Determination of the Median Levels of Triple Test Screenıng Parameters in Our Region

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Abstract

Objective: The purpose of this study was to determine the median values of the triple test screening parameters in pregnancy and reevaluate the risky pregnancies according to the classical cut-off values by the way of the new median values in our region.

Methods: In this study we evaluated the serum hormon values of 700 pregnant women who admitted to Biochemistry Laboratory for the prenatal triple test between 2003-2006.

Results: The median values of 1130 patients were calculated for each parameters between the gestational 16-19 weeks. We found that the alpha feto protein median values were significantly low according to the values used 16th-19th weeks ($p< 0.05$).

Conclusion: As a result we conclude that using the median values of a specific region during the evaluation of prenatal risk will be the matter of fact and further unnecessary evaluations can be prevented.

Keywords: Prenatal diagnosis, screening tests, median, Down syndrome.

Üçlü test tarama belirteçlerinin bölgemize ait medyan değerlerinin belirlenmesi

Amaç: Bu çalışmadaki amacımız,gebelik taramasında kullanılan üçlü test tarama belirteçlerinin bölgemize ait medyan değerlerini belirlemek ve kullanılan medyan değerlere göre saptanan riskli gebelikleri yeni medyanlara göre tekrar değerlendirmektir.

Yöntem: Başkent Üniversitesi Alanya Uygulama ve Araştırma Merkezi Biyokimya laboratuvarına 2003-2006 yılları arasında üçlü tarama testi yaptırmak için başvuran 16-19 gestasyonel haftalar arasındaki toplam 1360 gebede ölçülen üçlü test biyokimyasal belirteçlerin medyan değerleri retrospektif olarak incelendi. Kullanılan programda girilmiş olan medyan değerleri ile arasındaki farklar araştırıldı. Riskli kabul edilen gebeliklerin risk durumu yeni medyanlara göre tekrar değerlendirildi.

Bulgular: Her bir belirteç için 16-19 gestasyonel haftalar arasına ait medyan değerleri hesaplamayı etkileyecek veriler çıkarıldıktan sonra toplam 1130 gebe üzerinden belirlendi. Bulduğumuz alfa-fetoprotein medyan değerleri kullanılan medyan değerlerine göre 16-19. haftalarda anlamlı oranda düşük olarak tespit edildi ($p<0.05$).Human koryonik gonodotropin medyan değerlerinde 17. haftada anlamlı oranda düşüklük tespit edilirken ($p<0.05$),16,18 ve 19. haftalarda anlamlı bir artış olduğu gözlendi ($p<0.05$). Ankonjuge östriol medyan değerleri 18. haftada anlamlı oranda olmak üzere ($p<0.05$) 16,17 ve 19. haftalarda düşük olarak saptandı. Önceden riskli olarak belirlenen ve medyan hesaplaması sırasında analize dahil edilmeyen 156 gebenin %17.9'u (28 gebe) yeni medyan değerlerine göre riskli durumdan çıktığı tespit edildi.

Sonuç: Prenatal risk değerlendirmesi sırasında kullanılmakta olan programlara girilmiş veriler yerine bölgelere ait medyan değerlerinin kullanılması ile anneye ve fetüse risk getiren gereksiz invazif girişimlerin önlenebileceği kanısındayız.

Anahtar Sözcükler: Prenatal teşhis, tarama testleri, medyan, Down sendromu.

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Introduction

Genetic disorders are an important group of disorder giving rise to mental and physical defects and also social and economic problems together. As a result of having no way of treatment, the preventive prenatal diagnostic studies come out. One of the studies is a screening test performing at pregnant women between 16-20 weeks of pregnancy which is known as triple test.[1,2] Screening tests are used for revealing the frequency of a little group of patient carrying high risk for a specific anomaly in a big group of society. After the risk estimation, high and low risk groups can be defined by using an obvious cut-off value. There can be also false positive values in group of having positive screening test results.[2] It is important that this false positive result ratio should be in an acceptable level because these patients undergo invasive tests like amniocentesis and corion villus biopsy which has the risk of fetal exitus, spontane abortus and intrauterine exitus between 2.4-%5.2%[3] In 1988 Prof. Dr. Wald developed the Triple Screening test in London. Trizomi 21 patients can be determined by these tests as the ratio of 60-65% by evaluating the chemical indicators as maternal age, AFP, β-hCG and unconjugated ostriol (μE3) together. According to the metaanalysıs the negative sides of these tests are the low diagnostic (67%) and false positivity (5%) rates. However, suggesting advanced investigations Down Syndrome determination ratio changes 1/25 to 1/77 in pregnants. Determination of neural tube defects with the test is much higher.[3-7] The secretion levels of maternal alpha feto-protein (AFP), Human corionic gonadotrophin Beta (β-hCG) and μE3 are independent from gestational week and are giving more useful information about the risk than the maternal age. While AFP and μE3 levels are increasing in the second trimestr of pregnancy the β-hCG levels go down. Because of this, for the purpose of easiness and obtaining the equation between units, the values of all three parameters are converted to multiple of median (MOM) unit by the way of dividing the median values by matching values of the pregnancy week. To determine the gestational week, screening programs generally use the calculated gestational age with ultrasonographic (USG) biparietal diameter (BPD). For a numerical estimation it is statistically required to take into consideration of the risk with maternal age and some other factors. This evaluation and the determination of estimated risk values can be done by using a pocket computer programme. It has shown that the diagnostic ratio of triple test by using the measurement of only the maternal AFP is 33% where as including β-hCG this ratio rises to 53%, and also including μE3 it rises to 58%. The level of these markers which are effected with many factors like race, geographical distribution should be determined according to the region as many routine biochemical levels.[7-9] It is determined that the level of AFP MOM is decreasing (0.7 MOM) and β-hCG serum level is increasing (2.5 MOM) in Down Syndrome where as AFP MoM level is increasing (3 MOM) at fetüs with NTD.[5-7] Because of its being cheap and simple triple test takes an important place in prenatal diagnosis also in our country. The reliability of the risk estimation by the application of triple test is closely correlated with median values those using for a specific region. The purpose of this study is to determine the median values of triple test markers in our regional population and to reevaluate the population that tests results are positive according to the previous computer programme.

Methods

In our study we evaluate the data of 1360 pregnants who admitted to our hospital biochemistry laboratory to have a triple screening test between 2003 and 2006 retrospectively. Their gestational age was 16-19 (16+0 and 19+6) weeks and were living in Alanya and environment. We evaluated the levels of AFP, β-hCG and μE3 and also the gestational age according to the biparietal diameter (BPD) determined ultrasonographically. The serum levels of AFP, β-hCG and μE3 has included as data to evaluate those we obtained with IMMULITE ONE equipment (Diagnostic Products Corporation, ABD) which run with chemiluminesans method and belongs to BIO-DPC company. The MoM values were calculated comparing these three marker values obtained according to the gestational week with the median values of normal gestational population. The screening test positive pregnancies were determined by analizing of obtained MOM levels of AFP, β-hCG , μE3 and maternal age with other data as maternal weight, smoking, DM and twin pregnancies statistically with Prisca 4.0 (Prenatal Risk Calculation, TYPOLOG Software/ GmbH, Hamburg, Germany) programme. The MoM values were calculated by comparing these three hormone levels obtained according to the gestational week with the median values normal gestational population. During the calculation of medians, the screening positive pregnancies determined by previous programme, patients having risks related with hormones (β-hCG 2.5 MoM and higher, 0.4 MOM and lower, for AFP and μE3 0.4 MOM and lower), twin pregnancies and having demographic data affecting the evaluations were excluded from the study. Previous screening tests positive pregnancies were reevaluated according to the new median values. Triple test cut-off values were accepted as 1/250 for Down Syndrome and 1/100 for Trisomi 18. SPSS 11.0 (SPSS-11.00, Inc, Chicago, USA) programme was used for statistical analysis. To evaluate the normal distribution of data Kolmogorov ¡VSmirnov test was used. To evaluate the differences between the normal disrtributed data Student-t test and Mann Whitney-U test for the other differences between the datas. Values of $p<0.05$ were considered statistically significant.

Results

156 of 1360 pregnants were excluded because of having positive screening test previously. 60 of remaining 1204 patients had at least one MOM value which was out of the stated interval and 14 patient had twin pregnancies . These were also excluded from the study. Median values for each marker concerning 16-19 gestational week were calculated of 1130 pregnant. Seperately and determined the percentage difference and significantly comparing with the stated median values. The demographic data of pregnants and serum marker values distribution is summarized in table1. We found that according to the stated median values AFP median values were significantly low as 18.6% at week 16%., 21.4 at week %17, 18.6 at week 18 and 14.9% at weeks 19 ($p<0.001$). β-hCG median

Table 1. The demographic data of pregnants and serum marker values distribution.

	Minimum	Maksimum	Median ± SEM*
Age	18	43	28 ± 0.13
Gestational week	15	21	17.2 ± 0.30
Weight (kg)	46	116	64 ± 0.32
BPD	28	50	37 ± 0.11
AFP (IU/ml)	14.5	95	32.50 ± 0.35
β-hCG (mIU/ml	2260	60775	20961 ± 309
μE3 (ng/ml)	0.77	9.10	2.70 ± 0.03
AFP (MOM)	0.41	2.92	0.81 ± 0.00
β-hCG (MOM)	0.41	2.47	1.05 ± 0.01
μE3 (MOM)	0.42	7.08	0.93 ± 0.01

Table 2. AFP median values for gestational weeks, the percentage differences and significancies.

	Situated Median	AFP Living Median	Different %	P*
16. Week	30.10	36.96	-18.6	**0.000**
17. Week	31.80	40.47	-21.4	**0.000**
18. Week	38.10	46.81	-18.6	**0.000**
19. Week	44.40	52.20	-14.9	**0.000**

Table 3. β-hCG, median values for gestational weeks, the percentage differences and significancies.

	Situated Median	β-hCG Living Median	Different %	P*
16. Week	25000	23975	+4.3	**0.000**
17. Week	20803	20979	-0.8	**0.000**
18. Week	18026	16943	+6.4	**0.003**
19. Week	16340	14435	+13.2	**0.000**

Table 4. µE3 median values for gestational weeks, the percentage differences and significancies.

	Situated Median	E3µ Living Median	Different %	P*
16. Week	2.10	2.39	**-12.1**	0.521
17. Week	2.85	2.89	**-1.4**	0.687
18. Week	3.20	3.69	**-13.3**	**0.014**
19. Week	4.20	4.29	**-2.1**	0.233

values were significantly high as 4.3% at week 16. (0.000), 6.4% at week 18. (0.003) and 13.2% at week 19. (0.000) and significantly low as 0.8% at week 17. (0.000). Serum µE3 median values were significantly low as 13.3% at week 18. (0.014), and 12.1% at week 16. (0.521), 1.4% at week 17. (0.687) and 2.1% at week 19 (0.233).

The percentage differences and significancies between the stated and the values those we found are summarized in table 1,2,3 and 4. The median value distribution of AFP, β-hCG and µE3 concerning the gestational week and difference graphics are shown in fig. 1, 2 and 3 (A and B). 28 (17.8%) of 156 pregnant who were excluded from the study because of having positive screening test result for the stated median value were determined to be out of the positivity according to the new median levels.

Discussion

The aim of the measurements in clinical laboratory is to diagnose, to follow and to evaluate the health condition. It is observed that widespread application and to put the statement "

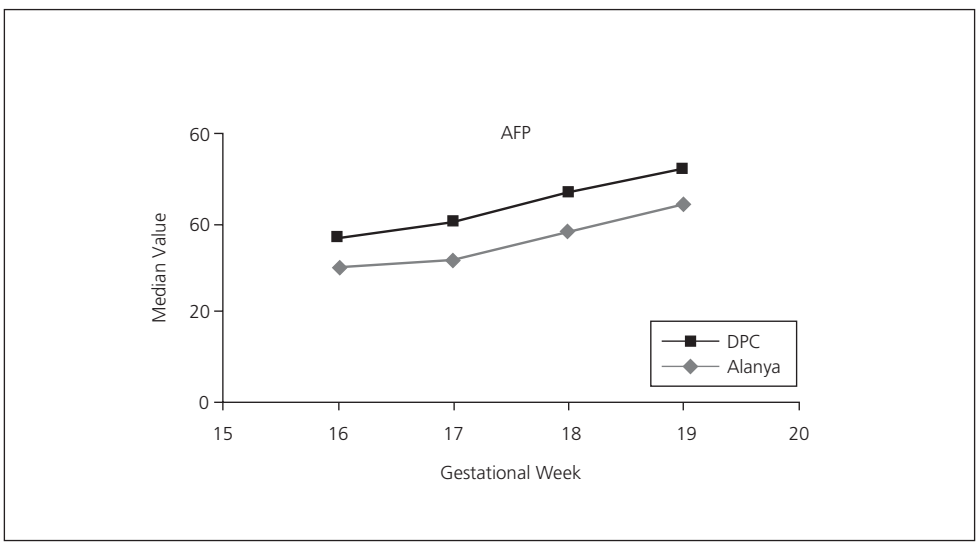

Figure 1a. The median values distribution for AFP levels in gestational weeks.

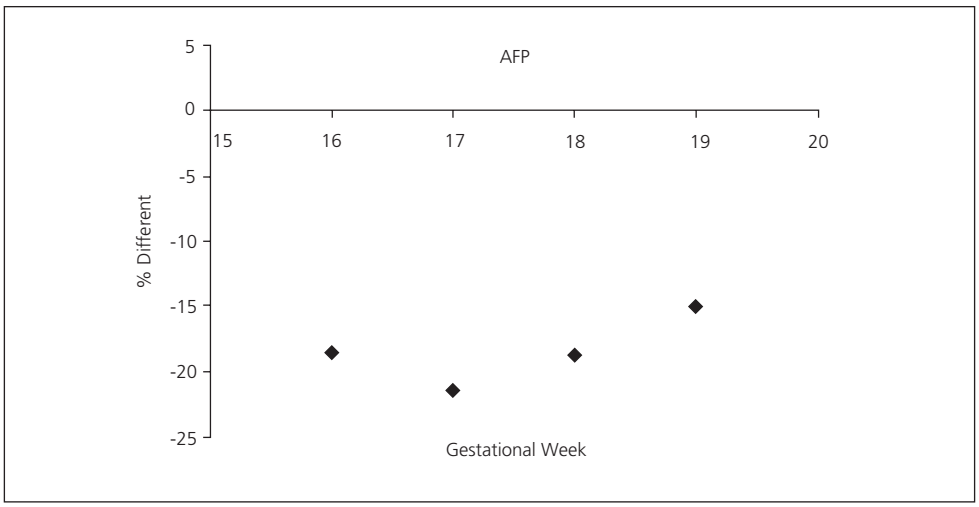

Figure 1b. AFP different figure.

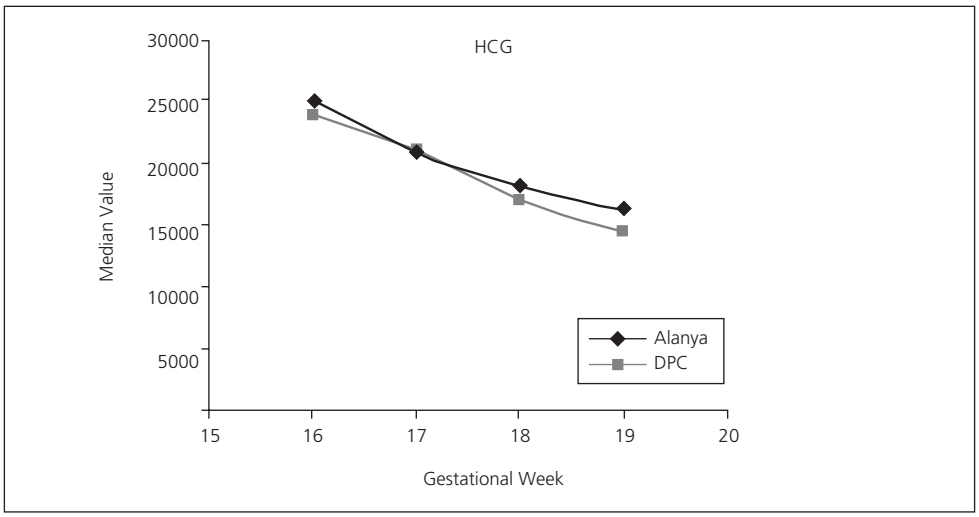

Figure 2a. The median values distribution for β-hCG levels in gestational weeks.

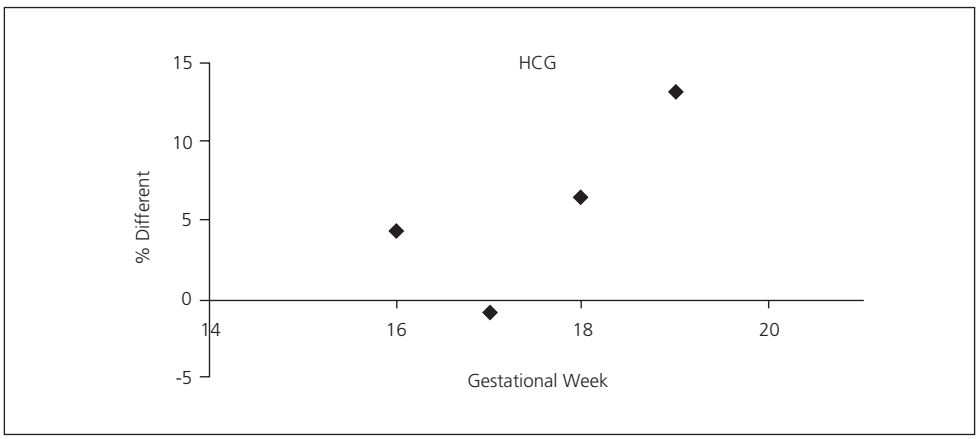

Figure 2b. β-hCG different figure.

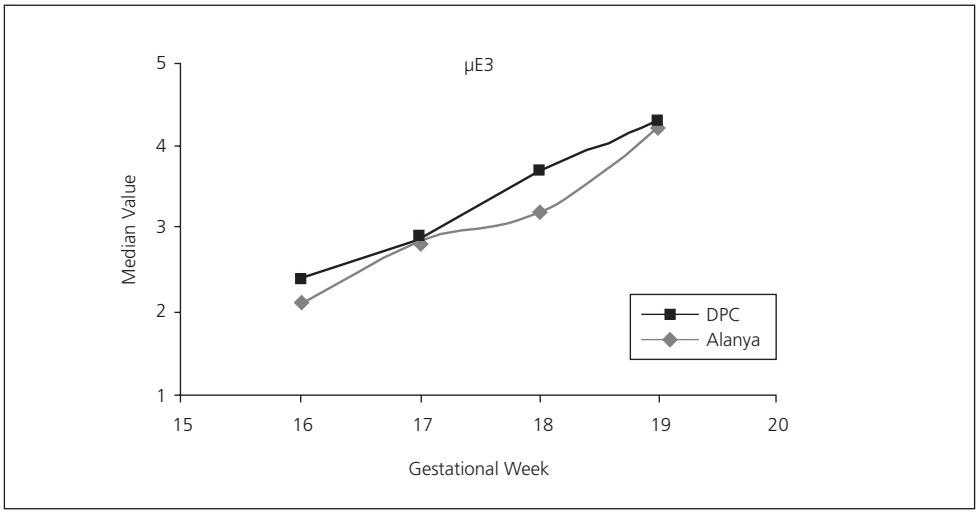

Figure 3a. The median values distribution for μE3 levels in gestational weeks.

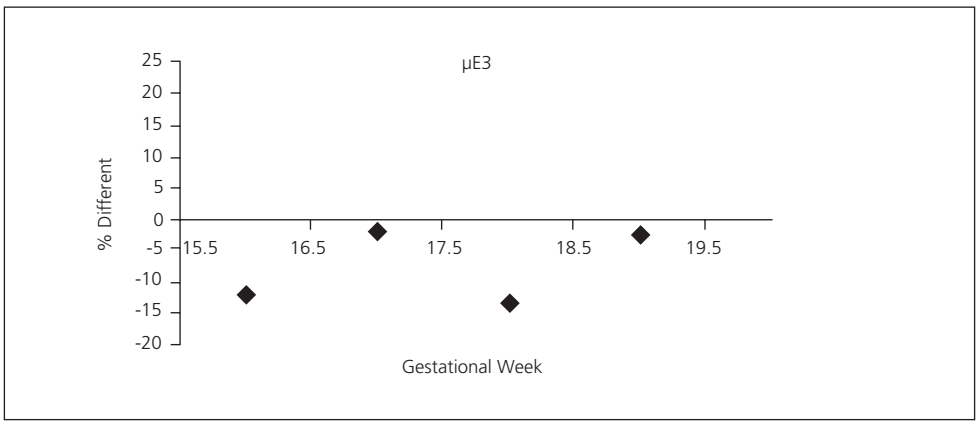

Figure 3b. μE3 different figure.

All laboratory should calculate its own reference interval" into practice is difficult because of the methodologic and regional differences although it is an accepted decision at international platform. In prenatal risk screening risk calculations are done by the MOM values so the detection of the local regional median values have more importance.[10] Laboratory test results should not be the cause of dilemma concerning especially the critical decision levels. Besides these values can affect the physician's judgement can also be the cause of negativeness in patients life. For these reasons the in order to evaluate the risk with triple screening tests application of the analysis should be reliable and determination of the median values should fit with the society and the laboratory conditions.[11,12] The excessive cost and the affects on taking extremely important decisions with the results make this subject actual. Following the evaluation some patients undergo invasive procedures (amniosentesis, corion villus biyopsy, fetal blood exam) unnecessarily while the other patients are being excluded from the risk group although they should be in. In our country the acceptance rate of amniosynthesis

among risky pregnancies is low. Low level of education and economical condition of the patient group can be the cause of this situation. Kaya et al. stated the necessity of suggesting screening tests also

to young pregnants preceeding the invasive procedures.[13] In recent years the studies concerning the regional median values arrangement and comparing the different measurement parameters revealed the changeable properties of the risk factors.[11-14] In their studies Johnson et al. considering the maternal weight and race they stated the necessity of using MOM values.[15] Raynaolds et al. revealed that differences in weight corrected MOM values estimated with the two approaches are highly significant ($p<0.001$).[16] Wald et al. showed in their study that MOM adjustment for values in a previous pregnancy improves overall screening performance and substentially reduces the high recurrent false-positive rate.[17] This adjustement can be routinely applied in screening programmes through the screening software used to interpret a women's screening results. Knight et al. stated that the percentages used for calculating the MOM values are sensitive to inaccurate and imprecise assays,inappropriate reference data and long term assay drift.[18] Xia et al. showed that screen positive pregnancies had increased risk of chromosomal abnormalities. Pregnancies with positive screening results had significantly higher risk of adverse outcomes than those with negative results ($p<0.05$).[19] According to the new median levels 28 (17.9%) of 156 previously risky pregnants were come out of the risky status and follow ups showed that there were no chromosomal abnormalities.

Conclucion

In our study we concluded that it is important to use the regional median values in screening tests instead of programmed for preventing the fetus and the pregnant from the risky and invasive procedures.

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