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A negative second trimester triple test and absence of specific ultrasonographic markers may decrease the need for genetic amniocentesis in advanced maternal age by 60%

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Objective A study was conducted to evaluate the sensitivity of combining a second trimester triple test and targeted ultrasound in order to detect Down syndrome in women undergoing amniocentesis over 35 years of age.

Methods Women over 35 years of age underwent a triple test and an ultrasound examination for chromosomal markers immediately prior to genetic amniocentesis.

Results One thousand and six women were examined. Four hundred and thirty seven were triple test-positive and in 195 cases ultrasonographic abnormalities were observed. Thirteen had Down syndrome and eight had other chromosomal abnormalities. All women with Down syndrome babies were triple test-positive and seven also had ultrasonographic markers. Three of eight women who had babies with chromosomal aberrations other then Down syndrome were also triple test-positive.

Conclusions The use of the triple test as a screening tool in our population would reduce the number of amniocenteses by 60%, while no cases of Down syndrome would be missed. Ultrasonographic markers have added little to this population. Three non-Down syndrome chromosomal abnormalities and two Down syndrome mosaic cases would be missed by this approach.

KEY WORDS: Down syndrome; amniocentesis; ultrasound; AFP

INTRODUCTION

Advanced maternal age (AMA), usually defined as greater than 35 years old at delivery, remains the most common indication for genetic amniocentesis. Since amniocentesis carries a risk of 0.25% to 0.5% of pregnancy loss many women now try to avoid the procedure. Non-invasive screening for aneuploidy risk has been used in the past decade mainly on women under 35 years of age, with biochemical markers and ultrasonographic markers serving as the main screening tools. Although several studies have suggested a relatively accurate risk calculation for women with AMA, based on a second trimester biochemical triple screen (triple test) or the presence of specific ultrasonic abnormalities (Haddow *et al.*, 1994; Bahado-Singh *et al.*, 1996a,b), genetic amniocentesis remains the standard of care for these women in Israel and in many other countries.

The present study has evaluated, prospectively, the sensitivity of combining a second trimester triple test and targeted ultrasound for the detection of Down syndrome in women undergoing amniocentesis due to an indication of AMA.

MATERIALS AND METHODS

The Genetic and Obstetric Units at the Sapir Medical Center, Kfar-Saba, Israel conducted the study between January 1995 and March 1997. All women with a singleton pregnancy who were scheduled to undergo genetic amniocentesis for AMA (age 35 years or more at conception) were asked, during counseling, to participate in the study. All women underwent amniocentesis between 16 and 18 weeks' gestation. Those women who agreed to participate in the study additionally had 10 ml of venous blood drawn prior to the amniocentesis, which was sent to the laboratory for the triple test. In addition, a targeted abdominal ultrasonographic scan was performed and the findings were recorded on a special data collecting form. The scans and amniocenteses were performed by one of three attending obstetricians (DJDR, HK or MT), all of whom have ultrasound expertise. The amniotic fluid was analyzed for chromosomal aberrations regardless of the screening tests' results. The referring obstetricians were notified of any screening test abnormalities.

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Based on the Center's past experience, a rate of 1.0–1.5% of chromosomal abnormalities was predicted in this population. The study was designed to include 1000 women.

Gestational age

Gestational age was based on the last menstrual period (LMP), and confirmed by a first trimester measurement of crown–rump length (CRL). When a discrepancy of 10 days or more was found between the LMP and the CRL, the ultrasonographic gestational age was used.

Triple-test screening (Laboratory procedure)

Alpha-fetoprotein (AFP) and total human gonadotrophin (hCG) were measured by fluoroimmunoassay (Wallace-DELFIA) and estriol (uE3) by radioimmunoassay (Amerlex-M Free Estriol 2nd Trimester RIA kit).

The risk of Down syndrome was calculated using software developed by the Israeli National Prenatal Screening Program, which takes into account maternal age and multiples of the medians (MoM) for all triple test markers, and corrects for weight and diabetes mellitus. Down syndrome risk is calculated for term, using the cutoff of 1/380, which is equal to the risk of a 35-year-old woman delivering a baby with Down syndrome.

Ultrasound scan

The following measurements were obtained: biparietal diameter (BPD), femur length (FL), nuchal thickness and both renal pelves (AP diameter in transverse section). Any of the following findings were considered to be screen-positive: femur length less than 0.91 of the expected length, nuchal thickness of 6 mm or more, a transverse renal pelvis diameter of 5 mm or more and/or the presence of choroid plexus cysts, echogenic bowel and double bubble stomach. In the original study design, the four-chamber view of the heart was included; however, since in more then 180 fetuses the picture was too difficult to interpret, it was decided not to include this in the analysis.

Cytogenetics

Amniotic cells were cultured on coverslips using the '*in situ*' method. The different cultures were harvested for 12–15 days, and 10–15 colonies from three preparations were analyzed after the G-banding technique.

RESULTS

One thousand and six patients were included in the study. Their age distribution is shown in Table 1.

Table 1 — Maternal age of study participants

Age (years)	Number of patients ($n=1006$)
35–36	305
37–38	372
39–40	197
41–42	87
43–44	35
>45	10

Sixty-seven percent were 38 years or younger. Seventy-one percent were Jewish and 29% were of Arab origin.

Triple-Test results

Four hundred and thirty-seven (43.4 %) women were screen-positive (a combined risk of 1/380 at term).

Chromosomal abnormalities

Twenty-one cases with a chromosomal aberration were detected (2.09%) by amniocentesis. Thirteen fetuses had trisomy 21 and two had trisomy 21 mosaic. The other aberrations are described in Table 2.

Chromosomal aberrations and triple test results

Table 2 shows the 21 chromosomal aberrations that were detected and their relationship to the mothers' triple test results. The triple test was positive in 16/21 patients whose fetuses had chromosomal aberrations. All 13 cases with trisomy 21 had an abnormal triple test. Both cases with trisomy 21 mosaic were triple test-negative. Of the six cases with other chromosomal abnormalities, three had a positive triple test. For Down syndrome the triple test had a sensitivity of 100%, specificity of 57.3% and a false-positive rate of 41.8%.

Table 2 — Abnormal karyotype, triple screen and ultrasonographic abnormality status

Karyotype	Total	Positive triple screen	Positive ultrasound
47,XY+21	4	4	1
47,XX+21	9	9	2
46,XY/47XY+21	2	0	1
46,XX,t(21:21)	1	1	1
17 p-	1	1	0
47,XYY	1	1	0
46,XX/47+13	1	0	0
47,XXY	1	0	0
46,XX,t(1:17)	1	0	0
All	21	16	5

Table 3 — Ultrasonographic abnormalities, triple screen status and karyotype

Ultrasonographic abnormalities	Total	Abnormal karyotype	
		Triple screen-positive	Triple screen-negative
Nuchal fold	100	3	0
Echogenic bowel	38	1 (trans)	1 (mosaic)
Choroid plexus cyst	24	0	0
Pyelectasis	49	0	0
BPD > FL	130	0	0

BPD, Biparietal diameter; FL, femur length.

Ultrasonography results

One or more abnormal ultrasonographic markers were seen in 195 women (19.3% of the population) (Table 3). A nuchal fold of 6 mm or more was measured in 100 patients. Three of these fetuses had Down syndrome, all of which were also triple test-positive. Echogenic bowel was observed in 38 fetuses. One fetus had chromosome 21 translocation [46,XX,t (21 : 21)] and also had a positive triple test and one had trisomy 21 mosaic that was triple test-negative. A choroid plexus cyst was observed in 24 fetuses, pyelectasis in 49 fetuses, and in 130 fetuses the femur length was less than 0.91 of the expected length. None of these fetuses had a chromosomal aberration. The prevalence of abnormal ultrasonographic markers was almost equal among triple test-negative and triple test-positive patients (20.2% and 18.3%, respectively, $p = \text{NS}$).

Nuchal fold ≥ 6 mm had a sensitivity of 23% for trisomy 21 with a specificity of 90.2% and a false-positive rate of 9.6% (Table 4). Other ultrasonographic markers had similar or lower sensitivities and the presence of any marker had a sensitivity of 53.8%, specificity of 81.9% and a false-positive rate of 18.7%.

DISCUSSION

Maternal age is the oldest and most frequently utilized screening tool used to detect the risk of trisomy 21. In most Western countries amniocentesis is offered to women over 35 years of age, at delivery or at conception – a policy that allows a false-positive rate of 99% and a low sensitivity of 25%. Non-invasive screening methods, such as biochemical and ultrasonographic, have been used in the last two decades to select women younger than 35 years of age whose pregnancies are at increased risk for chromosomal aneuploidy. These women are also offered amniocentesis. Different investigators have suggested implementing a combined risk calculation policy in older women by utilizing triple test and/or targeted ultrasonography screening as an alternative to universal amniocentesis. In Israel, the triple test is offered to women under the age of 35 years at conception while all women over 35 years of age are eligible for free amniocentesis.

Conde-Agudelo and Kafury-Goeta (1998), in a meta-analysis of 20 large studies, examined the value of triple-marker biochemical tests as a screening tool for Down syndrome using a cutoff of 1 : 190–200 at 16 weeks' gestation. They concluded that in women over 35 years of age, the test had a sensitivity of 89% with a false-positive rate of 25%. Beekhuis *et al.* (1994), Haddow *et al.* (1994) and Suzumori *et al.* (1997) also reported similar results. Suzumori *et al.* and Haddow *et al.* pointed out that several cases of different aneuploidies were missed including a trisomy18, a trisomy 13, all sex chromosome abnormalities and one case of an additional marker chromosome. Haddow calculated that should the US adopt this as policy, it would mean missing 320 Down syndrome babies and a saving of $250 million and 14 400 fetal lives annually. These data are very similar to the present data. In the present population, using a cutoff of 1 : 380 at delivery, 100% sensitivity was achieved with a false-positive rate

Table 4 — Detection of trisomy 21[a] ($n = 13$)

	Sensitivity	Specificity	PPV	NPV	False-positive	False-negative
Nuchal fold	23	90.2	3	90.2	9.6	76.9
Echogenic bowel	0	96.1	0	98.7	3.8	100
CPC	0					
Pyelectasis	0					
CRDS	100	57.3	2.7	100	42.1	0
AMA	100				98.7	0
CRDS or US	100	45.7	2.3	100	53.5	0
Any US	53.8	81	3.6	99.2	18.7	46.1

[a]All values are given as percentages.
PPV, Positive predictive value; NPV, negative predictive value; CPC, choroid plexus cyst; CRDS, combined risk for Down syndrome (triple test); AMA, advanced maternal age; US, ultrasound.

of 40% and a false-negative rate of 50% for non-Down syndrome chromosomal abnormalities.

This re-emphasizes the need for comprehensive counseling before a decision is made to avoid amniocentesis, based on triple test results only.

Various ultrasonographic findings have been described in association with chromosomal aneuploidy (Wladimiroff *et al.*, 1995; Williamson *et al.*, 1987; Benacerraf *et al.*, 1996, 1996; Gagnon *et al.*, 1992; Nyberg *et al.*, 1993). However, because of its low sensitivity and the high level of expertise required, ultrasonography is not usually considered to be a good single-screening method, even in a low-risk population.

Several studies have tried to evaluate the use of ultrasound alone, or in addition to the triple test, to detect trisomy 21 in a high-risk population. While Vinzileos *et al.* (1997) and Verdin and Economides (1998) reported very high sensitivities of 80–90%, others, such as Borrell *et al.* (1996), Bahado-Singh *et al.* (1992, 1996a,b) and Nyberg *et al.* (1993, 1995) reported sensitivities of 33–70%. These studies differ in the choice of ultrasonographic markers for Down syndrome, which range from detailed and lengthy sonography using multiple markers (Vinzileos *et al.* with 92.8% sensitivity) to more basic scans, using one or two markers only (Borrell *et al.* with 33.3% sensitivity for nuchal thickness of 6 mm). Most studies, however, have reported that the presence of even a minor ultrasonographic abnormality greatly increased the probability of an abnormal karyotype detected by amniocentesis by 6–40-fold (Nyberg *et al.*, 1995; Drugan *et al.*, 1996, Verdin and Economides, 1998).

In the present population of women over the age of 35 years the presence of any sonographic marker had a sensitivity of 53.8%, specificity of 81.9% and a false-positive rate of 18.7% for trisomy 21. These results compare well with most studies that did not involve a detailed and lengthy sonogram, which requires high expertise and superior equipment. The presence of an ultrasonographic marker in the present study increased the likelihood of trisomy 21 in a triple screen-positive patient by little more than three-fold (2.74% to 8.75%). This was lower than most studies and was probably due to the fact that the rate of detection of suspicious ultrasonographic markers in the present population was 19.3% versus 9.8% and 7.2% in the Verdin and Nyberg studies, respectively, (higher false-positive rate).

It is possible that a more detailed scanning in the present population could have yielded better results. However, this form of scanning is very time consuming, requires great expertise and is, therefore, very costly. The present study employed a more basic scanning program. The issue of cost must be taken into consideration when scanning is considered as a possible alternative to amniocentesis.

In the present population of women over the age of 35 years, the risk for Down syndrome was 1.19%. A triple test risk of $>1:380$ raised the risk to 2.74%, while the presence of any ultrasonographic marker was associated with a risk of 3.59%. When both screening methods were positive the risk was 8.75% and when both were negative [combined risk for Down syndrome (CRDS) $>1:380$ and no ultrasonic markers] the risk was 0%, since in all cases of Down syndrome the combined triple test risk was above 1 : 380. If amniocentesis had been performed on triple test-positive patients only, almost 60% of amniocenteses could have been avoided without missing a single case of Down syndrome. In this group, the presence of ultrasonographic markers added no new detected cases. Had amniocentesis been offered only to women who had both positive ultrasound and triple test markers, 92% of procedures would have been avoided but only 54% of Down syndrome cases would have been diagnosed – an unacceptably low detection rate.

Furthermore, the two trisomy 21 mosaics and three of the six other chromosomal anomalies would have been missed by both screening methods. Adding ultrasound screening to the triple testing yielded no new cases of Down syndrome in 115 patients. Among these patients one trisomy 21 mosaic was detected.

Based on the literature, and on our experience, we can now reach a 90% detection rate for Down syndrome in the advanced maternal age population by performing amniocentesis on only 30–40% of the population. These numbers are high enough to justify a discussion regarding a change of policy to selective amniocentesis based on a combination of age, triple test and ultrasonographic markers, in Israel and in other countries where routine amniocentesis is still offered to all women over 35 years of age. The main obstacle to this process will be our inability to detect the chromosomal abnormalities that are not related to maternal age and are currently detected as a by-product of universal screening.

In 1994, Pauker and Pauker published an editorial in the *New England Journal of Medicine* questioning the age of 35 years as the magic number. They examined the possibility of changing the policy of voluntary amniocentesis at age 35 and stated that the main issue that this raises is, of course, moral. Women over 35 years of age are currently routinely offered amniocentesis and changing this policy to one based on age and triple test markers would result in the birth of up to 10% more Down syndrome babies that would have otherwise been detected. Conversely, if we offer amniocentesis to younger women only when their combined risk is high, should we not apply the same criteria to older women?

Pauker and Pauker asked: 'Do older women deserve more of society's resources just because they now have access to them?' They felt that the real issue is resource allocation – applying the same criteria to all ages would increase the number of trisomy 21 babies detected without increasing by much the rate of amniocentesis for the whole population. We strongly support this notion.

In conclusion, in the present population of women over 35 years of age, triple testing can prevent 60% of genetic amniocentesis that are carried out for trisomy

21 detection. Ultrasonographic markers had a much lower sensitivity and had little value in trisomy 21 detection where there was no abnormal biochemistry. More and more women over 35 years of age are questioning the necessity of routine amniocentesis while many women under the age of 35 years undergo amniocentesis following an abnormal screening. Thus, it seems to us that the magic number of 35 should be replaced by combined non-invasive screening for all patients and that amniocentesis should be performed only in patients with a positive combined screening result.

This would increase the cost–benefit rate of the procedure and allow for better allocation of healthcare resources. Such a change in policy raises political, ethical and legal questions, which are beyond the scope of this paper. The development of better screening tools in the future may make this issue redundant.

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