

TO EVALUATE THE EFFICIENCY OF THE SECOND-TRIMESTER SCREENING (QUAD TEST) FOR FETAL CHROMOSOMAL ABNORMALITIES IN AHVAZ, IRAN

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ABSTRACT

Objective: To evaluate the efficiency of quad test screening for chromosomal abnormalities in Ahvaz (Iran). **Materials And Methods:** Maternal serum concentrations of α -fetoprotein, human chorionic gonadotropin, unconjugated estriol, and inhibin-A were measured among 907 pregnant women from 15 to 20 weeks of gestation during March 2013 to July 2014. Risk calculation, screen-positive rate and detection rate for chromosomal abnormalities were determined. **Results:** 190 women with high risk and 18 women with intermediate risk in the initial screening test decided to have invasive procedure for genetic diagnosis. Three cases of Down syndrome and one case of trisomy 13 were detected prenatally. Also, one case with Down syndrome was detected in follow-up despite a low estimated risk in screening test. The detection rate for Down syndrome was 75%

(three out of four cases), with a 12% false-positive rate. **Conclusion:** The detection rate of quad test for Down syndrome in Ahvaz is very similar to the previous studies. But, false-positive rate is higher than found in previous studies. Therefore, a study with a larger sample size is needed to determine standard multiple of medians (MoMs) for serum markers among Iranian women for use in risk assessment software.

KEYWORDS: Down syndrome (trisomy 21), quad test, second-trimester, trisomy 18, trisomy 13, Amniocentesis.

INTRODUCTION

Aneuploidy, a deviation from the normal number of 46 chromosomes in humans, is associated with significant morbidity and mortality, particularly in infancy and childhood.^[1] Down syndrome (DS) is the most common chromosomal aneuploidy in live born infants. The overall incidence of DS is approximately 1 in 800 births in the general population,^[2] In 2007, the American College of Obstetricians and Gynecologists (ACOG) recommended that all pregnant women, regardless of their age, should be offered screening for chromosomal aneuploidy. Those with high risk should be confirmed by invasive diagnostic procedures like amniocentesis or chorionic villus sampling.^[3] Research during the past 30 years has focused on developing testing options to assess a woman's risk of having a child with aneuploidy.^[1] Noninvasive tests that are associated with a better prediction of pregnancies complicated by aneuploidy than the use of maternal age alone have become widely available, leading to new strategies for screening and a decrease in the number of births of infants with Down's syndrome in the United States, particularly among women younger than 35 years of age.^[4] The use of screening tests can decrease the need for invasive diagnostic testing (i.e., amniocentesis or chorionic villus sampling) and reduce the risk of invasive procedures that may cause loss of a normal pregnancy. However, screening tests have limitations, including both false positive and false negative results.^[23]

Anatomical ultrasound has been used since the 1980s to provide health care practitioners and expectant mothers with information regarding a pregnancy.^[5] Bricker et al have defined some markers as structural changes detected at ultrasound scan which may be transient and also these markers have little or no pathological significance, but are thought to be more commonly found in fetuses with congenital abnormalities.^[5,6,7] The most important marker of these sonographic markers is the nuchal translucency (NT) that is measured in the first trimester. First trimester screening is including: NT, serum hCG, serum PAPP-A. The detection rate of first trimester screening is 79%-90% with a 5% false positive screen rate.^[8,9] Second trimester screening is based on the triple test or quad test. Triple test (serum AFP, HCG, UE3) can detect 61 to 70 percent of Down syndrome cases.

The quad test has a trisomy 21 detection rate of approximately 80% (74-81) and a false-positive rate of 5% (10). Second-trimester maternal serum screening can be performed

between 15 and 20 weeks of gestation but is most accurate between 16 and 18 weeks. Serum screening is used primarily to detect other fetal aneuploidies, except for possibly trisomy 18. Thus, serum screening misses both lethal (e.g. trisomy 13) and sex chromosomal abnormalities that are not associated with severe physical or developmental, limitations or profound mental retardation.^[11]

The quad test is the most commonly used second-trimester serum screening test for aneuploidy. As a stand-alone test, it is generally used if women do not begin care until the second-trimester or if first-trimester screening is not available.^[10]

The Nuchal Translucency (NT) measurement is not available in many areas of Iran. Thus, the quad test is a useful option in pregnancies with this situation.

Thus this study designed to evaluate the efficiency of quad test for screening of an Iranian pregnant women for the first time.

MATERIALS AND METHODS

From March 2013 to July 2014, 982 pregnant women that all of them were in their 15th to 20th week of gestation were studied. 52 women because of maternal diabetes and 21 women because of multiple gestation were excluded and the remaining 907 cases were studied.

These women screened for chromosomal abnormalities using the quad test. Only singleton and healthy pregnancies were enrolled in the study. The exclusion criteria included multiple pregnancies, maternal diabetes, and known fetal abnormality according to the sonography. Ten milliliters of venous blood sample were taken from each eligible subject for study. All basic pregnancy data, including age, body weight, delivery history, smoking history, assisted reproductive technologies used and ethnicity, were recorded. The MoMs for hCG, UE3, AFP and Inhibin-A were analyzed using the chemiluminescent enzyme-linked immunoassay system. Risk calculation was performed using Alpha prenatal risk assessment software (Alpha, England, version 7). Maternal age, body weight, ethnicity, cigarette smoking and the level of the four markers were factored in by the software.

The high risk cut-off level for trisomy 21 and trisomy 18 was defined as a risk greater than 1 in 300 and 1 in 100, respectively. The intermediate risk was between 1:300 and 1:1000. These women were offered to perform diagnostic invasive test such as amniocentesis.

The final results recorded in the central laboratory. All women were followed up to the end of the pregnancy.

The collected data were analyzed by SPSS version 22 statistical software using independent t-test. The p-values of less than 0.05 were considered as statistically significant.

RESULTS

After exclusion of the unsuitable subjects, 907 pregnant women were remained in this study. The quad test revealed 190 high risk pregnancies (20.9%), which 113 women (12.5%) were positive for trisomy 21, 51(5.6%) were positive for trisomy 18, and 26(3%) were positive for neural tube defect(NTD).

Mean maternal age and maternal body weight were 30.42 ± 5.42 years and 70.6 ± 12.26 kg, respectively. Mean maternal age in high risk women for Down syndrome and trisomy 18 were 33.6 and 33, respectively. 286 women (31.4%) were nulliparous, 426(46.9%) were gravid 2 and 196 (21.6%) were multigravida.

All of these 190 women with high risk pregnancies decided to have an invasive procedure for a genetic diagnosis. 62 women (6.8%) had intermediate risk that 18 women of them (29%) decided to have amniocentesis. All of the 62 women had normal fetuses without chromosomal abnormalities.

According to the results four chromosomal abnormalities were detected, including three Down syndrome and one trisomy 13. In other words, from the cases who had invasive procedure, 97.8% had normal karyotype. 1.57% of this group had Down syndrome and trisomy 13 was detected in 0.52% of this group. This result shows that 2% of high risk pregnancies in this study, had chromosomal abnormalities. During the study period, one pregnancy with Down syndrome at 28 weeks gestational age was reported that the initial risk assessments indicated low risk in this case. The diagnosis of Down syndrome in this case was detected by sonography and amniocentesis confirmed it.

There was no case of trisomy 18 in this study. The detection rate for Down syndrome was 75%(three of four) with a 12% false-positive rate. Total fetal loss rate after amniocentesis was 1.4% (3 of 208).

Table 1 demonstrates mean maternal age, body weight, gestational age and average MoM for each Down syndrome marker.

Table 1: The characteristics of women who had a fetus with Down syndrome

| Case | Maternal age | Gestational age(week) | Weight (kg) | AFP (mom) | UE3 (mom) | hCG (mom) | Inhibin-A(mom) | Estimated risk | outcome |
|------|--------------|-----------------------|-------------|-----------|-----------|-----------|----------------|----------------|------------|
| 1 | 32 | 16 | 75 | 0.45 | 0.39 | 0.82 | 0.84 | 1:1200 | terminated |
| 2 | 41 | 16 | 93 | 0.72 | 0.37 | 0.79 | 2.04 | 1:12 | terminated |
| 3 | 44 | 16 | 72 | 0.47 | 0.7 | 1.16 | 1.21 | 1:9 | terminated |
| 4 | 31 | 16 | 72 | 0.14 | 0.42 | 0.22 | 0.35 | 1:170 | terminated |
| Mean | 37 | 16 | 77.88 | 0.45 | 0.47 | 0.75 | 1.11 | | |

Patient of case 1 born a fetus with trisomy 21 despite low risk results for the four-marker screening test. The overall risk for Down syndrome of this case was extremely low, with normal values of serum markers.

Table 2: Comparison of unaffected and affected group

| | Unaffected n=903 | Affected n=4 | P |
|--------------------------|---------------------|-----------------|------|
| Mean maternal age (yr) | 30.39±5.39 | 37±6.48 | 0.04 |
| Mean maternal weight(kg) | 70.03±12.24 | 77.88±10.09 | 0.11 |
| AFP (MoM) | 1.19±1.17 | 0.45±0.23 | 0.32 |
| hCG(MoM) | 1.53±1.34 | 0.75±0.38 | 0.39 |
| UE3(MoM) | 0.86±0.66 | 0.47±0.15 | 0.23 |
| Inhibin-A(MoM) | 1.083±0.98 | 1.11±0.79 | 0.07 |

There was significant difference between maternal age in affected women and unaffected group ($p<0.05$). But, there was no significant difference for body weight and serum markers.

DISCUSSION

This study designed to evaluate the risk of aneuploidy using the quad test in Iranian pregnant women.

Women with Down syndrome fetuses were older than of women with normal fetuses. We found four cases of DS which all of them were over 30 years old. Two cases of them belonged to the maternal age less than 35 years. So, it seems that it is essential to use screening tests in all pregnant women.

We found significant difference between the maternal age of DS group and non-DS group ($p<0.05$); But there was no significant differences between maternal weight of DS group and

non-DS group. Also, There was no significant difference between serum marker values in DS group and non-DS group.

None of the mothers had the history of previous DS fetus. From all of the cases, two women were treated with Assisted Reproductive Technology (ART) methods that both of them had increased risk of DS in this study, but their karyotype reported normal. From the cases that performed amniocentesis ($n=208$ cases), three mothers (1.4%) lost their fetus. Their karyotype were normal. These results are comparable with the previous studies,^[12,13] Because all of the 51 positive for trisomy 18 were initially recognized normal karyotype, there was no possibility to evaluate the accuracy of this screening test for Edwards syndrome. Regarding to the low prevalence of trisomy 18 in general population (1 per 2000 recognised pregnancies_ including abortions, stillbirths, and live births, and approximately 1 per 6600 live-born infants)^(14,15), further studies with larger sample size are necessary to investigate the accuracy of quadruple test for trisomy.^[18]

Because nuchal translucency (NT) scanning is not available in many hospitals, a reliable, easy, simple blood test is the best test methodology for both patients and physicians in such situations.^[16]

In this study our detection rate was 75% and specificity was 88%, which are comparable to most of the quad test studies in the world,^[17,18,19]

The false-positive rate in our study was 12%. Which in similar studies it was approximately 5% (10). This is mostly because of the fact that there was no available previous data for MoMs of serum markers among Iranian women to use in the risk assessment software. The high false-positive rate led to more unnecessary invasive procedures and therefore more complications.

Also, false-positive results may led to ongoing anxiety.^[20] that even after the birth of unaffected child, these worries can persist.^[21] Younger women with false-positive results often have very high levels of anxiety, even after normal karyotype results.^[22]

The study by Shahbazian N et al, demonstrated that the rate of adverse fetal outcomes was 5.79% in the cases with first trimester screen positive tests.^[24] In the study by Barati M et al, the rate of aneuploidy was increased with increasing NT thickness. The prevalence of abnormal karyotype in cases with increased NT was 6%.^[25] In the our study, 2% of cases

with high risk pregnancies had chromosomal abnormalities. So we can conclude that increased NT is more accurate than first trimester screening and both are more useful than second trimester screening to detection of adverse outcome of pregnancy.

CONCLUSION

In conclusion, our study demonstrates quad test in an Iranian population is an appropriate test for prenatal screening of chromosomal abnormalities. However, for prevention of high false-positive results, an extensive study is needed to determine standard MoMs for serum HCG, AFP, inhibin-A, UE3 in Iranian 15th to 20th weeks pregnant women. Also according to the results of this study and the previous studies, we advise to perform NT screening and first trimester screening in pregnant women in initial time of pregnancy.

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