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ORIGINAL ARTICLE

Reliability of second trimester triple screening for Down syndrome in rhesus-negative women

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Objective: To find out whether there is considerable influence on second trimester serum concentrations owing to the rhesus status.

Study design: This retrospective cohort study was performed at the Perinatology Unit of the GATA Haydarpasa Teaching Hospital. During the study interval, 2265 pregnancies met inclusion criteria. The blood samples were collected in 117 pregnancies with a maternal rhesus-negative blood group status. The control group consisted of 2148 pregnancies with a maternal rhesus-positive blood group status. Statistical analysis was performed by SPSS 11.0 statistical software.

Results: Pregnancies with a maternal rhesus-negative blood group status were identified in 117 patients. The overall prevalence of pregnancies with a maternal rhesus-negative blood group status were 5.1% in our study. Only unconjugated estriol multiples of the median values were significantly decreased in rhesus-negative women ($P<0.001$). Alpha-fetoprotein and human chorionic gonadotrophin multiples of the median values did not differ significantly ($P>0.05$).

Conclusion: We conclude that if second trimester screening test to be used in Rh negative pregnancies, either the corrected value should be referred or double test result should be considered ignoring the unconjugated estriol result. Another option is the first trimester Down syndrome screening test.

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Introduction

Prenatal screening for Down's syndrome and other chromosomal abnormalities is conducted in the second trimester of pregnancy with a combination of maternal serum biochemical markers and maternal age. The most commonly used biochemical markers are human chorionic gonadotrophin (hCG), alpha-fetoprotein (AFP) and unconjugated estriol (uE3). Second-trimester maternal serum screening (triple screening) have become a part of routine prenatal care. This screen assigns each woman a risk of having a Down syndrome affected pregnancy by combining her age-specific risk with the likelihood ratios based on her serum marker levels. The concentrations of these parameters are significantly altered in pregnancies affected by fetal trisomy 21 and other chromosomal disorders.

The effectiveness of this screening is based on the association of fetal Down syndrome with decreased second-trimester levels of AFP[1,2] and uE3[3] and increased levels of human chorionic gonadotrophin.[4] Following the introduction of maternal serum screening for fetal Down syndrome, it was noted that fetal trisomy 18 (Edward syndrome) is associated with decreased levels of all three screening analytes,[3] making screening for trisomy 18 feasible within a Down syndrome screening program.[5,6]

Besides the chromosomal constitution of the fetus, a variety of other factors are known or thought to influence marker concentrations, for example maternal weight, smoking habits[7] ethnic origin,[8] twins,[9] insulin-dependent diabetes mellitus[10] and multigravidity.[11,12] Adjustment for such factors is necessary to avoid erroneous individual risk estimates.

Estriol is a steroid hormone derived from cholesterol and is the major estrogen produced during pregnancy. Functions attributed to estrogens include regulation of uteroplacental blood flow, development of placental vascularization and regulation of the maternal cardiovascular system.[13] Estriol is secreted into the maternal circulation where its concentration increases gradually throughout pregnancy. In the maternal liver, estriol undergoes glucuronide or sulfate conjugation and is excreted in the maternal urine in the conjugated form. Around 10% of circulating estriol in the maternal compartment remains in the unconjugated form. Low mid-trimester uE3 alone or in association with other mid-trimester markers has been associated with adverse pregnancy outcome.[14–17]

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The present study focused on measurements of hCG, AFP and uE3 in the serum of women with a rhesus-negative blood group in order to find out whether there is considerable influence on serum concentrations owing to the rhesus status.

Material and methods

This retrospective cohort study was performed at the Perinatology Unit of the GATA Haydarpasa Teaching Hospital, a tertiary referral center in Istanbul, Turkey, from 1 January 2002 to 31 December 2005. The study used a cohort design (i.e. a cohort study from which cases and controls were sampled), which is an effective method of evaluating potential screening tests that avoid biases owing to interventions performed on the basis of screening test results. During the study interval, 2265 pregnancies met inclusion criteria. The blood samples were collected in 117 pregnancies with a maternal rhesus-negative blood group status. The control group consisted of 2148 pregnancies with a maternal rhesus-positive blood group status. Multiple pregnancies, pregnancies with chromosomal or structural abnormalities and a history of (known) systemic disease were excluded from the study groups. In addition, the study also excluded pregnancies suggestive of fetal abnormality. Pregnancies of both groups were selected for normal outcome confirmed by follow-up. No information of fetal rhesus type was available for both the groups.

Serum analysis

Blood for maternal serum triple marker screening analysis was drawn between 15 and 20 weeks of gestation with a single viable fetus. Serum analysis of hCG, uE3 and AFP was performed using the immulite 1000 immunoassay system (CA, USA). Biochemical analysis was performed at clinical biochemistry laboratories (GATA Haydarpasa Teaching Hospital, Uskudar, Istanbul). The risk of Down syndrome, trisomy 18 and open spina bifida was calculated using the program Prisca 4.0 software (Typolog Software GmbH, Munich, Germany), which incorporates analyte multiples of the median (MoMs), maternal age, gestational age at the time of sampling, smoking habits, maternal weight, ethnicity and insulin-dependent diabetes mellitus status into the calculation. Ultrasound dating was used for gestational age calculation.

We performed a thorough obstetric ultrasound examination using a curvilinear 4.2 MHz transducer (Toshiba Powervision 6000, SSA-370A, Tokyo, Japan). Gestational age was estimated by fetal biparietal diameter or by the date of last menstrual period. Standard fetal biometric data were obtained. A second trimester risk for Down syndrome greater or equal to 1:270 was used to define the population of women that were screen-positive.

Statistical analysis

Statistical analysis was performed by SPSS 11.0 statistical software (SPSS Inc, Chicago, IL, USA). The independent samples *t*-test was used to compare the mean maternal age, gravidity, parity gestational age at sampling date and maternal weight at sampling date between the study and control groups. Comparison of the analyte MoM's between the two groups was based on the mixed-effects model. Comparison of the proportion of screen-positive results for Down syndrome was based on Pearson's χ^2 test. For all comparisons, a *P*-value of <0.05 was considered statistically significant.

Results

Pregnancies with a maternal rhesus-negative blood group status were identified in 117 patients. The overall prevalence of pregnancies with a maternal rhesus-negative blood group status were 5.1% (117 of 2265 cases). The mean ages of pregnant women with a maternal rhesus-negative blood group status and control group were found to be 28.50 ± 3.96 (range 19–40) and 27.92 ± 4.31 (range 17 to 40), respectively. There were no statistically significant differences in maternal age, gravidity, parity, gestational age at sampling date and maternal weight at sampling date between the two groups (each $P>0.05$). Mean maternal weight in the study and control groups were found to be 61.62 ± 9.63 and 61.37 ± 9.78 kg, respectively ($P>0.05$).
A comparison of the clinical characteristics and second trimester triple test levels between pregnancies with a maternal rhesus-negative blood group and a maternal rhesus-positive blood group are shown in the Table 1.

Means, s.d. are given for the natural logarithms of hCG, AFP and uE3 MoM values. Only uE3 MoM values were statistically significantly decreased in rhesus-negative women ($P<0.001$). AFP and hCG MoM values did not differ significantly.

Figure 1 summarizes the distribution of the triple test results in the study population. False-positive screening rates were 13/117 (11.1%) and 118/2148 (5.4%) for rhesus-negative blood group and rhesus-positive blood group, respectively. There were statistically significant differences between two groups ($P=0.01$).

The detection rates of the two groups were not compared in this study, as the number of the pregnancies of rhesus-negative mothers was too small to validate this analysis. None of the pregnancies with a maternal rhesus-negative blood group status were affected with Down syndrome, other chromosomal abnormalities or neural tube defects.

Discussion

Multiple-marker maternal serum screening for Down syndrome is well established in the second trimester of pregnancy. Statistical modelling predicts a 60 to 70% detection rate for a 5% false-positive rate and several prospective studies have confirmed this.

The present study focused on measurements of hCG, AFP and uE3 in the serum of women with a rhesus-negative blood group.

Table 1 Demographic information and the distribution of hCG, AFP, and uE3 MoM values for both study groups

	Rh (−) group (n = 117) Mean ± s.d.	*Rh (+) group (n = 2148) Mean ± s.d.*	*Significance (P)*
Maternal age (year)	28.50 ± 3.96	27.92 ± 4.31	0.16
Gravidity	1.60 ± 0.85	1.58 ± 0.80	0.85
Parity	0.61 ± 0.85	0.59 ± 0.79	0.77
Gestational age at sampling date (week)	16.99 ± 1.09	16.97 ± 1.03	0.85
Maternal weight at sampling date (kg)	61.62 ± 9.63	61.37 ± 9.78	0.78
AFP (MoM)	1.06 ± 0.45	1.04 ± 0.41	0.67
hCG (MoM)	1.04 ± 0.45	1.06 ± 0.51	0.66
uE3 (MoM)	0.87 ± 0.31	1.08 ± 0.38	0.001

Abbreviations: AFP, alpha-fetoprotein; hCG, human chorionic gonadotrophin; MoM, multiple of the medians; Rh, rhesus; s.d., standard deviation; uE3, unconjugated estriol.

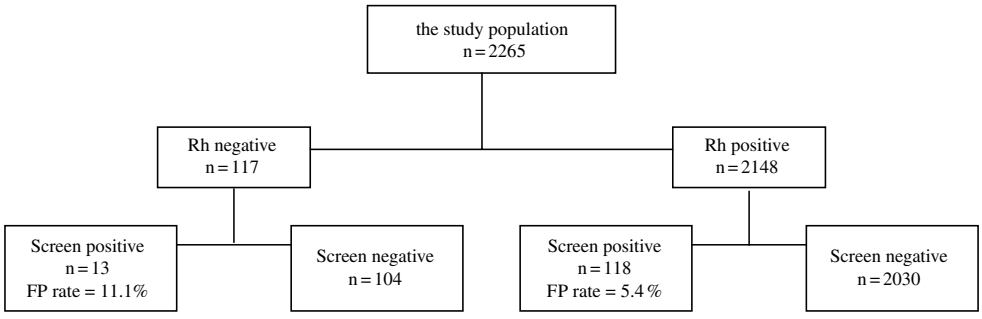

Figure 1 Summary of the triple screening results. FP rate: false-positive rate.

AFP and hCG MoM values did not differ significantly. Only uE3 MoM values were significantly decreased in rhesus-negative women compared with rhesus-positive controls. The reason for this statistically significant decrease of uE3 in rhesus-negative pregnant women is not clear.

Estriol levels are reported to be 4 to 7 times higher in fetal blood than maternal blood.[18] A lower level of synthesis could be due to low levels of the precursor DHEAS, or to reduced enzymatic conversion of the substrate. Any disruption to the catalytic activity of one of these enzymes or to the supply of DHEAS would therefore have a marked effect on the ability of the placenta to synthesize estriol.[19]

Previous studies have reported that if Rh incompatibility, which is expected to affect only a very small part of the pregnancies of rhesus-negative mothers, is the factor that modifies uE3 levels, a bimodal distribution should be assumed. Therefore, an immunological sensibilization is unlikely to be the cause of decreased uE3 levels. As a conclusion, the relation between the rhesus-negative status of the mother and reduced uE3 concentrations remains unclear. A decrease in uE3 MoM value leads to a falsely increased risk value in triple screening test. In our study, false-positive screening rates were 13/117 (11.1%) and 118/2148 (5.4%) in rhesus-negative blood group and rhesus-positive blood group, respectively. The difference between the two groups was statistically significant ($P = 0.01$). For this reason, a coefficient should be defined in order to decrease the false-positive rate of the triple test. Accordingly, Sancken *et al*.[20] suggested a simple formula as follows: $uE3 - MoMadj = 1.1765 \times uE3 - MoM$, where uE3 − MoM adj is the uE3 MoM value corrected for the rhesus-negative status.

Further studies with larger sample sizes are necessary to decide if adjustment for negative rhesus status should be recommended. In addition, the influence of paternal and fetal rhesus type on the test results should be investigated in well-designed prospective studies. We conclude that if second trimester screening test to be used in Rh negative pregnancies, either the corrected value should be referred or double test result should be considered ignoring the uE3 result. Another option is the first trimester Down syndrome screening test.

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