Is there a Need for Own Median Calculation in the Second Trimester Biochemical Markers Screening?

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Screening of fetal aneuploidies in early pregnancy is a well-established method in the materno-fetal medicine. The aim of our study was to analyze if the medians recommended by the manufacturers are adequate to perform an accurate screening or if there is a need for own laboratory medians calculation in second trimester biochemical marker screening.Sera were collected between 14 wp and 22 wp from 3374 singleton pregnancies. We analyzed three second trimester biochemical markers (AFP, hCG and free Estriol) concentration in all pregnant women and in a subgroup of pregnant women in which gestational age was determined based on crown-rump length. Our results showed that for all biochemical markers the difference between the manufacturer and the own calculated median was lower than 10% excepting the hCG value in the group of pregnant women in which the gestational age was determined on basis of crown-rump-length. Our results show it is recommended to replace the values of the median for hCG measurement with the own laboratory calculated medians. This does not seem to be necessary in the case of AFP and free Estriol measurement.

Keywords: second trimester, biochemical markers, median values, own laboratory audit

Implementation of a screening program based on integration of first trimester ultrasound markers and second trimester biochemical markers implies a good compliance of pregnant women. Previous studies showed that only around 60% of pregnant women have such a good compliance and this is one of the reasons why combined first trimester screening test is preferred by physicians and pregnant women [1,2]. Another reason is that first trimester combined test allows evaluation of trisomy risk in the first trimester while the integrated test allows it only in the second trimester. The advantage of an integrated approach is that second trimester ultrasound evaluation allows the gathering of more fetal anatomical details compared to the first trimester ultrasound and these details could adjust the calculated risk [2]. Development of technology and of ultrasound machines in the last decade has reduced this gap and gives nowadays the possibility for an earlier fetal ultrasound evaluation [3,4]. The aim of the integrated screening test is to obtain fetal nuchal translucency (NT) and crown-rump length (CRL) values in the first trimester and to collect maternal serum in the second trimester. We used the integrated risk evaluation algorithm parallel to the first trimester combined screening algorithm in our hospital between 2007 and 2012. According to the fetal-medicine-foundation protocol the ultrasound measurement of CRL and NT was performed between 11 weeks of pregnancy (wp) + 4 days and 13 wp + 6 days [2]. The evaluation of the three second trimester biochemical markers (alfafetoprotein, hCG, and free Estriol) was performed in sera of pregnant women between 15 wp and 22 wp.

Human Alpha-fetoprotein (AFP) is a single-chain glycoprotein with 609 residues [5], with a molecular mass of 69 kDa or greater depending on carbohydrate content [6]. AFP is sometimes named alpha-1-fetoprotein, alpha fetoglobulin or alpha fetal protein and is encoded by a gene located on the q arm of chromosome 4 (4q25) [5,6]. The fetal form of AFP was first detected in 1957 as a fetal-associated protein and later in 1963 as a tumor-associated protein. During pregnancy AFP is produced by the yolk sac and the liver and decreases in Down syndrome fetuses and increases in fetal disorders (neural tube defect, omphalocele) or maternal disorders (tumors, hepatoma, etc.) [7].

Human chorionic gonadotropin (hCG) is a hormone usually produced by trophoblastic cells in placenta after implantation, [8,9] although sometimes, besides pregnancy, cancerous cells produce hCG [10]. hCG consists of two subunits: α subunit identical to that of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and β subunit that is

unique to hCG. hCG values levels correlate with maturation grade of placenta and embryo [7]. Deviation of hCG concentration compared to the expected value allows the estimation of a risk of certain fetal chromosomal abnormalities and birth defects. Huge hCG values are related to Down Syndrome and germ cell and throphoblastic tumors [7].

Chemical structure and synthesis of estriol was presented elsewhere [7]. Since free estriol is produced by both fetus and placenta the estriol concentration reflects both the fetal wellbeing and the placenta activity. Variation of free estriol values are observed in chromosomal or congenital anomalies such as Down syndrome or Edward's syndrome [7].

The aim of our study was to analyze if the medians recommended from the manufacturers are adequate to perform an accurate screening or if there is a need for own laboratory medians calculation in second trimester biochemical marker screening. As far as we know this is the first study from our country that analyzes this important topic.

Experimental part
Patients and sera
Non-smoking pregnant women (n=3374) with single spontaneously conceived pregnancies, without diabetes, who came for aneuploidy screening to our hospital from 2007 to 2016, where recruited for our study. Sera were collected between 14 wp and 22 wp 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. There were different ways to determine the gestational age based on: last menstrual period, date of conception, first trimester crown-rump length measurement, second trimester ultrasound or biparietal diameter measurement.

Measurement of second trimester biochemical markers
Second trimester biochemical markers (AFP, hCG and E3) 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, 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) [11,12].

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 was used for statistical analysis.

Results and discussions
Identification of early markers predictive of pregnancy complications is a goal of materno-fetal medicine [7,13-17]. The algorithm of aneuploidy risk calculation needs a correct evaluation of gestational age. It is expected that the pregnant women in which gestational age determination is performed according to the CRL will have the lowest deviation from the median values recommended by the manufacturers of the kits. We present below our results about the demographic features of the screened pregnant women and the deviation of the own laboratory calculated median from the manufacturers recommended ones. The analysis was performed both in all pregnant women and in the group of pregnant women in which the gestational age determination was done based on crown-rump-length measurement.

Demographic features of pregnant women in our study
The age of pregnant women at the time of screening was 28.9 ± 0.08 years, the gestational age was 16 weeks and 4 days $\pm$ 1 day and the weight was 62 ± 0.2 kg (table 1). The pregnant women (n=3374) were investigated by 45 physicians. The gestational age was determined by DBP measurement (n=439), CRL measurement (n=1078), date of conception (n=11), last menstrual period (n=1544), scan (n=208), and unknown criteria (n=94).

Parameter	Age of pregnant women	Gestational age (days)*	Weight (Kg)
Minimum	15.19	98.00	36.5
Median	28.91	117.00	62
Maximum	46.54	160.00	142
Standard error of mean	0.08	0.13	0.20
Mean	29.17	117.74	63.86
Standard deviation	4.69	7.85	11.99
sample size	3374	3374	3374

Table 1
DEMOGRAPHIC FEATURES OF PREGNANT WOMEN

Gestational age at the time of second trimester biochemical screening

Parameter	AFP (MoMc)	hCG (MoMc)	free Estriol (MoMc)
Minimum	0.3	0.15	0.06
Median	0.94	0.92	0.99
Maximum	11.47	5.66	7.18
Standard error of mean	0.007	0.01	0.007
Mean	1.00	1.05	1.04
Standard deviation	0.42	0.59	0.42
sample size	3374	3374	3374

Table 2
SECOND TRIMESTER BIOCHEMICAL MARKERS CONCENTRATION IN SERA OF ALL PREGNANT WOMEN (n=3337) INDEPENDENT OF THE MODE OF GESTATIONAL AGE DETERMINATION. DATA ARE EXPRESSED IN CORRECTED MULTIPLE OF MEDIAN (MoMc)

Parameter	AFP (MoMc)	hCG (MoMc)	free Estriol (MoMc)
Minimum	0.3	0.17	0.06
Median	0.99	0.85	0.98
Maximum	5.4	4.70	7.18
Standard error of mean	0.01	0.01	0.01
Mean	1.05	0.97	1.03
Standard deviation	0.42	0.55	0.44
sample size	1078	1078	1078

Table 3
SECOND TRIMESTER BIOCHEMICAL MARKERS CONCENTRATION IN SERA OF PREGNANT WOMEN FOR WHOM THE GESTATIONAL AGE WAS DETERMINED ONLY ACCORDING TO THE CROWN-RUMP-LENGTH (CRL). DATA ARE EXPRESSED IN CORRECTED MULTIPLE OF MEDIAN (MoMc)

Accuracy of measurement of second trimester biochemical markers in all pregnant women

The median values of the corrected Multiple of Medians of all pregnant women were calculated for AFP (0.94±0.007), hCG (0.92±0.01) and free Estriol (0.99±0.007). Each of the calculated medians showed lower values than the median value recommended by the supplier. The nearest value to the manufacturer median was calculated for the free estriol measurement (table 2).

Accuracy of measurement of second trimester biochemical markers only in pregnancies dated according to crown rump length

The median value of corrected Multiple of Medians was calculated for AFP (0.99±0.01), hCG (0.85±0.01), and free Estriol (0.98±0.01) in pregnant women in which the gestational age was determined according to the crown-rump length measurement. In this group of pregnant women, too, the calculated medians showed lower values than the median value recommended by the supplier. The nearest value to the manufacturer median was obtained for the alfafetoprotein measurement (table 3).

Since recent studies showed that early pregnancy ultrasound evaluation is not dangerous to the embryo, there is no contraindication to perform early and first trimester ultrasound [3,4] as well as later in life [17]. Although it is expected that the majority of pregnant women had a first trimester ultrasound in our study only around 30% of them underwent this kind of examination. Since the majority of pregnant women screened by us carry a healthy fetus, it is expected that the median of corrected multiples of medians will be equal to 1. Our results showed that for all biochemical markers the difference between the manufacturer and the own calculated median was lower than 10% excepting the hCG value in the group of pregnant women in which the gestational age was determined on basis of crown-rump-length. Since it is known that the CRL measurement is a more accurate mode of gestational age determination than other methods (excepted date of conception) we expected that the deviation from the manufacturer median would be lowest in these group. These hypothesis was confirmed for the two biomarkers AFP (1%) and free Estriol (2%), but not for the hCG measurement, where the difference was higher (15%). Because the deviation from the median is a factor that directly influences the calculation, we recommend the performance of routine audit of the screening program and, in the case of hCG measurement, the replacement of the value of the median recommended by the manufacturers with the own laboratory calculated median.

Conclusions

Our results show it is recommended to replace the values of the median for hCG measurement with the own laboratory calculated medians. This does not seem to be necessary in the case of AFP and free estriol measurement.

References
1. WALD NJ, RISH S, HACKSHAW AK.. Prenat Diagn. 2003 Jul;23(7):588-92. doi: 10.1002/pd.627
2. NICOLAIDES, K.H. Fetal Diagn Ther, 29(3), 2011, p. 183. doi: 10.1159/000324320
3. NEMESCU D, BERESCU A, ONOFRIESCU M, NAVOLAN DB, ROTARIU C. (2015). PLOS ONE 10(5), article number: e0127570, doi: 10.1371/jurnal.pone.0127570
4. NEMESCU D, BERESCU A. (2015). Ultrasound Med Biol 41(1), pp.35-39. doi: 10.1016/j.ultrasmedbio.2014.08.010
5. MINGYU ZHU, BO LIN, PENG ZHOU AND MENGSEN L. Molecular analysis of AFP and HSa interactions with PTEM protein. Biomed Res Int 2015;2015:256916. doi> 10.1155/2015/256916
6. MIZEJEWSKI GJ. New insights into AFP structure and function: Potential biochemical applications. Academic Press, 1985, Orlando.
7. NAVOLAN, D., VLADAREANU, S., CIOHAT, I, STOIAN, D., BADIU, D., CRAINA, M., TOMOVICI, M., BIRSASTEANU, F., CRACIUNESCU., M., A preliminary study over second trimester biochemical markers and their clinical utility. Rev. Chim. (Bucharest), **67**, no. 6, 2016, p. 1224 WOS:000385510300043.
8. COLE LA (2009). Reprod. Biol. Endocrinol. 7: 8. doi:10.1186/1477782778..
9. GREGORY JJ, FINLAY JL (April 1999). Alphafetoprotein and betahuman chorionic gonadotropin: their clinical significance as tumour markers. Drugs. 57 (4): 463–7. doi:10.2165/0000349519995704000001.
10. NAVOLAN D, CIOHAT I, DRAGOI V, CONSTANTINESCU S, BADIU D, TIMAR R, ONOFRIESCU M, DENK R, VLADAREANU R. (2013). Gineco.eu 9(2), pp. 80-82. DOI: 10.18643/gieu.2013.80
11. NAVOLAN D, VLADAREANU S, DENK R, CRACIUNESCU M, KLEIST C, RATIU A, LAHDOU I, BADIU, D, CRAINA M, SAS I, CIOHAT I, HANGAN T, NICODIN O, PAINAITE B, GRIGORAS D, IONESCU C, BACALABASA N, ONOFRIESCU M, VLADAREANU R, NEMESCU D. (2016). Gineco.eu 12(1), pp. 12-14. DOI:10.18643/gieu.2016.12
12. NAVOLAN D, VLADAREANU S, CIOHAT I, BADIU D, NEMESCU D, VLADAREANU R, SAS I. (2015). Proceedings of the 49TH annual scientific meeting of the European Society for Clinical Investigation, Pages 249-252.
13. NAVOLAN D, SAS I, BADIU D, VLADAREANU R, CIOHAT I, NEMESCU D, VLADAREANU S. (2015). Proceedings of the 49TH annual scientific meeting of the European Society for Clinical Investigation, Pages 253-256.
14. NAVOLAN DB, VLADAREANU S, LAHDOU I, CIOHAT I, KLEIST C, GRIGORAS D, VLADAREANU R, TERNESS P, SAS I. (2016). Journal of Perinatal Medicine 44(1), pp. 517-522. DOI: 10 1515/jpm-2015-0081
15. NAVOLAN, D., CRACIUNESCU, M., CIOHAT, I., STOIAN, D., BADIU, D., CRAINA, M., BIRSASTEANU, F., NEMESCU, D., VLADAREANU S.., Rev. Chim. (Bucharest), **67**, no. 5, 2016, p. 991
16. STOIAN D, PANTEA S, MARGAN M, TIMAR B, BORCAN F, CRAINA M, CRACIUNESCU M (2016). Interntaional Journal of Molecular Sciences 17(1), article number 88. DOI: 10.3390/ijms17010088.
17. STOIAN D, TIMAR B, DERBAN M, PANTEA S, VARCUS F, CRAINA M, CRACIUNESCU M. (2015) Medical Ultrasonography 17(3), pp. 327-332. doi: 10.11152/mu.2013.2066.173.dst.