluation. The software calculates the risk based on medians and formulas that are not calculated specific for a certain country [6]. Previous research showed that different countries require sometimes different values of medians [6]. Since no audit of Romanian aneuploidies screening program has been run at a national level we have no proof that median values from providers are applicable to our population. Our results highlight the differences between the own calculated medians and medians suggested by providers for hCG corrected MoM values [1,121.

As expected the weight of pregnant women inversely correlates with absolute concnetration values respectively MoM STBM values. We don't know why the absolute uE3 values showed only a weak inverse correlation with the weight of pregnant women.

The fetal aneuplodies risk calculation software aims to exclude the influence of weight on STBM concentration. Thereby there should not be an inverse correlation between weight and MoMc values of STBM. Our results showed that the software works properly for AFP and hCG MoMc. However, a weak but significant inverse correlation between uE3 MoMc values and the weight of pregnant women is still present after correction.

weight vs. hCG	absolute value	Multiple of Median (MoM)	Multiple of Median corrected (MoMc)
Rho	- 0.2154	- 0.2142	- 0.0231
p- value	< 0.0001	< 0.0001	0.41 (NS)

Table 4
CORRELATION BETWEEN SECOND TRIMESTER SERA HCG VALUES AND WEIGHT OF PREGNANT WOMEN

weight vs. uE3	Absolute value	Multiple of Median (MoM)	Multiple of Median corrected (MoMc)
Rho	- 0.0715	- 0.1704	- 0.0562
p- value	< 0.0117	< 0.0001	0.04 (NS)

Table 5
CORRELATION BETWEEN SECOND TRIMESTER SERA UE3 VALUES AND WEIGHT OF PREGNANT WOMEN

Conclusions

Our results confirms that there is a difference between the own calculated medians and medians suggested by providers for hCG corrected MoM values. The weight of pregnant women inversely correlates with STB values. The software used for aneuploidies risk evaluation corrects the influence of weight of pregnant women on STBM, but a minimal influence on uE3 MoM corrected values is still present.

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