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# Determination of the median levels of first trimester screening test parameters in our region

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## Abstract

**Objective:** In this study our purpose was to determine the median values of the first trimester screening parameters in pregnancy in our region to decide whether Prisca medians are appropriate or not and reevaluate risky pregnancies according to prisca medians by the way of the new regional median values and compare results.

**Methods:** In this study we evaluated serum free beta-human chorionic gonadotropin (free β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) values of 1,613 pregnant women who admitted to our biochemistry laboratory for the first trimester screening test between 2005-2010.

**Results:** β-hCG and PAPP-A median values were calculated for each week between 11-13th gestational weeks. When regional medians calculated by Prisca median values on 11-13th weeks are compared, statistically no significant difference was found on free β-hCG levels ($p>0.05$) as there was statistically a significant difference on PAPP-A levels ($p<0.05$).

**Conclusion:** We believe that calculating regional median values or determining appropriateness of used medians can decrease the need for invasive diagnostic procedures which carries risk for both mother and fetus.

**Key words:** Down syndrome, first trimester screening, regional median.

## Bölgemizde ilk trimester tarama testi parametrelerinin medyan düzeylerinin belirlenmesi

**Amaç:** Bu çalışmada amacımız gebelikte kullanılan ilk trimester tarama testi belirteçlerinin bölgemize ait medyan değerlerini belirleyerek mevcut meydanların uygun olup olmadığını saptamak ve şu anda kulanılan Prisca medyan değerlerine göre riskli saptanan vakaları yeni belirlenen medyanlara göre tekrar değerlendirerek sonuçları karşılaştırmak.

**Yöntem:** Bu çalışmada biyokimya laboratuvarımıza 2005-2010 yılları arasında ilk trimester tarama testi için başvuran, 11-13 gebelik haftaları arasında bulunan toplam 1,613 gebede serbest beta insan koryonik gonadotropini (serbest β-hCG) ve gebelikle ilişkili plazma protein A (PAPP-A) değerleri geriye dönük olarak incelendi.

**Bulgular:** Serbest β-hCG ve PAPP-A için 11-13 haftalar arası medyan değerler her bir haftaya göre yeniden hesaplandı. 11-13. haftalarda Prisca medyan değerleri ile yeni hesaplanan bölgesel medyanlar karşılaştırıldığında; serbest β-hCG düzeylerinde istatistiksel olarak anlamlı fark görülmedi ($p>0.05$), PAPP-A düzeylerinde ise istatistiksel olarak anlamlı fark görüldü ($p<0.05$).

**Sonuç:** İlk trimester tarama testlerinde bölgesel medyanların hesaplanması ya da mevcut meydanların uygunluk açısından değerlendirilmesinin anne ve fetüs açısından riskli olabilecek girişimsel işlemlere ihtiyacı azaltacağına inanmaktayız.

**Anahtar sözcükler:** Down sendromu, ilk trimester tarama testi, bölgesel medyan.

## Introduction

The major advantage of first-trimester screening is the earlier gestational age of detection so that further diagnostic testing can be made available for patients considered at higher risk for chromosome abnormalities.[1] Antenatal screening for fetal chromosomal abnormalities was improved over last years. These improvements have increased chance of couples for more effective

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screening options. In 1970's maternal age was a single indication for invasive testing. Maternal age alone is a poor screening method which determines 1/3 of fetuses affected by Down syndrome. Risk of a patient aged 35 or older can be lower than a 20-year-old woman with new screening protocols.[2] Maternal age combined with biochemical markers improved accuracy of screening tests. First trimester screening including NT and maternal serum parameters is now offered to women of all ages.[3]

First trimester screening protocols include maternal serum analytes and ultrasonographic examination. Free β-hCG and PAPP-A are most important serum analytes for first trimester screening.[1] PAPP-A is decreased in Down syndrome pregnancies, with a mean value of 0.4 MoM, on the other hand free β-hCG is elevated with a mean value of 1.8 MoM. Studies on risky pregnancies showed that increased risk of aneuploidy associated with increased nuchal translucency (NT). Nuchal translucency is a sonoluscent fluid filled space beneath the skin at the back of the neck. It can be measured between 11 and 14 gestational weeks by transabdominal ultrasonography.[4] NT was the best single marker with a detection rate more than 70 percent.[5] Combining NT measurements as multiples of median (MoM) with serum analytes and maternal age will improve detection rate of Down syndrome in the first trimester.[1] Amniocentesis, chorion villus biopsy and cordosynthesis are specific diagnostic tests used to determine fetal aneuploidy with near %100 accuracy but they also carry risk of fetal exitus or spontaneous abortus. In order to determine fetal aneuploidy combined biochemical and NT screening is used preferentially.[6] The risk of invasive testing, availability of resources and a cost-to-benefit ratio are important factors considered to use more effective screening methods. New screening protocols have resulted in a decrease in the ratio of amniocentesis in women over 35 years of age. Also screening has become available for younger patients who carry a risk of birth defects about 2% to 3%. More effective screening methods mean more effective use of sources and the decrease in normal fetus loss associated with interventional evaluations.[2]

It is required to understand MoM statistics which is used to normalize analyte values used for screening tests in order to understand clinical applications of screening tests and published literature. A median value set is constituted to calculate MoM values for each week of gestation. Each test result divided by the median for the relevant gestational week to define MoM value for that test result. MoM values also could be corrected by other factors (e.g; maternal age, weight, smoking and maternal race) which affect analyte values. MoM values are used extensively for converting biochemical analyte values into an interpretable unit in calculating the risk of fetal aneuploidy.[3] Incorrect median values commonly affect laboratory performance in screening tests. One of the fundamental duties of the prenatal screening laboratory is to reveal that stated median values obtained from another source are appropriate.[3]

In this study our purpose was to determine the median values of the first trimester screening parameters in pregnancy in our region to decide whether Prisca medians are appropriate or not and reevaluate risky pregnancies according to Prisca medians by the way of the new regional median values and compare results.

## Methods

In our study we evaluate the data of 1,613 pregnants who admitted to the laboratory of Eskişehir Maternity and Children Hospital to have a first trimester screening test between 2005 and 2010 retrospectively. Their gestational ages were 11-13 (11w+0d and 13w+6d) weeks and were living in Eskişehir and environment. We determined serum levels of free β-hCG and PAPP-A by IMMULITE® 2000 device (Diagnostic Products Corporation, Los Angeles, CA, USA) which run with chemiluminescence method and belongs to BIO-DPC company. Gestational age defined according to crown rump length (CRL) determined ultrasonographically. The MoM values were calculated comparing these two marker values obtained according to the gestational week with the median values of normal gestational population. The cases were determined by analyzing of obtained MoM levels of free β-hCG, PAPP-A, maternal age and NT with other data (such as maternal weight, smoking, diabetes mellitus (DM) and twin pregnancies etc.) statistically by Prisca 4.0 (Prenatal Risk Calculation, TYPOLOG Software / GmbH, Hamburg, Germany) software. MoM values were calculated by comparing free β-hCG and PAPP-

A hormone levels measured in each case with β-hCG and PAPP-A median values determined according to normal population in Prisca software for the same gestational week. In order to calculate regional median values, twin pregnancies and cases with risky evaluation results according to Prisca software were excluded from the study. Data of remaining 1,613 pregnants were used in the study. First trimester screening test cut-off values were accepted as 1/250 for Down syndrome and 1/100 for trisomy 18. SPSS 15.0 (SPSS-15.0, Inc, Chicago, USA) software was used for statistical analysis. MoM values of screening test positive pregnancies according to Prisca medians compared with newly defined MoM values according to regional medians by nonparametric Mann-Whitney U test since our data not normally distributed. In order to compare Eskişehir region first trimester screening test medians with Prisca medians Minitab 15 software Sign Test used and differences among data were evaluated by sign test for median. Values of $p<0.05$ were considered statistically significant.

## Results

Demographic values of pregnants (n=1,613) included in this study are shown in **Table 1.** Regional median values for each marker concerning 11-13 gestational week were calculated. We found regional medians for PAPP-A significantly higher than Prisca medians at weeks 11, 12 and 13 ($p<0.05$, $p<0.001$, $p<0.001$, respectively). We found that regional free β-hCG medians for each week was not significantly different than Prisca free β-hCG medians ($p>0.05$) (**Table 2**). Risky pregnancies according to Prisca medians reevaluated according to regional medians values for free β-hCG and PAPP-A and MoM values recalculated for each week. We found that new MoM values was not significantly different than MoM values defined according to Prisca medians for each week ($p>0.05$) (**Table 3**).

## Discussion

The aim of any screening program is to identify a small group of patients among a healthy population that has sufficient risk of a disorder for specific diagnostic examination.[1] Screening tests are used to select the women who may be offered amniocentesis and other invasive obstetrical interventions during pregnancy. Fetuses at risk for neural tube defects or fetal chromosome abnormalities as well as women at risk for third-trimester obstetrical complications can be defined by prenatal screening tests. Maternal serum screening has the benefit of earlier diagnosis by this way decrease fetal mortality, morbidity and also help couples to decide about appropriate delivery strategies.[7] Couples with positive screening test results should be informed about Down syndrome and complications of invasive procedures for specific diagnosis.[8] Prenatal screening laboratories should define kit specific and population specific median values for each analyte used in screen-

**Table 1.** The demographic data of pregnancy and serum parameters.

| N=1,613 | Minimum | Maximum | Mean | Standard deviation |
|---|---|---|---|---|
| Age | 15.95 | 41.14 | 27.15 | 4.688 |
| Gestational week | 11w 0days | 13w 6days | 12.48 | 0.705 |
| Body weight (kg) | 38 | 130 | 62.51 | 11.569 |
| CRL | 39 | 83 | 61.90 | 9.566 |
| NT | 0.10 | 3.50 | 1.58 | 0.482 |
| NT (MoM) | 0.07 | 2.33 | 1.00 | 0.305 |
| Free β-hCG | 6.43 | 181 | 42.09 | 24.464 |
| Free β-hCG (MoM) | 0.19 | 3.84 | 1.07 | 0.593 |
| PAPP-A | 0.39 | 15.5 | 3.08 | 2.003 |
| PAPP-A (MoM) | 0.15 | 3.95 | 1.06 | 0.610 |

**Table 2.** Comparison of Eskişehir region first trimester screening test medians and Prisca medians.

| Test | Week | Group | N | Median | p |
|---|---|---|---|---|---|
| Free β-hCG | 11 | Eskişehir<br>Prisca | 403<br>403 | 42.9<br>42.8 | p>0.05 |
| Free β-hCG | 12 | Eskişehir<br>Prisca | 722<br>722 | 37.2<br>37.8 | p>0.05 |
| Free β-hCG | 13 | Eskişehir<br>Prisca | 488<br>488 | 29.9<br>30.9 | p>0.05 |
| PAPP-A | 11 | Eskişehir<br>Prisca | 403<br>403 | 1.64<br>1.53 | p>0.05 |
| PAPP-A | 12 | Eskişehir<br>Prisca | 722<br>722 | 2.54<br>2.32 | p<0.001 |
| PAPP-A | 13 | Eskişehir<br>Prisca | 488<br>488 | 3.72<br>3.19 | p<0.001 |

ing and decide whether median values obtained from another source are appropriate or not. Small differences in median values can affect accuracy of calculated risk and number of screen-positive women.[3] Performance of the screening tests can be improved by using the regional median values and prenatal risks can be calculated more accurately.[9] In our laboratory we noticed that our initial positivity rate was very high. Since the most important reason for high initial positivity rate is inappropriate median values, we decided to define our own regional medians. We found statistically significant difference between regional median values of PAPP-A and Prisca medians (p<0.05). We did not find statistically significant difference for free β-hCG median values compared to Prisca medians (p>0.05). Free β-hCG and PAPP-A levels of risky pregnancies according to Prisca medians which were excluded from the study for calculation of new median values, were revaluated according to new regional medians. Difference in PAPP-A medians not affected new MoM calculations and risk level not changed. The detection rate is about 90% for a 3% false-positive rate in combined screening for trisomy 21 based on maternal age, fetal NT, free β-hCG and PAPP-A.[10]

**Table 3.** MoM values of the free β-hCG and PAPP-A in risky pregnancies according to first trimester screening test Prisca medians compared with MoM values calculated according to new regional medians.

| Test | Week | Group | N | MoM | 25% | 75% | p |
|---|---|---|---|---|---|---|---|
| Free β-hCG | 11 | Eskişehir<br>Prisca | 61<br>61 | 1.720<br>1.724 | 1.159<br>1.163 | 2.611<br>2.621 | p>0.05 |
| Free β-hCG | 12 | Eskişehir<br>Prisca | 110<br>110 | 1.736<br>1.709 | 1.169<br>1.151 | 2.639<br>2.597 | p>0.05 |
| Free β-hCG | 13 | Eskişehir<br>Prisca | 55<br>55 | 1.438<br>1.391 | 0.806<br>0.779 | 2.585<br>2.501 | p>0.05 |
| PAPP-A | 11 | Eskişehir<br>Prisca | 61<br>61 | 0.493<br>0.496 | 0.371<br>0.397 | 0.728<br>0.781 | p>0.05 |
| PAPP-A | 12 | Eskişehir<br>Prisca | 110<br>110 | 0.404<br>0.441 | 0.266<br>0.291 | 0.643<br>0.702 | p>0.05 |
| PAPP-A | 13 | Eskişehir<br>Prisca | 55<br>55 | 0.362<br>0.423 | 0.229<br>0.258 | 0.568<br>0.647 | p>0.05 |

Appropriate NT measurement is necessary for high screening performance; overestimate or underestimate in NT measurements reduces the detection rate of trisomy 21. Overestimate in NT increases the false-positive rate whereas an underestimate in fetal NT reduces the detection rate at a fixed screen-positive cut-off.[11] Wortelboer et al stated that over the years performance of the firs-trimester test has improved. Main reason was more precise NT measurements. Appropriate determination of medians for the biochemical parameters may cause a higher detection rate.[12] Ardawi et al. examined distribution of MoM values of fetal NT, free β-hCG and PAPP-A in Saudi singleton pregnancies and they found that maternal body weight, smoking, twin pregnancy and ethnicity are important factors for first trimester screening test results.[13] Different study groups have examined whether there is a relationship between abnormal serum levels of free β-hCG, PAPP-A in first trimester and subsequent pregnancy complications like fetal growth retardation or preterm labour and they found conflicting results.[14] Goetzinger et al. demonstrated that low first-trimester PAPP-A levels are associated with the development of preeclampsia.[15] Spencer et al. showed that in the preeclampsia group, compared to the controls, maternal serum levels of PAPP-A, free β-hCG, activin A and inhibin A were significantly increased.[16] Kirkegaard et al. revealed that low serum levels of PAPP-A and free β-hCG are independent biomarkers associated with preterm delivery (<37 week).[17] Although low serum PAPP-A levels are significantly associated with preterm delivery. Additional studies are needed to use PAPP-A as a screening parameter.[18] Improvements in ultrasound resolution and multiplanar 3D ultrasound have resulted in earlier detection of structural defects in the first trimester.[2]

## Conclusion

Since risky pregnancies according to first trimester screening test were offered invasive procedures like amniocentesis and chorion villus biopsy which carries risks both for mother and fetus, determination of population specific medians in large case groups or researching the convenience of current medians is very important in order to improve performance of screening tests and to reduce the frequency of invasive procedures which are risky for mother and fetus.

**Conflicts of Interest:** No conflicts declared.

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