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ÖZGÜN ARAŞTIRMA / RESEARCH STUDY

Determining The Influence of Regional Differences and Maternal Factors on The Triple Test

Bölgesel Farklılıkların ve Gebeye Bağlı Faktörlerin Üçlü Test Üzerine Etkileri

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ABSTRACT

Objective: Second-trimester maternal serum screening has become a routine part of prenatal care. We aimed to identify if triple test measurements were affected by regional differences and maternal factors. We compared our results with the results of a previous study on Turkish population, and with the values of the manufacturer firm.

Material and Methods: The study group consisted of 984 pregnant women between 14–22 weeks of gestation who had undergone a triple test. The original PRISCA software was used for calculating the statistical risk.

Results: Comparison of median values for serum AFP, between published Turkish population, manufacturer values and Gaziantep population were: 1% between Gaziantep population and Turkish population, and 3 % between Gaziantep population and manufacturer values in 16. gestational week; 5 % and 4 % in 17. gestational week; 13 % and 14 % in 18. gestational week, respectively. 19. gestational week showed no difference. Same comparison for β-hCG showed; 5 % and 1 % in 16. gestational week; 4 % and 2 % in 17. gestational week; 13 % and 1 % in 18. gestational week; 1 % and 24 % in 19. gestational week, respectively. Comparison for uE3 values showed; 36 % and 95 % in 16. gestational week; 39 % and 89 % in 17. gestational week; 25 % and 67 % in 18. gestational week; 34 % and 72 % in 19. gestational week, respectively.

Conclusion: This study showed that, screening tests may vary based on regional differences. Our study presents a potential risk factor that may be an explanation for some problems with the triple test evaluations in some laboratories.

Keywords: Triple test; risk factors; regional differences; PRISCA.

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ÖZET

Amaç: İkinci trimester tarama testleri prenatal bakımın rutin bir parçasıdır. Çalışmamız bu testin anneye bağlı etkenler ve bölgesel farklılıklardan etkilenip etkilenmediğini bulmayı amaçlamıştır. Gaziantep bölgesini temsil eden sonuçlarımızı, Türkiye genelini gösteren bir çalışma ve üretici firma verileriyle karşılaştırdık.

Gereç ve Yöntem: Çalışma grubu 14-22 gebelik haftalarında üçlü test yaptıran kadınları kapsamaktadır. İstatistiksel risk hesaplamada PRISCA® programı kullanılmıştır.

Bulgular: Median verileri AFP için karşılaştırıldığında: 16. gebelik haftasında, sırasıyla Gaziantep populasyonu ve yayınlanmış Türkiye populasyonu verileri arasında % 1, Gaziantep populasyonu ve üretici firma verileri arasında % 3; 17. gebelik haftasında % 5 ve % 4; 18. gebelik haftasında % 13 ve % 14, fark bulunmuştur. 19. gebelik haftasında fark saptanmadı. Benzer karşılaştırma β-hCG için yapıldığında sırasıyla 16. gebelik haftasında % 5 ve % 1; 17. gebelik haftasında % 4 ve % 2; 18. gebelik haftasında % 13 ve % 1; 19. gebelik haftasında % 1 ve % 24 fark bulunmuştur. uE3 karşılaştırmasında: 16. gebelik haftasında % 36 ve % 95; 17. gebelik haftasında % 39 ve % 89; 18. gebelik haftasında % 25 ve % 67; 19. gebelik haftasında % 34 ve % 72, fark bulunmuştur.

Sonuç: Bu çalışma üçlü test sonuçlarının bölgesel farklılıklara bağlı değişiklik gösterebileceğini işaret etmektedir. Bu durum bazı laboratuarlarda üçlü test değerlendirmelerinde yaşanabilecek sorunlar için açıklayıcı olabilir.

Anahtar Kelimeler: Üçlü test; risk faktörleri; bölgesel farlılıklar; PRISCA.

INTRODUCTION

Second-trimester maternal serum screening for Down syndrome (DS), open neural tube defects (NTD) and trisomy 18 have become a routine part of prenatal care in recent years. A screening test is not equal to a diagnostic test. It helps to identify which pregnancies may benefit from additional testing. A normal screening test means the risk of certain birth defects being present is greatly reduced. Currently, second trimester serum screening is generally carried out at 15-18 weeks gestational age but the testing is considered to be valid up to 22 weeks (1).

Primarily, the developing liver produces alpha fetoprotein (AFP) in the fetus. AFP is present in fetal plasma. By means of fetal urination, it is also present in the amniotic fluid, although at much lower concentrations. If a developmental defect, such as failure of the neural tube to close exists more AFP escapes into the amniotic fluid. Fetal AFP enters the maternal circulation by placental transfer and by diffusion across placental membranes (2).

Fetal AFP peaks between 10 weeks to 13 weeks gestation, then declines during second and third trimesters. Maternal serum (MS) AFP levels rise gradually and peak between 28 weeks and 32 weeks of gestation. After that time, MSAFP declines until delivery. Lower than expected MSAFP levels are seen when fetal gestational age is overestimated, or in maternal Type 1 diabetes, fetal Trisomy 21 (Down Syndrome) or Trisomy 18 (Edward's syndrome), and in pseudopregnancy (2). Depending upon the cutoff thresholds used by the laboratory, measurement of MSAFP detects most NTDs, 70 percent to 80 percent of open spina bifida (OSB), and 95 percent to 98 percent of anencephaly (1). The sensitivity of MSAFP for DS is low even when maternal age is considered, but it is a simple test that is widely available.

Cells of the placenta synthesize hCG. hCG consists of two subunits — an alpha and a beta. Both subunits are required for biological activity, but it is the beta subunit that determines molecular specificity (3). Less than 1 percent of the beta subunit exists in the free form. β-hCG appears in the maternal circulation shortly after implantation and increases rapidly until about eight weeks gestation. The concentration of maternal serum β-hCG then declines slowly to plateau at about 18 weeks to 20 weeks gestation, and then remains fairly constant. Multiple hCG-related molecules can be measured in both maternal serum and urine (4). hCG and its related molecules have been found to be sensitive maternal screening markers for detection of DS. Clinical results may be reported as multiples of the median (MoM) or in conventional units of measure. Human chorionic gonadotropin elevation of at least 2,5 MoM is associated with a DS fetus. Measurement of β-hCG levels is not useful in predicting the occurrence of a NTD. Hyperglycosylated hCG, also known as invasive trophoblast antigen, is a large oligosaccharide variant of hCG. It is the main form of hCG produced in the weeks following implantation and can be measured in the expectant mothers urine as well as her serum (5). In Down Syndrome cases, the median hyperglycosylated hCG value has been reported to be 9,9-fold higher than in unaffected pregnancies (6).

Free beta hCG is the sole biochemical marker presently known to be useful in both first and second trimester screenings. Maternal serum levels of free beta hCG are elevated in DS pregnancies and dramatically reduced in cases of Trisomy 18. The median concentration for free beta hCG in DS is 1,9 MoM compared to a MoM of 1,0 in an unaffected pregnancy. Intact hCG does not discriminate as well since its median MoM is less than 1,3 (7).

Early in the second trimester, the fetus, through a complex series of enzymatic reactions involving the fetal adrenal glands, liver and placenta, synthesizes large amounts of unconjugated estriol (uE3). A portion of the uE3 diffuses across the placenta and can be measured in the maternal serum. Concentration of uE3 in the maternal circulation rises progressively throughout gestation. A decreased level early in the second trimester is an independent predictor for NTD. Elevated MSAFP and low MSuE3 concentrations are highly predictive for NTD (8).

Prenatal testing has certain advantages and in some situations can be a great help. Screen negative results do not require amniocentesis which is an invasive approach. Related risks of amniocentesis include miscarriage, fetal puncture, bleeding and possible infection (7). Thus, amniocentesis is not offered to everyone, but typically offered to women who are 35 years old or older and to women with screen positive Triple Test results. The rate of miscarriage is expected to be 2 percent to 3 percent, and the procedure of amniocentesis carries with it an additional increase in risk of 0,5 percent to 0,8 percent (9).

A variety of situations can lead to elevation of risk, including fetal open NTD, underestimation of gestational age, the presence of multiple fetuses, racial background, placental changes, fetal abdominal wall defects, congenital nephrosis, and fetal death. Correction factors should be used for maternal diabetes mellitus, twin pregnancies, smoking and ethnical origin. The standard adjustment procedure is based on a linear relationship between the marker concentration, expressed as the log of the multiple of the median (MOM) and maternal weight on a linear scale. It has been proposed that maternal weight adjustment may better be performed using a linear relationship between marker concentration expressed in MOM and reciprocal of maternal weight (10).

Accurate estimation of gestational age is essential for valid interpretation of laboratory results. This is more difficult than expected**,** because between 25 percent and 45 percent of women cannot provide a reliable menstrual cycle history, and the prospective mother may inaccurately estimate gestational age. Ultrasound examination is recommended to verify gestational age. A difference of 10 days or more between the estimated gestational age based on patient recollection of her last menstrual period and ultrasound dating requires the laboratorian to recalculate MoMs and modify the previous interpretation of laboratory results.

Even fetal gender appears to influence the maternal serum markers, AFP and β-hCG . In pregnancies with an unaffected female fetus, MSAFP has been reported to be significantly lower (3 percent) than in the presence of a male fetus; free beta hCG levels are higher (7 percent) if the fetus is female (11).

In this study, our aim was to identify if triple test measurements were affected by regional differences. We compared our results with the results of a previous study on Turkish population, and with the values of the manufacturer.

MATERIAL and METHODS

The study group consisted of 984 pregnant women between 14–22 weeks of gestation who present to the hospital in two years time. The mean maternal age for the study group was 26-28 years. Serum samples were obtained at 14 to 22 weeks of gestation.

All three maternal serum analytes had been analyzed in the same time period. Maternal levels of AFP, β-hCG and uE3 were measured immediately using commercially available chemiluminescence immunoassay kits in Immulite analyzer (DPC, Los Angeles, CA, USA). Gestational age was determined from the date of definite last menstrual period and by ultrasound examination (the measurement of biparietal diameter). The original PRISCA software was used for calculating the statistical risk (PRISCA, Prenatal Risk Calculation, TYPOLOG Software/GmbH, Hamburg, Germany).

The calculation algorithms in the PRISCA program are based on the results of Norgaard-Pedersen. Norgaard-Pedersen used discriminant analysis to obtain the risk value. This program combines the biochemical analysis with maternal age and is corrected for race, maternal weight, and maternal smoking. PRISCA does not use a fixed cut-off level. Triple test cut-off levels between 1:100 and 1:400 are permissible in the PRISCA program. A false-positive rate of 5 % has been preset in PRISCA as a predefined value. If the false-positive rate differs from the preset value, PRISCA suggests changing cut-off values in order to achieve the required false-positive rate. Analyte levels were expressed as multiples of the median (MoM) determined by comparison with our own median values.

We used the data obtained by the manufacturer for comparison. These values were representative of different regions of the world and Turkey (12). AFP, β-hCG and uE3 were measured using same chemiluminescence immunoassay kits, used in our study.

STATİSTİCAL ANALYSİS

All statistical analyses and illustrations were obtained with SPSS® (USA) and MedCalc®(Belgium) statistical software. Regression analysis was done by using median values of AFP, β-hCG and uE3 due to age between 14-22 gestational weeks. Correction factor was 1.0 when calculating median values. MOM values were calculated by the following formulae:

MOM= Maternal Serum Marker/Median serum marker (according to gestational age)x Applicable correction factors (1,0)

RESULTS

At the end of data collection, we observed high frequency of subjects (>100 subjects) in gestational weeks 16, 17, 18, and 19; while gestational weeks 14, 20, 21 and 22 were with a lower frequency(<100 subjects). We used the data obtained from high frequency weeks in comparison, and presented low frequency weeks data in sake of acknowledgement.

Table I summarizes the overall results of 245 women (gestational week=16), 267 women (gestational week=17), 205 women (gestational week=18) and 108 women (gestational week=19). Median values of maternal serum AFP were 31,4 IU/ml; 33,8 IU/ml; 35,2 IU/ml and 47,0 IU/ml respectively. Median values of β-hCG were 24390 mIU/ml; 20145 mIU/ml; 16850 mIU/ml and 17514 mIU/ml respectively. Median values of uE3 were 2,6 ng/ml; 3,2 ng/ml; 3,6 ng/ml and 4,7 ng/ml, respectively.

The groups that contained less than 100 subjects were as follows: 50 women in 14 weeks; 68 women in 20 weeks; 36 women in 21 weeks and 10 women in 22 weeks of gestational age. Serum AFP, β-hCG and uE3 values have been shown at Table II. Median values of maternal serum AFP were 20,9 IU/ml; 47,0 IU/ml; 50,0 IU/ml and 73,0 IU/ml respectively. Median values of β-hCG were 18576 mIU/ml; 16668 mIU/ml; 14789 mIU/ml and 19880 mIU/ml, respectively. Median values of uE3 were 2,3 ng/ml; 4,7 ng/ml; 5,3 ng/ml and 6,2 ng/ml, respectively (Table II).

Comparison of median values according to gestational weeks, between Turkish population, manufacturer values and Gaziantep population were demonstrated at Table III. There were differences at median values of serum AFP levels in 16. gestational week between Gaziantep population and Turkish population as 1 %; Gaziantep population and median values by manufacturer as 3 % . In 17. gestational week differences were calculated as 5 % and 4 %; in 18. gestational week as 13 % and 14 %, respectively. 19. gestational week measurements showed no difference.

Same comparison was done for β-hCG; difference at median values of serum β-hCG levels in 16. gestational week between Gaziantep population and Turkish population was 5 % and between Gaziantep population and median values by manufacturer 1 % . In 17. gestational week differences were calculated as 4 % and 2 %; in 18. gestational week as 13 % and 1 %; in 19. gestational week as 1 % and 24 %, respectively.

When we compared the uE3 values; differences at median values of serum uE3 levels in 16. gestational week between Gaziantep population and Turkish population were 36 %, and between Gaziantep population and median values by manufacturer as 95 % . In 17. gestational week differences were calculated as 39 % and 89 %; in 18. gestational week as 25 % and 67 %; in 19. gestational week as 34 % and 72 % respectively.

Table I: Median values of triple test values according to gestational weeks.

Gestational week	AFP (Iu/ml) Median	β-hCG(mIu/ml) median	uE3(ng/ml) median
16 (n=245)	31,4	24390	2,6
17 (n=267)	33,8	20145	3,2
18 (n=205)	35,2	16858	3,6
19 (n=108)	47,0	17514	4,7

Table II: Median values of triple test values according to gestational weeks (number of subjects less than 100).

Gestational week	AFP (Iu/ml) Median	β-hCG(mIu/ml) median	uE3(ng/ml) median
14 (n=50)	20,9	18576	2,3
20 (n=68)	47,0	16668	4,7
21 (n=36)	50,0	14789	5,3
22 (n=10)	73,0	19880	6,2

Table III: Comparison of median values between our results and other studies. Difference respectively (%).

	Gestational week	Gaziantep Population	Turkish population	DPC (Manufacturer)
AFP(Iu/ml)	16	31,4 (1 %, 3 %)	31,5	30,47
	17	33,8 (5 %, 4 %)	35,4	35,16
	18	35,2 (13 %, 14 %)	40,4	40,59
	19	47 (0 %, 0 %)	46,4	46,85
β-HCG(mIu/ml)	16	24390 (5 %, 1 %)	23 100	24 624
	17	20145 (4 %, 2 %)	20 890	20 426
	18	16858 (13 %, 1 %)	19 300	16 943
	19	17514 (1 %, 24 %)	17 250	14 054
uE3(ng/ml)	16	2,6 (36 %, 95 %)	1,9	1,33
	17	3,2 (39 %, 89 %)	2,3	1,69
	18	3,6 (25 %, 67 %)	2,86	2,15
	19	4,7 (34 %, 72%)	3,5	2,73

DISCUSSION

Our results clearly present the regional variation in parameters of maternal triple test. Of concern, was the percentage differences in uE3, reaching 95 % and 89 % respectively in gestational weeks 16 and 17. Apparently, uE3 was the most affected parameter among the parameters used in screening test, in means of regional variations. AFP and β-hCG did also showed quite important differences between groups, although not so prominent as uE3. The common sense holds the suggestion that users should use their local values at best. In addition, we suggest, extreme caution on uE3 when dealing such an exercise.

Screening programs are encouraged to calculate their weight correction formulae, based on data from their own population and to monitor the mean maternal weight to detect when modifications in the weight correction formula might be indicated. Hsu et.al. reported that there was no discernible effect in maternal weight adjustment. It was worth making weight corrections for serum marker levels in order to reduce individual variance. In their study, the mean maternal weight was 54,95 $\pm$ 7,36 kg in Taiwanese pregnant women during the second trimester (13). We observed the mean maternal weight as 65,13 $\pm$ 11,10 kg in Gaziantep population. Significant differences in analyte medians existed after adjustments were made for patient weight. The differences observed in various studies may be attributable to the inconsistent use of weight correction, heterogeneity in the gestational ages of the screened populations, maternal age differences and regional differences in the subpopulation of women.

The reliability of gestational age determination may also be a source of variability in establishing medians for different groups. Gestational age determination on the basis of time from the last menstrual period can show a bias towards overestimation and the preferential use of ultrasound biometry to determine gestational age could lead to minor differences in median values (14). The standard adjustment procedure is based on a linear relationship between the marker concentration, expressed as a log of multiple of the median(MOM) and maternal weight on a linear scale. It has been proposed that maternal weight adjustment may be better performed using a linear relationship between marker concentration expressed in MOM and reciprocal of maternal weight (15).

Chromosomal abnormalities increase with advancing maternal age. The most common chromosome abnormality is trisomy 21 or Down Syndrome, which occurs in 1 of 800 live births. An extra copy of the long arm region q22.1 to q22.3 of chromosome 21 results in a phenotype consisting of mental retardation, hypotonia, congenital heart defects and flat facial profile. Neural tube defects occur early in embryonic development and cause very serious birth defects. The incidence of neural tube defects varies with geography and ethnicity. Almost all cases of

neural tube defects are open and therefore in direct communication with the amniotic fluid. Thus, fetal serum proteins penetrate into the amniotic fluid. Elevated AFP concentration in amniotic fluid leads to increased amounts in the maternal circulation. About 90 % of open neural tube defects can be detected using maternal serum AFP testing (9). Trisomy 18 is a severe chromosomal abnormality with an incidence of about 1 in 8000 infants born alive. Two thirds of cases diagnosed at the time of amniocentesis are spontaneously aborted before delivery. Growth restriction and other problems have led to a cesarean section rate of approximately 50 % in cases not diagnosed before delivery. The prognosis after delivery is poor, with at most 10 % survival for 1 year. For this reason, serum screening for trisomy 18 represents an attractive option (10, 15- 18).

In conclusion, this study showed that, screening tests may vary based on regional differences. PRISCA already corrects biochemical analysis results for race, maternal weight, and smoking. However, all precautions are fallible, and it is sometimes impossible to include all variables that bare a potential to affect the results. To the utmost importance, the users must closely follow-up their false-positive rate, and when a significant shift is observed elaborate investigations must be performed. The clinician should not be reluctant to provide feedback about test results that will alleviate the stress on the laboratory. Our study presents regional differences as a potential risk factor that may be an explanation for some problems with the triple test evaluations in some laboratories.

REFERENCES

1. UK Collaborative Study. Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of UK collaborative study on alpha-fetoprotein in relation to neural-tube defects. Lancet 1977:1(8026):1323-32.

2. Alldred SK, Deeks JJ, Guo B, Neilson JP, Alfirevic Z. Second trimester serum tests for Down's Syndrome screening. Cochrane Database Syst Rev 2012;6:CD009925.

3. Bredaki FE, Poon LC, Birdir C, Escalante D, Nicolaides KH. First-trimester screening for neural tube defects using alpha-fetoprotein. Fetal Diagn Ther 2012;31(2):109-14.

4. Ashwood ER. Evaluating health and maturation of the unborn: The role of the clinical laboratory. Clin Chem 1992;38(8):1523-9.

5. Chard T. Biochemistry and endocrinology of the Down's syndrome pregnancy. Ann N Y Acad Sci 1991;626:580-96.

6. Cole LA. Immunoassay of human chorionic gonadotropin, its free subunits, and metabolites. Clin Chem 1997;43(12):2233-43.

7. Butler SA, Khanlian SA, Cole LA. Detection of early pregnancy forms of human chorionic gonadotropin by home pregnancy test devices. Clin Chem 2001;47(12):2131-6.

8. Cole LA, Shahabi S, Oz UA, et al. Hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen) immunoassay: a new basis for gestation Down Syndrome screening. Clin Chem 1999;45(12):2109-19.

9. Genecare. Screening for Down Syndrome, Trisomy 18 syndrome and open neural tube defects in the first trimester. At http://www.genecare.com/ 1st Tri Scr PT.html Accessed on: 06.12.2001.

10. Knight CJ, Palomaki GE, Neveux LM, et al. HCG and the free beta-subunit as screening tests for Down syndrome. Prenat Diagn 1998:18(3):235-45.

11. Leshin L. Prenatal testing for Down Syndrome. At http://www.DownSyndrome-health.com/prenatal. htm. Accessed on: 21.03.2000.

12. http://www.typolog.de/welcome.html Turkish language files update: 12.07.2011.

13. Hsu JJ, Hsieh TT, Soong YK, Kuo B. The influence of maternal weight correction formulae in Asian Down Syndrome screening using alpha-fetoprotein and free beta-human corionic gonadotropin. J Maternal Fetal Investig 1998;8(2):66-70.

14. Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry. 2nd Edition. Philadelphia: Saunders, 1994:2121–2.

15. Yankowitz J, Fulton A, Williamson R, Grant SS, Budelier WT. Prospective evaluation of prenatal maternal serum screening for trisomy 18. Am J Obstet Gynecol 1998;178(3):446-50.

16. Watt HC, Wald NJ. Alternative methods of maternal weight adjustment in maternal serum screening for Down Syndrome and neural tube defects. Prenat Diagn 1998;18(8):842-5.

17. Spencer K. The influence of fetal sex in screening for Down Syndrome in the second trimester using AFP and free beta-hCG. Prenat Diagn 2000;20(8):648-51.

18. Benn BA, Clive JM, Collins R. Medians for second-trimester maternal serum α-fetoprotein, human corionic gonadotropin, and unconjugated estriol; differences between races or ethnic groups. Clin Chem 1997;43(2):333-7.