Orjinal Araştırma Makalesi/Original Paper

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Determination of Median Values of First Trimester Screening Tests: A Tokat Scale Retrospective Study

Birinci Trimester Tarama Testlerinin Medyan Değerlerinin Belirlenmesi: Tokat Ölçekli Retrospektif Bir Çalışma

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ÖZET

Amaç: Birinci trimester tarama testinde bakılan maternal serum gebelikle ilişkili plazma protein A (PAPP-A) ve serbest beta-human koryonik gonadotropin (free ß-hCG) değerlerinin daha sonraki gebelik haftalarında gelişebilen komplikasyonları tahmin edebilme kapasitesini ve ikili tarama testlerimizin performansını artırabilmek için hastanemize ait medyan değerlerini hesaplamayı amaçladık.
Gereç ve yöntem: Çalışmaya Tokat Gaziosmanpaşa Üniversitesi Tıp Fakültesi Eğitim ve Araştırma Hastanesi Biyokimya Laboratuvarına başvuran 16-46 yaş arası, gebelik yaşları 10 hafta 6 gün ile 13 hafta 6 gün arasında tekil canlı gebeliği olan, kötü obstetrik öyküsü ve sistemik bir hastalığı bulunmayan ve sigara kullanmayan 3166 gebenin sonuçları retrospektif olarak değerlendirildi. Hastane laboratuvarına özel medyan değerleribelirlenerek, kullanılan yazılım programının değerleriyle karşılaştırıldı.
Bulgular: Hastaların yaşlarının ortalaması 27,43±5,46 ve ağırlıkları 65,66±13,13 kilogramdı. Baş popo mesafeleri (CRL) 60,71±8,56 mm olarak belirlendi. Serbest ß-hCG değerleri, 55,1±132,07ng/mL, PAPP-A değerleri ise 3683,53±2486 mIU/l olarak ölçüldü. Ense kalınlığı ölçümü (NT) ise 1,38±0,37mm olarak saptandı. PAPP-A ve Serbest ß-hCG MoM değerleri sırasıyla 1,23±0,68 ve 1,23±0,88 idi. β-hCG'nin yeni medyan değerlerinin, programdaki medyan değerlerinden anlamlı olarak düşük olduğu tespit edilirken ($p<0.05$), PAPP-A değerlerinin ise anlamlı şekilde yüksek olduğu görüldü ($p<0.05$).
Sonuç: Sonuç olarak, nöral tüp defekti ve kromozomal anomalilerin tanısında kullanılan ve ileri girişimsel işlemler için yol gösterici olan, birinci Trimester tarama testlerinin doğruluğunun ve performansının artırılmasının önemliolduğu, bölgeye ve hatta laboratuvara özel medyan değerlerinin belirlenmesinin de artık kaçınılmaz bir hale geldiği kanısına varılmıştır.

Anahtar kelimeler: Free ß-hCG, PAPP-A, İkili tarama testi, Birinci Trimester

ABSTRACT

Objectives: The aim of this study was to determine the ability of maternal serum plasma protein A (PAPP-A) and free beta-human chorionic gonadotropin (free ß-hCG) values measured in the first trimester screening test to predict the complications that may develop in later gestational weeks, calculate the median values of these parameters and compare them with those of software we use.
Materials and methods: The study included 16-46 years old women who applied to biochemistry laboratory of Tokat Gaziosmanpasa University School of Medicine with gestational ages of 10 weeks and 6 days to 13 weeks and 6 days. The results of 3166 pregnant women were evaluated retrospectively.
Results: The mean age of the patients was 27.43 ± 5.46 and their weight was 65.66 ± 13.13 kilograms. Crown rump lengthes (CRL) were determined as 60.71 ± 8.56 mm. Free ß-hCG levels were 55.1 ± 132.07 ng / mL and PAPP-A values were 3683.53 ± 2486 mIU / l. Nuchal translucency measurements (NT) were determined as 1.38 ± 0.37mm. PAPP-A and Free ß-hCG MoM values were 1.23 ± 0.68 and 1.23 ± 0.88, respectively. New median values of β-hCG were found to be significantly lower than those of the program ($p <0.05$), while PAPP-A values were significantly higher ($p <0.05$).
Conclusion: The accuracy and performance of first trimester screening tests should be improved. Determination of median values specific to the region and even to the laboratory is now inevitable

Key words: Free ß-hCG, PAPP-A, Binary screening test, First Trimester

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INTRODUCTION

Screening tests are performed to identify a specific group that carries a certain level of risk for specific diagnostic evaluation in a healthy population (Şanlı and Kartkaya, 2011). Hereditary diseases such as Down (Trisomy 21), Edward (Trisomy 18), Patau (Trisomy 13) syndromes and Neural Tube Defect cause physical and mental disorders that lead to both social and economic problems. The most common chromosomal anomaly in the newborn is Down Syndrome and its prevalence is 1/800. In the 1970s, only maternal age was used for prenatal screening of hereditary diseases. Therefore, all mothers over the age of 35 were considered to be at risk and were referred for amniocentesis. However, only one third of the cases could be detected (James et al., 2008). Maternal age was found to be an inadequate screening method and in the 1980s. In the screening method developed by N. J. Wald, in the first trimester, various analytes detected of maternal serum were combined with maternal age. It has been increasingly used in the last 30 years as the average gestational age increases.

In the 1990s, when the population of women aged thirty-five years and over is examined, It was determined that increase in nuchal translucency (NT) thickness determined by ultrasonography between 10-15 weeks of gestation was related to increase in maternal serum free beta-human chorionic gonadotropin (freeβ-hCG) and decrease in pregnancy related plasma protein-A (PAPP-A) (Nicolaides et al., 1992; Brizot, Snijders et al., 1994). As a result, dual-marker screening captures 75% or more of pregnancies affected by trisomy 21 and other aneuploidies (Kappel et al., 1987), while the rate of detecting false positives decreases to 5% (Cuckle 2001; Kagan et al,. 2017).

Depending on the result of the selected screening test, it is then decided whether interventional tests are necessary for diagnosis. Combining screening tests and diagnostic tests ensures that the maximum number of patients can obtain accurate information about their personal risk status (James et al. 2008). With the development of prenatal screening tests, the need for interventional procedures such as chorionic villus sampling and amniocentesis has decreased. Interventional diagnostic procedures can cause serious complications; such as bleeding, preterm labor and fetal loss (Marteau et al., 1992; Ananth et al., 2017). The psychological dimension of all tests and interventional procedures that can affect both pregnant and fetus is also important (Marteau et al., 1992). Interventional diagnostic tests are known to have fetal loss rates of 1.5% in chorionic villus biopsy, 2% in amniocentesis performed in first trimester and 1% in amniocentesis performed in the second trimester. According to the results of the second trimester screening test, the risk of Down Syndrome must be 1/250 and higher in order to recommend interventional tests (Creasy et al., 2004). Laboratories unitize the values of the measured biochemical parameters in multiples of median (MoM) calculated according to the gestational week to be standard, more understandable and easier to evaluate. Mom value is calculated by dividing the analysis result by the median value of that analyte for the week of gestation (Assessment, 2000).

In calculating these values, each geographic region, even each clinical laboratory in the region, should estimate its own median values and evaluate the screening tests according to this median average (Alp et al., 2018).

Furthermore, when calculating MoM values, adjustments can be made by taking into account other maternal factors such as maternal age, weight and race that affect analyte levels. Today, MoM values; Down Syndrome, Trisomy 18 and neural tube defect risk are commonly used to standardize biochemical analyte values and convert them into a more interpretable unit.

In this study, we aimed to evaluate retrospectively the data of binary screening tests that we have worked in our hospital laboratory within two years and to calculate the median values of our screening tests, especially to improve the performance of our double screening tests.

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MATERIAL AND METHODS

In this study, the results of pregnants (n=3166) who are living in or around Tokat city and applied to the Tokat Gaziosmanpaşa University Medical Faculty Hospital Central Laboratory for the double screening test, between January 2017 and December 2018 were evaluated retrospectively.

They were between 16-46 years of age, their gestational ages were between 10 weeks and 6 days to 13 weeks and 6 days. They had a live single pregnancy, no poor obstetric history and no systemic disease and who did not smoke were evaluated. Diabetic pregnants, smoking pregnant women, twin pregnancies and those who became pregnant by in vitro fertilization (IVF) method were excluded from the study.

Fetal NT values, serum PAPP-A and free β-hCG values of pregnant women between the 11 and 14th weeks of gestation were used for statistical analysis.

All of the biochemical parameters in blood samples taken for paired screening tests were measured on the IMMULITE 2000 device (Diagnostic Product Corporation, USA), which was operated by chemiluminescence immunoassay.

The SsdwLab 5 program is used in the laboratory to determine the risk in a double-screening test. The risk of Down Syndrome must be 1/250 and higher in order to define as high risk.

Descriptive analyzes were conducted to give information about the general characteristics of the study groups. Data of continuous variables were expressed as mean ± standard deviation; categorical variables are given as n (%).

When comparing the averages of the quantitative variables between the groups, the significance test of the difference between the two means and the one-way analysis of variance are used. Pearson correlation coefficient is used for correlation between quantitative variables. p values less than 0.05 were considered statistically significant.

In the calculations, ready-made statistical software was used (IBM SPSS Statistics 19, SPSS inc., An IBM Co., Somers, NY).

RESULTS

Demographic data of the pregnant women participating in the study, the values of biochemical tests and MoM values of these tests are summarized in Table 1.

Comparison of quantitative variables according to gestational week was given in Table 2. In Table 3, qualitative variables are evaluated. In Table 4, the risk status of trisomy 21 is compared with qualitative variables.

The reports given to all pregnant women included in the study were evaluated and the rates of pregnant women reported at high risk were evaluated. 1.9% (59 pregnant) of 3166 pregnant women included in the dual screening test was reported to be at high risk for Down Syndrome using the median values available in the program. 0.1% (2 pregnant) of the pregnant women was found to be at high risk for trisomy 18.

In the correlation studies between the quantitative variables, a negative correlation was found between the weight of the pregnant women and the PAPP-A values, a positive correlation between CRL and PAPP-A, and a negative correlation between CRL and NT MoM.

There was a weak positive correlation between β-hCG and β-hCG MoM. There was a very strong positive correlation between NT and NT MoM and a weak positive correlation between NT and the risk of trisomy 21. There was a weak positive correlation between NT MoM and the risk of trisomy 21.

In Table 5, the median values obtained from the double screening results were compared with the median values of SsdwLab5 software in pregnant women admitted to our hospital, and it was estimated that the new median values of β-hCG were significantly lower than those of the program ($p < 0.05$) and PAPP-A values were significantly found to be high ($p < 0.05$).

Table 1: Quantitative variable distribution

	n	Mean	Standard Deviation	Minimum	Maximum
Age (Years)	3166	27,43	5,46	16,00	46,00
Weight (Kilograms)	3166	65,66	13,13	,00	144,00
CRL (mm)	3166	60,71	8,56	41,00	84,00
β-HCG(ng/mL)	3166	55,10	132,07	3,43	3514,42
PAPPA(mIU/l)	3166	3683,53	2486,00	363,00	25501,00
NT (mm)	3166	1,38	,37	,50	3,70
β-HCG MoM	3166	1,23	,88	,16	8,81
PAPPA MoM	3166	1,23	,68	,09	6,13
NT MoM	3166	,89	,25	,26	2,74
Age Risk (%)	3166	,0016	,0026	,0006	,0609
Trisomy 21 Risk (%)	3166	,0006	,0052	,0000	,1780
Trisomy 18 Risk (%)	3166	,0001	,0040	,0000	,2265

Table 2: Distribution of quantitative variables according to gestational week

	Gestational Week				p
	11	12	13	14	
Age (Years)	27,86±5,69 (ab)	27,15±5,37 (a)	27,71±5,47 (b)	27,85±5,68 (ab)	0,017
Weight (Kilograms)	65,88±12,7	65,15±13,27	66,18±12,98	67,63±13,26	0,063
CRL (mm)	47,55±1,9 (a)	57,27±3,56 (b)	68,9±3,51 (c)	79,29±2,16 (d)	<0,001
β-HCG(ng/mL)	55,2±95,8 (ab)	60,6±136,5 (a)	48,9±141,1 (ab)	30,46±77,17 (b)	0,018
PAPPA(mIU/l)	2271,4±1555,5 (a)	3269,1±2124,6 (b)	4599,2±2666,9 (c)	5956,7±3378,1 (d)	<0,001
NT (mm)	1,31±0,41 (a)	1,36±0,36 (a)	1,44±0,35 (b)	1,48±0,39 (b)	<0,001
β-HCG MoM	1,17±0,83 (a)	1,27±0,91 (b)	1,23±0,9 (b)	0,96±0,57 (a)	<0,001
PAPPA MoM	1,31±0,77 (a)	1,26±0,71 (a)	1,17±0,59 (b)	1,09±0,56 (b)	<0,001
NT MoM	1,01±0,31 (a)	0,91±0,25 (a)	0,83±0,21 (b)	0,77±0,2 (b)	<0,001
Age Risk (%)	0,0019±0,0042	0,0015±0,0026	0,0015±0,0019	0,0017±0,0021	0,158
Trisomy 21 Risk(%)	0,0016±0,012 (a)	0,0005±0,0035 (b)	0,0005±0,0036 (b)	0,0002±0,0011 (b)	0,002
Trisomy 18 Risk(%)	0±0,0002	0±0,0003	0,0002±0,0073	0±0,0001	0,561

One way analysis of variance was used. (abcd): The common letter as a line indicates statistical insignificance.

Table 3: Distribution of qualitative variables

		n	%
Gestational Week	11	362	11,4
	12	1689	53,3
	13	975	30,8
	14	140	4,4
Age Risk Category	Low Risk	3166	100,0
	High Risk	0	0,0
Trisomy 21 Category	Low Risk	3107	98,1
	High Risk	59	1,9
Trisomy 18 Category	Low Risk	3164	99,9
	High Risk	2	0,1

Table 4: Distribution of Trisomi21 Risk by qualitative variables

	Trisomy21 Risk		p
	Low Risk (n=3107) Mean	**High Risk (n=59) Mean**	
Age(Years)	27,32±5,34	33,27±7,83	<0,001
Weight(Kilograms)	65,66±13,16	65,78±11,15	0,944
CRL(mm)	60,75±8,53	58,5±9,52	0,045
β-HCG(ng/mL)	53,22±129,04	154,46±223,38	<0,001
PAPPA(mIU/l)	3708,9±2489,01	2347,26±1906,91	<0,001
NT(mm)	1,37±0,35	1,89±0,87	<0,001
β-HCG MoM	1,2±0,84	2,78±1,59	<0,001
PAPPA MoM	1,24±0,68	0,9±0,77	<0,001
NT MoM	0,88±0,23	1,28±0,66	<0,001
Age Risk(%)	0,0015±0,0019	0,0077±0,0116	<0,001
Trisomy 21 Risk(%)	0,0002±0,0004	0,023±0,0307	<0,001
Trisomy 18 Risk(%)	0,0001±0,0041	0,0002±0,0005	0,796

Significance test of difference between two means was used.

Table 5: Comparison of newly estimated median values with the software program

Gest. Week	**Free β-hCG(ng/mL)**				**PAPP-A (mIU/l)**		
	CaseNumber	**New Estimated Median**	**Software Median**	**p value***	**New Estimated Median**	**Software Median**	**p value***
11	362(11,4)	36,76	51,79	<0,001	1836	1337	<0,001
12	1689(53,3)	33,64	41,75	<0,001	2719	1919	<0,001
13	975(30,8)	28,51	35,70	<0,001	4052	2926	<0,001
14	140(4,4)	20,89			5370	4358	<0,001

Wilcoxon test used.

DISCUSSION

Dual screening tests are now in routine use with increasing gestational age and they lead to interventional application when high risk is reported. So those, accuracy of their results are very important. False positive and false negative results can negatively affect the life of the pregnant and the baby (Assessment, 2000).

Due to the advanced procedures performed in pregnant women due to false positivity in the double screening test; the risk of obstetric complications such as preterm labor, pre-eclampsia, low birth weight, intrauterine growth retardation, perinatal fetus death increases. While analytical performance is within acceptable limits, variations in measurements are not effective in predicting risk in low-risk groups, but are highly effective in predicting risk in high-risk groups and high maternal age groups. The analytical accuracy of the measured biochemical parameters for the combined test is important and can lead to high variations in risk calculation. These variations result in repetition of the test or carry the patient to amniocentesis, which is an invasive procedure and causes anxiety in the patient.

Many studies have shown a relationship between Down Syndrome and low levels of maternal serum PAPP-A and high levels of free β-HCG levels in the first trimester (Spencer, Macri et al., 1992). In the first trimester, a combined test is obtained by evaluating two biochemical parameters measured in maternal serum and NT measurement, an ultrasound data (Wald and Hackshaw, 1997). The combined test has a risk detection rate of 82-87%, with a false positivity of 5%. The performance was evaluated

as better than the triple test performed in the second trimester (Ananth et al., 2017). According to the 2007 clinical guidelines for ACOGs (American Congress of Obstetricians and Gynecologists), the combined test is an effective screening test for Down Syndrome in the general population (Goetzl, 2002). In terms of the effect of analytical variations on risk calculation, there are many studies related to the second trimester tests, but there are no studies related to the first trimester tests (Holding, 1991).

The use of devices of different brands and models and the analysis kits of different manufacturers may cause the laboratories where prenatal screening tests are performed to contain different analytical processes (Alp et al., 2018). The important factorsthat increase the variability of the risk analysis are the use of different biochemical markers in different screening protocols and the calculation of different components with various software in risk calculation. The result of the analysis are affected by the algorithms used by the software program used in the laboratory and the factors used in the steps of the calculation (such as accuracy of biochemical analysis, demographic data [gestational age and gestational age and BPD measurement] and / or USG date).

Atak et al. (2014) performed a retrospective study of 5820 singleton pregnant women in the Adıyaman region using dual screening data obtained with the Beckman-Coulter Unicel DxI 800 device and compared the median values of the Benetech PRA package program with the median values. Both β-hCG and PAPP-A were significantly lower than those of the program at all weeks ($p < 0.05$). Sucu et al. (2018) conducted a retrospective study of triple screening data of 1572 singleton pregnant women using the Immulite One device in Istanbul and compared the median values of the Prisca4.0 Typolog software with those of the firm software program. Values were significantly different in all weeks except 11th gestational week. In addition, Alp et al. (2018) in their retrospective study using the double screening data obtained by the Immulite 2000 device in 1413 single pregnant women for Van region, compared with those of the Prisca 5.0 Typolog software. New median values of β-hCG was found to be significantly lower than those of the program ($p < 0.05$), but no difference was found for PAPP-A ($p > 0.05$).

In our study; the data obtained from Immulite 2000 device in 3166 single pregnant women admitted to our hospital with the median values of SsdwLab5 software. Estimated median values of β-hCG were found to be significantly lower than those of the program ($p < 0.05$), while PAPP-A values were significantly higher ($p < 0.05$).

Estimation of different median values is due to the influence of different kits, devices, software programs, laboratories and regions. This makes it necessary for each laboratory to estimate its own median values. In all of the aforementioned studies, this is a common opinion. Our study has also supported this view. Since first trimester screening test is performed in other hospitals in our province, our results reflect only the results of patients admitted to our hospital.

These results once again showed that the analytical performance of the 1st Trimester screening tests should be kept at optimum levels. It is important to have experienced laboratory personnel, high quality and the highest level of laboratory equipment. This also requires a strict internal and external quality control programs. Additionally, it is inevitable to estimate the region-specific and even laboratory-specific median values.

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REFERENCES

Alp HH, Huyut Z, Çokluk E, Şekeroğlu MR. İkili ve üçlü prenatal tarama testi medyan değerleri: Van ölçekli retrospektif bir çalışma. Turk Biyokimya Derg 2018;16(1):17-24.

Ananth CV, WapnerRJ, AnanthS, D'Alton ME, Vintzileos AM. First-trimester and second-trimester maternal serum biomarkers as predictors of placental abruption. Obstet Gynecol2017;129(3):465.

Assessment P R. Down syndrome: prenatal risk assessment and diagnosis. Am Fam Physician2000; 62(4): 825-32.

Atak PG, Arpacı A, Seydal G. Adıyaman iline ait ikili ve üçlü prenatal tarama testlerinin medyan değerlerinin belirlenmesi. Turk Biyokimya Derg 2014;39(2): :231-7.

Brizot ML, SnijdersRJ, BersingerNA, Kuhn P, Nicolaides KH. Maternal serum pregnancy-associated plasma protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. Obstet Gynecol 1994;84(6): 918-22.

Creasy RK, Resnik R, Iams JD. Maternal-fetal medicine: principles and practice, Gulf Professional Publishing, 2004.

Cuckle H. Integrating antenatal Down's syndrome screening. Curr Opin Obstet Gynecol 2001;13(2): 175-81.

Goetzl L. ACOG Practice Bulletin. Clinical Management Guideline for Obstetrician-Gynecologists, Number 36, July 2002. Obstetric analgesia and anesthesia. Obstet Gynecol2002;100: 177-91.

Holding S. Biochemical screening for Down's syndrome. Bri Med J1991;302(6787):1275.

James D, Steer P, Weiner C, Gonik B. Yüksek Riskli Gebelikler Yönetim Seçenekleri. Erken prenatal bakım (Derleyen: Güner H), Güneş Tıp Kitabevleri, İstanbul. 2008. S65.

Kagan KO, Sonek J, Wagner J, HoopmannM. Principles of first trimester screening in the age of non-invasive prenatal diagnosis: screening for chromosomal abnormalities. Arch Gynecol Obstet2017;296(4): 645-51.

Kappel B, Nielsen J, Hansen KB, Mikkelsen M, Therkelsen AJ. Spontaneous abortion following mid-trimester amniocentesis. Clinical significance of placental perforation and blood-stained amniotic fluid. Int J Obstet Gynaecol1987; 94(1): 50-4.

Marteau TM, Cook R, Kidd J, Michie S, Johnston M, Slack J et al. The psychological effects of false-positive results in prenatal screening for fetal abnormality: a prospective study. Prenatal Diag 1992;12(3):205-14.

Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. BR Med J 1992;304(6831): 867-9.

Spencer K, Macri J, Aitken D, Connor J. Free β-hCG as first-trimester marker for fetal trisomy. The Lancet 1992;339(8807): 480.

Sucu V, Yıldırmak S, Vardar M, Mihmanlı V. İkili ve üçlü tarama testi biyokimyasal parametrelerinin hastanemize ait medyanlarının değerlendirilmesi. Abant Tıp Derg2018;7(2): 35-40.

ŞanlıDB, Kartkaya K. Eskişehir bölgesinde üçlü test tarama parametrelerinin medyan düzeylerinin belirlenmesi. Turk Biyokimya Derg 2011;36(1):50–4.

Wald N, Hackshaw A. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. Prenat Diagn 1997;17(9): 821-9.