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Triple Marker Screening for Fetal Chromosomal Abnormalities in Korean Women of Advanced Maternal Age

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The purpose of this article is to assess the value of maternal serum triple marker screening of alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol (uE_3) for the prenatal diagnosis of fetal chromosomal abnormalities in Korean women of advanced maternal age. Maternal sera were collected from 458 pregnant Korean women aged 35 between 15 and 20 weeks gestation before amniocentesis. A patient- specific second trimester risk for fetal Down's syndrome was calculated using the median values for AFP, hCG, uE_3 and maternal age. Twelve fetal chromosomal abnormalities were identified. These included six cases of trisomy 21, one case of 46,XY/47,XY,+21, two cases of trisomy 18, one case of trisomy 13, and two cases of 45, X. A cutoff level of 1:200 detected 85.7% (6/7) of the cases of Down's syndrome and 20% (1/5) of the other aneuploidies, with a 27.3% false positive rate. However, a cutoff level of 1:270 did not result in any gains in detecting Down's syndrome or other aneuploidies at the expense of a false positive rate of 34.3%. Second trimester triple marker testing is an effective screening tool for detecting fetal Down's syndrome in Korean women $\geq$ 35 years old. However, it is not an effective screening tool for non-Down's chromosomal abnormalities.

Key Words: Triple marker screening, chromosomal abnormalities, advanced maternal age.

INTRODUCTION

It has been well established that second trimester maternal serum levels of certain analytes are altered when the fetus has Down's syndrome.[1-3] Second trimester maternal serum triple marker screening using a combination of AFP, hCG, uE_3, and maternal age in women younger than 35 years will detect approximately 60% of fetal Down's syndrome cases with a 5% to 7% false positive, or amniocentesis, rate.[4,5]

Although maternal serum screening for Down's syndrome using multiple markers is becoming increasingly popular in women aged younger than 35 years, currently there is insufficient data to reach a conclusive screening policy for Down's syndrome in women aged 35 years and older. Several recent studies from Western countries have demonstrated that maternal serum triple screening (AFP, hCG, and uE_3) can help obstetricians reliably revise the individual risk of Down's syndrome in women aged $\geq$ 35.[6-11]

The aim of this study was to evaluate whether second trimester maternal serum triple marker screening is an effective predictor of fetal chromosomal abnormalities in Asian women of advanced maternal age.

MATERIALS AND METHODS

Our study population consisted of 458 Korean women with singleton pregnancies aged 35 years and older whose sera were sent for triple marker testing at Yonsei University Medical Center from January 1995 to December 1999. This study was approved by the Institutional Review Board of our hospital.

Women with multiple gestations or fetal deaths in utero were excluded. Serum samples were obtained prior to amniocentesis at 15 to 20 weeks' gestation, and analyzed within one week. Amniocentesis was offered to all of the women with the indication of advanced maternal age, regardless of the serum screening results. Gestational age was

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determined by last menstrual period (LMP) or ultrasonic examination (biparietal diameter) if the value differed from the LMP- derived gestational age by ≥ 10 days.

AFP and hCG were measured using commercially available immunoradiometric assay kits (Diagnostic Products Corp., Los Angeles, CA, USA). A radioimmune assay was used to measure uE_3 levels (Diagnostic Products Corp., Los Angeles, CA, USA). Median values for the three analytes were determined for each gestational week from unaffected singleton pregnancies. The results obtained from each woman were expressed as multiples of the median (MoM). All three values were adjusted for maternal weight, and the AFP values were additionally adjusted for maternal insulin-dependent diabetes.

The second trimester risk for Down's syndrome was calculated using a commercially available software program (AFP Expert, Benetech Medical Systems, Toronto, Canada). This software program produced a patient-specific risk estimate for Down's syndrome based on maternal age and trivariate gaussian frequency distribution from affected and unaffected pregnancies for the three analytes.[12,13] We obtained blood karyotypes from all of the infants born to women who had declined amniocentesis.

RESULTS

The mean age of the women screened was 36.9 years, and the median age was 36 years. Four hundred and four of the 458 (88.2%) women actually underwent amniocentesis. Table 1 shows the number and percentage of positive screenings according to different cutoff levels and maternal age groups in 446 unaffected pregnancies. One hundred and fifty-three of these 446 (34.3%) women were identified as having a second trimester Down's syndrome risk ≥ 1:270 (the general risk of a 35-year-old woman). At any cutoff level chosen, the proportion of women designated as false positive increased with advancing maternal age.

Table 2 shows the second trimester Down's syndrome risk according to maternal age alone or a combination of maternal age and three serum markers in 12 affected pregnancies. These included six pregnancies with trisomy 21, one with 46,XY/47,XY,+21, two with trisomy 18, one with trisomy 13, and two with 45,X. The sera of six of the seven cases of Down's syndrome were screen positive at the 1:200 cutoff level. The rate of positivity was the same at the 1:270 level.

Among the five fetuses with aneuploidies other than Down's syndrome, only one with 45, X was screen positive at 1:270 cutoff level. This case, which had a Down's syndrome risk of 1:30, had ultrasound findings of cystic hygroma and hydrops.

Table 3 summarizes the results of the detection and false positive rate of Down's syndrome and non-Downs chromosomal abnormalities on the triple marker test at three different cutoff levels. At a cutoff level of 1:200 the detection and false positive rates for Down's syndrome were 85.7% (6/7) and 27.3%, respectively. At the same cutoff level, the detection rate for other aneuploidies was 20% (1/5). However, raising the cutoff level to 1:270 did not result in any further improvement in the detection rate of either Down's syndrome or other aneuploidies at the relatively great

Table 1. Maternal Age Distribution and False Positive Rates of Maternal Serum Triple Marker Screening at Various Risk Cutoff Levels in Unaffected Pregnancies

Maternal age(yr)	No screened	Risk cutoff level		
		≥1:100	≥1:200	≥1:270
35-36	236	21 (8.8)	53 (22.4)	69 (29.2)
37-38	130	23 (17.6)	35 (26.9)	42 (32.3)
39-40	52	11 (21.1)	15 (28.8)	19 (36.5)
≥41	28	12 (42.8)	19 (67.8)	23 (82.1)
All	446	67 (15)	122 (27.3)	153 (34.3)

Numbers in the parenthesis indicate %.

Table 2. Triple Marker Levels and Second Trimester Risk for Down's Syndrome in 12 Affected Pregnancies

Karyotype	MA (yr)	GA (wk)	AFP (MoM)	hCG (MoM)	uE_3 (MoM)	Down's syndrome risk	
						MA	MA+AFP+ hCG+uE_3
Down's syndrome							
Trisomy 21	35	16	0.33	3.08	0.9	1:270	1:17
Trisomy 21	36	18	0.77	1.49	1.76	1:206	1:129
Trisomy 21	44	15	1.1	2.34	0.8	1:28	1:12
Trisomy 21	46	16	0.86	1.33	0.45	1:16	1:7
Trisomy 21	40	16	0.66	0.81	0.83	1:100	1:165
Trisomy 21	37	15	1.67	0.64	1.62	1:174	1:666
46, XY/47, XY, +21	36	15	0.75	1.26	0.64	1:206	1:139
Other chromosomal Abnormalities							
Trisomy 18	36	17	0.67	0.24	0.72	1:206	1:280
Trisomy 18	38	17	0.61	0.27	0.17	1:148	1:2000
Trisomy 13	38	16	1.29	0.24	0.51	1:148	1:3700
45, X	36	17	0.97	0.71	0.84	1:206	1:1190
45, X	36	16	0.43	1.31	0.47	1:206	1:30

MA, maternal age; GA, gestational age; AFP, alpha fetoprotein, HCG, human chorionic gonadotropin; uE_3, unconjugated estriol.

Table 3. Detection and False Positive Rates of Maternal Serum Triple Marker Screening for Down's Syndrome and Other Chromosomal Abnormalities

Risk cutoff	False positive rate(%)	Detection rate(%)		
		Down's syndrome (n=7)	Others (n=5)	Total (n=12)
≥ 1:100	15	42.8	20	33.3
≥ 1:200	27.3	85.7	20	58.3
≥ 1:270	34.3	85.7	20	58.3

expense of a false positive rate of 34.3%.

DISCUSSION

Maternal serum screening for fetal chromosomal abnormalities in women aged 35 years and older has been the subject of much investigation. To our knowledge, this is the first report investigating the triple marker test for Korean women in this age group.

In this study, 12 (2.6%) out of 458 fetuses had chromosomal abnormalities. Six of the seven (85.7%) cases of fetal Down's syndrome could be detected using a second trimester Down's syndrome risk of 1:200 with a 27.3% false positive rate. This result is clinically significant because by using the triple marker test 72.7% of our study population would have been counseled against undergoing amniocentesis, at the expense of missing 14.3% of the Down's syndrome cases.

Several recent studies have assessed the triple marker test on patients aged ≥ 35. Three of these studies used the cutoff of 1:190-200.[8,9,11] Their sensitivities varied between 78% and 100%, and their false positive rates varied between 19.7% and 29.3%. Two studies chose a cutoff of 1:270.[7,10] The sensitivities for their false positive rates of 20.6%

and 20.7% were 75% and 80%, respectively. Only one study used a cutoff of 1:250, and its sensitivity and false positive rate were 100 and 23%, respectively.[6]

In this study, at a cutoff of 1:270 the false positive rate increased to 34.3% without any gains in detecting Down's syndrome. Therefore, the cutoff that best predicted Down's syndrome in our study was 1:200, which could detect 85.7% of the cases of fetal Down's syndrome with a 27.3% of false positive rate. Although our detection rate for Down's syndrome was similar to the detection rates reported in other studies, our results may be criticized because of a relatively great false positive rate. This might be explained by the small number of subjects enrolled in this study.

Interestingly, one case of Down's syndrome, which was caused by mosaicism, was detected using a risk cutoff of 1:200. Amniocentesis revealed a 46,XY/47,XY,+21 complement, with the 46, XY line comprising 32% of the total and the other line comprising 68% of the total. This result suggests that the level of second trimester maternal serum triple markers in mosaic Down's syndrome may be similar to those in trisomy 21.

It is well known that women over 35 are also at increased risk for fetal trisomies besides Down syndrome. However, it has been suggested that triple marker screening is quite inconsistent in detecting aneuploidies other than Down's syndrome.[12,14] Haddow et al. reported that in their subjects aged 35 years and older, only seven out of 15 fetuses (47%) with other trisomies, 11 out of 25 (44%) with sex aneuploidies, and one out of nine (11%) with miscellaneous chromosomal abnormalities would have been detected using triple marker screening with the same cutoff level as for Down's syndrome (1:200).[9]

In our study, neither of the two cases of trisomy 18, would have been detected through Down's syndrome screening at a risk cutoff of 1:270. In addition, neither of these two cases would have been detected with ultrasonography. Canick et al. first reported that trisomy 18 in the second trimester was associated with a unique maternal serum screening pattern(very low AFP, very low uE_3, and very low hCG).[15] Our findings in these two cases were consistent with this observation.

One case of trisomy 13 in our study had a Down's syndrome risk of 1:3700, and triple marker testing would have failed to detect this abnormality. Blitzer et al. reported that unless an open neural tube defect is present, the median levels of the three markers were not dramatically different from that of the general gravid women.[16] Rose et al. found that maternal serum AFP levels were not decreased (0.96 and 1.99 MoM) in their two cases of trisomy 13 in women aged 35.[17] Our finding was consistent with these two reports. In our opinion, it appears that trisomy 13 pregnancies cannot be detected by the currently used triple marker screening.

Two cases of 45,X occurred in our study population. One case with cystic hygroma/hydrops would have been detected by serum screening if a cutoff level of 1:100 had been used. Seller et al. suggested that Turner's syndrome was associated with a similar pattern to Down's syndrome in serum screening (low AFP and low uE_3).[18] The hCG levels were markedly elevated in cases of Turner's syndrome associated with hydrops. In contrast, when Turner's syndrome was not associated with hydrops, hCG levels were decreased. The screening results of the two cases of 45,X in our study were in agreement with this report.

The role of prenatal serum screening in women aged 35 years and older is currently controversial. This screening can provide more accurate information about an individual's risk of Down's syndrome, and the performance of fewer amniocentesis procedures can reduce costs and procedure-related fetal losses. Second trimester genetic amniocentesis has been routinely offered to all gravid women aged $\geq$ 35 in our institution. However, so far 11.8% of these women have wished to avoid the risk of an invasive procedure. Thus, we believe that the triple marker test could be used effectively for further delineation of the risk of chromosomal abnormalities in these women.

One disadvantage of triple marker screening is that a proportion of Down's syndrome and other chromosomal abnormalities will not be detected. Our results demonstrate that even at a risk cutoff of 1:270, the triple marker test failed to detect 14.3% of Down's syndrome and 80% of other aneuploidies. The American College of Medical

Genetics currently advises against replacing amniocentesis with multiple serum marker screening in women aged 35 years and older.[19]

In conclusion, our data indicate that maternal serum triple marker screening is an effective method of detecting fetal Down's syndrome in Korean women aged 35 years and older. However, it does not seem to be useful for the identification of non-Down's chromosomal abnormalities. Therefore, adequate counseling about the limitations of serum screening is important. Further studies in a larger population will be necessary in order to develop a more definitive protocol for this age group.

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