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A RETROSPECTIVE EVALUATION OF MATERNAL SERUM SCREENING FOR THE DETECTION OF FETAL ANEUPLOIDY

KAORU SUZUMORI[1]*, MITSUYO TANEMURA[1], ISAMU MURAKAMI[1], SETSUO OKADA[1], MICHIYA NATORI[2], MAMORU TANAKA[3], TSUKASA TAKAGI[4] AND AKIO SATO[4]

[1]*Department of Obstetrics and Gynaecology, Nagoya City University Medical School, Nagoya, Japan*
[2]*Department of Clinical Research, National Ohkura Hospital, Tokyo, Japan*
[3]*Department of Obstetrics and Gynaecology, Keio University School of Medicine, Tokyo, Japan*
[4]*Esoteric Testing, SRL Inc., Tokyo, Japan*

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SUMMARY

A retrospective evaluation of alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated oestriol (uE3) levels in maternal blood in the second trimester was conducted for cases of aneuploid pregnancies identified from a series of women who underwent amniocentesis. Blood samples were collected from 1078 women just before genetic amniocentesis was performed, mainly for individuals of advanced maternal age (greater than 35 years). Twenty-five maternal serum samples from pregnant women with an aneuploid fetus, including 14 with Down's syndrome, were available for analysis of all three parameters. An algorithm to detect Down's syndrome was used for this analysis with a risk of $\geqslant$1:299 classified as screen-positive, this being found for 20·4 per cent of the cases (220/1078). The actual Down's syndrome detection rate was 85·7 per cent (12/14), whereas the detection rate for all aneuploidies was 72·0 per cent (18/25). Those that were not detected were two cases of trisomy 21, one trisomy 18, two trisomy 13, three sex chromosome abnormalities, and one case of an additional marker chromosome. The data indicate that this tri-analyte test should be provided after thorough genetic counselling and informed decision-making regarding maternal serum screening for women who wish for a prenatal diagnosis.

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INTRODUCTION

Since the early 1970s, second-trimester maternal serum alpha-fetoprotein (MSAFP) has been used as a screening test for fetal anomalies such as neural tube defects, and after Merkatz *et al.* (1984) reported the association of low MSAFP with fetal aneuploidy, this parameter was rapidly adopted for mass screening for Down's syndrome in the United States and western Europe. Studies aimed at improving the test have resulted in the introduction of two further second-trimester markers of fetal aneuploidy, human chorionic gonadotropin (hCG) (Bogart *et al.*, 1987) and unconjugated oestriol (uE3) (Canick *et al.*, 1988). The resultant triple-marker screening test has now largely replaced MSAFP alone as a second-trimester screening test for fetal aneuploidy, identifying approximately two-thirds of cases with fetal Down's syndrome.

Maternal serum markers are measured in mass units and then expressed as multiples of the

*Correspondence to: K. Suzumori, MD, Department of Obstetrics and Gynaecology, Nagoya City University Medical School, 1-Kawasumi, Mizuho-Cho, Mizuho-Ku, Nagoya 467, Japan.

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median (MOM) for normal pregnancies at the same gestational age. Expressing each value in this way allows for adjustment of biological variables known to affect the maternal serum levels of AFP, hCG, and uE3, such as maternal weight, insulin-dependent diabetes, and race. Maternal weight was first identified as influencing the AFP concentration, with an inverse correlation being observed by a number of workers (Haddow *et al.*, 1981; Crandall *et al.*, 1983; Baumgarten, 1986). Recently, the serum level of hCG has also been discovered to be linked to maternal weight, so that adjustment is necessary on this account (Wenstrom *et al.*, 1995). Diabetes affects the serum levels of AFP, hCG, and uE3, with AFP in particular showing 20 per cent lower values on average than in the general pregnant population (Wald *et al.*, 1979, 1988, 1992).

Since a pregnant woman's race has also been shown to influence her serum levels of AFP, hCG, and uE3 (Bogart *et al.*, 1991; Muller *et al.*, 1994), median values in native Japanese pregnant women must be established for reference.

In 1994, Natori *et al.* reported the serum levels of AFP and hCG obtained for 1964 unaffected pregnant Japanese women whose gestational age and singleton pregnancy were confirmed by ultrasonograms during the first trimester. They also carried out log-linear regression statistical analysis to calculate MOM. The levels of the two analytes were found to decrease noticeably with increasing maternal weight. However, maternal age did not have any significant influence on either parameter. The authors thus concluded that gestational age and maternal weight should be considered as regression functions for the adjustment of serum levels in the risk estimation of fetal aneuploidies in Japan. Using the same sample, uE3 levels were also measured and median values obtained.

As far as we know, however, maternal serum screening for fetal aneuploidies based on the data of Japanese pregnant women has not yet been reported. We therefore describe here the results of a retrospective study of maternal serum with screening of AFP, hCG, and uE3, along with an evaluation of the clinical significance of these markers.

MATERIALS AND METHODS

One thousand and seventy-eight blood samples were obtained just before genetic amniocentesis from women between 15 and 18 weeks of gestation. Venous blood was placed in serum separator tubes and aliquots of each serum sample were frozen and sent for analysis of the three markers at SRL Inc. Laboratory from April 1994 to March 1996. The patient information included maternal age, body weight, diabetic status, number of fetuses, and gestational age calculated from the first day of the last menstrual period or ultrasonographic measurements (crown–rump length or biparietal diameter). We excluded from the study multiple gestations and pregnancies complicated by diabetes. All samples were coded so that the chromosome results remained unknown until all assays had been completed. Biochemical analyses were performed using commercially available kits (AFP, Abbott Lab., Abbottpark, IL, U.S.A.; hCG, Wallak Oy., Turku, Finland; uE3, Diagnostic Products Corp., Los Angeles, CA, U.S.A.). Median values were established for AFP, hCG, and uE3 using the results for stored sera from 4256 normal singleton pregnancies at each week of gestation. The values for each of the three analytes in the study population were then expressed as multiples of the median (MOM). Adjustments for maternal weight were made for each analyte according to the method described by Natori *et al.* (1994).

With the three MOM values and the patient's age at blood sampling, a patient-specific second-trimester risk for Down's syndrome was produced based on the maternal age and the trivariate Gaussian frequency distributions from affected and unaffected pregnancies for the three markers (Cuckle *et al.*, 1987; Wald *et al.*, 1988). A patient was considered to be screen-positive when the combined risk was 1:299 or greater, which represents the term risk at the maternal age of 35 years based on the data from the Foundation for Blood Research, Scarborough, Maine, U.S.A.

RESULTS

We determined the median maternal serum AFP, hCG, and uE3 levels for each gestational week from week 15 to week 19 using samples from 4256 uncomplicated normal singleton pregnancies in Japanese women (maternal age 19–49 years; gestational weeks 14–20). The number of samples at each week of gestation and the median AFP, hCG, and uE3 values are shown in Table I. Maternal weight-adjusted median values for AFP

Table I—Median levels of AFP, hCG, and uE3 at 14–20 weeks' gestation

Week	No.	AFP (ng/ml)	hCG (mIU/ml)	uE3 (ng/ml)
14	71	35·74	49 000	0·98
15	1127	41·82	41 000	1·59
16	1452	48·93	34 000	2·19
17	994	57·25	29 000	2·80
18	548	66·99	24 000	3·41
19	39	78·38	20 000	4·02
20	25	91·71	17 000	4·62

$n = 4256$. Maternal weight adjusted to 50·0 kg.

Table II—Levels of the three analytes by maternal age

Maternal age (years)	No.	AFP (MOM)	hCG (MOM)	uE3 (MOM)
19–22	34	1·05	1·07	1·19
23	38	0·94	1·31	0·92
24	62	0·97	0·94	0·93
25	96	0·98	1·00	1·04
26	150	0·97	1·05	0·95
27	240	0·96	0·97	1·06
28	297	0·99	1·01	1·03
29	399	0·98	1·06	1·00
30	398	0·97	0·99	1·02
31	411	1·00	1·01	1·00
32	356	1·00	1·03	0·99
33	325	1·01	0·97	0·94
34	304	1·04	0·99	0·91
35	261	1·02	1·01	0·96
36	244	1·05	1·05	0·93
37	169	1·04	1·02	1·00
38	154	1·05	0·95	0·89
39	121	1·04	0·94	1·00
40	80	1·05	0·88	0·95
41	61	1·08	1·08	0·90
42	27	1·05	0·85	0·96
43	22	1·12	1·28	1·00
44–49	16	1·14	1·10	0·90
Total	4265	1·01	1·01	0·98

and uE3 increased, whereas hCG levels decreased steadily as the pregnancy progressed. The levels of all three analytes decreased noticeably with increasing maternal weight. However, maternal age did not have any significant effect (Table II).

The risk for Down's syndrome was calculated for 1078 women, of whom 843 (78·2 per cent) were over 35 years of age at amniocentesis and 235 (21·8 per cent) were under 35. The overall screen-positive rate was 20·4 per cent (4·3 per cent for women under 35 years and 24·9 per cent for women over 35 years). Screen-positive rates grouped according to maternal age are given in Table III.

Table III—Maternal age distribution and screen-positive rate

Maternal age (years)	No. screened	Screen-positive rate (%)
20–24	6	0
25–29	84	1 (1·2%)
30–34	144	9 (6·2%)
35–39	626	108 (17·3%)
>40	218	102 (46·9%)

We detected 25 cases of fetal aneuploidies confirmed by amniocyte analysis, of which 18 (12 cases of trisomy 21, one case each of trisomy 13 and an extra marker chromosome, and four sex chromosome abnormalities) were included in the screen-positive group. The other seven (two cases of trisomy 21, one case of trisomy 13, two cases of trisomy 18, one case of a sex chromosome anomaly, and one case of an extra marker chromosome) were in the screen-negative group. Table IV summarizes the screening results for trisomy 21 confirmed by amniocentesis. Applying a risk for Down's syndrome of 1:299 or more, 12 of 14 actual cases (85·7 per cent) were detectable. The maternal ages of the two false-negative cases (Nos 12 and 14) were 28 and 37 years, respectively, while all the positive cases were over 35 years. In addition to Down's syndrome, there were 11 other chromosomal abnormalities: two cases of trisomy 13, two trisomy 18, two extra marker chromosomes, and five sex chromosome anomalies. The results are summarized in Table V. Except for four cases, one trisomy 13, two trisomy 18, and one XYY, all had abnormal maternal serum test results. One of the trisomy 18 cases showed both low hCG and low uE3 levels, of 0·19 and 0·22 MOM, respectively, in line with the results reported by Leporrier *et al.* (1996), but the other had only a low uE3 level (0·26 MOM). Of the total cases (1078), 220 (20·4 per cent) were screen-positive and 858 (79·6 per cent) were screen-negative. In the <35-year-old group, there was a 4·3 per cent screen-positive rate with an incidence

Table IV—Women with Down's syndrome fetuses detected by amniocentesis

Case No.	Maternal age (years)	Age-related risk	MSAFP (MOM)	hCG (MOM)	uE3 (MOM)	Estimated risk of Down's syndrome
1	37	1:189	1·86	0·70	1·22	1:195
2	40	1:87	0·57	2·66	0·49	1:4
3	43	1:39	1·58	11·43	0·64	1:2
4	35	1:299	0·74	3·03	0·60	1:19
5	43	1:39	0·74	0·82	1·01	1:128
6	43	1:39	0·67	2·71	1·23	1:32
7	41	1:67	1·36	2·64	0·68	1:17
8	36	1:239	0·64	1·56	0·33	1:41
9	39	1:114	1·12	2·01	0·23	1:24
10	40	1:87	0·50	4·63	0·42	1:1
11	40	1:87	0·45	1·05	0·30	1:21
12	28	1:865	1·00	1·16	0·63	1:1048
13	43	1:39	0·97	1·57	0·41	1:14
14	37	1:189	0·79	0·70	0·73	1:536

Table V—Women with fetuses with other aneuploidies detected by amniocentesis

Case No.	Maternal age (years)	Age-related risk	Fetal chromosome	MSAFP (MOM)	hCG (MOM)	uE3 (MOM)	Estimated risk of Down's syndrome
1	29	1:788	Trisomy 13	1·28	2·42	0·70	1:253
2	26	1:992	Trisomy 13	0·54	0·42	0·40	1:2643
3	28	1:865	Trisomy 18	0·93	0·77	0·26	1:1852
4	42	1:51	Trisomy 18	1·22	0·19	0·22	1:4949
5	42	1:51	XXX	0·92	1·46	1·03	1:89
6	40	1:87	XXX	1·08	1·88	0·78	1:53
7	39	1:114	XXY	1·20	1·30	0·86	1:235
8	43	1:39	XXY	1·22	0·64	0·70	1:298
9	34	1:369	XYY	2·45	1·51	1·08	1:2788
10	36	1:239	+marker	1·19	1·36	0·64	1:268
11	30	1:704	+marker	1·16	0·85	0·65	1:2410

of actual aneuploidies of 10 per cent within these. In women $\geqslant$35 years old, there was a 24·9 per cent screen-positive rate. Using 1:299 as the second-trimester risk cut-off level, our false positives for all women constituted 18·7 ($220 - 18/1078 \times 100$) per cent with a detection rate of 72·0 ($18/25 \times 100$) per cent. Women less than 35 years old had a false-positive rate of 3·8 ($10 - 1/235 \times 100$) per cent and a detection rate of 16·7 ($1/6 \times 100$) per cent, whereas women 35 years old or older had a false-positive rate of 22·9 ($210 - 17/843 \times 100$) per cent with an 89·5 ($17/19 \times 100$) per cent detection rate.

The concepts of sensitivity and positive predictive value are valuable for understanding the principles and practical application of this test in Japanese pregnant women.

If a second-trimester risk for Down's syndrome greater than 1:299 is regarded as a screen-positive for fetal aneuploidies, then from our data 72·0 per cent of the actual cases were detectable (sensitivity = 0·720). The total of only 80·8 per cent

of normal fetuses with screen-negative results (specificity = 0·808) means that 19·2 per cent of normal pregnancies are screen-positive. Thus, 18 affected and 202 normal fetuses had screen-positive results (i.e., positive predictive value = [18/18 + 202] = 0·08, or 1 in 13 fetuses with a screen-positive result will be affected). Similarly, seven cases with aneuploidy and 851 normal fetuses had screen-negative results (i.e., 851/7 + 851 = 0·99, or 99 of 100 fetuses with a screen-negative result will be normal).

DISCUSSION

Maternal serum alpha-fetoprotein (AFP) levels are elevated in cases with neural tube defects (Tyrrell *et al.*, 1988) and this forms the basis for maternal serum screening for congenital anomalies including spina bifida, anencephaly, umbilical hernia, gastroschisis, and the limb body complex, which was introduced for routine obstetric practice in western countries in the 1980s. Observations on the association between low AFP and fetal chromosome trisomies allowed for the development of prenatal screening protocols for these abnormalities during the second trimester of pregnancy. Because the most common chromosomal abnormality in live-born infants is trisomy 21, maternal serum screening was primary directed at the detection of this disorder. Soon after, the addition of intact human chorionic gonadotropin (hCG) (Bogart *et al.*, 1987) and unconjugated oestriol (uE3) (Canick *et al.*, 1988) to screen for Down's syndrome improved the detection efficiency from the 21 per cent level with maternal serum AFP alone (Cuckle *et al.*, 1984) to 64 per cent with these multiple analytes (Haddow *et al.*, 1992).

Our present results indicate that maternal serum screening for Down's syndrome using three analytes can in fact achieve a higher performance than expected from recent reviews (Benn *et al.*, 1995; Kellner *et al.*, 1995). This is ascribable to the tested women being predominantly of advanced age having high detection rates as well as high false-positive rates (Wald *et al.*, 1988; Haddow *et al.*, 1994).

With this triple-marker screening applied to older women, Haddow *et al.* (1994) estimated that approximately 50 per cent of other aneuploidies would be identified. In our study, we obtained essentially this detection rate (54·5 per cent; 6/11 cases). The screening indicates the possibility of detecting sex chromosomal aneuploidies such as 47,XXX and 47,XXY (4/5 cases). All fetuses with autosomal aneuploidy (trisomy 13 or 18) showed ultrasonographic abnormalities, including hydrocephaly or hydrops. This imaging, in conjunction with the triple-marker test, might therefore increase the detection of fetal autosomal irregularities.

The risk of a fetal aneuploidy increases with maternal age and prenatal diagnosis, amniocentesis or chorionic villus sampling has traditionally been directed at women aged 35 years or older. In Japan, 8 per cent of all pregnant women, or around 100 000 cases a year, are 35 years or older. However, it is impossible to offer invasive techniques like amniocentesis in all cases and the figure for actual performance is more in the region of 10 000. In the light of this and considering our present data, it is clear that screening for Down's syndrome with maternal serum markers should also be initially directed at women aged 35 years or older.

For maternal serum tests to become common practice at general clinics (Onda *et al.*, 1996), it is necessary to carefully train genetic counsellors with a sufficient understanding of the significance of maternal serum tests and not simply ordinary genetics, as well as to thoroughly educate doctors themselves. At the time of testing, doctors must explain to pregnant women that the purpose of the test is to determine whether the fetus is at risk of a chromosome abnormality (mainly Down's syndrome), but that it cannot offer a diagnosis with the certainty of such tests as genetic amniocentesis or chorionic villus sampling. Furthermore, if, as in this report, the cut-off value for risk is 1:299, almost 25 per cent of pregnant women over 35 years old will screen-positive. Even when screen-positive, however, the proportion of women in which a chromosome abnormality of the fetus will actually be discovered is only 8 per cent. Women should thus be advised not to abandon their pregnancies but should instead be introduced to a facility where genetic screening can be performed and be encouraged to undergo amniocentesis. They should also be made to understand that even if they are screen-negative, 0·8 per cent may still have some chromosome abnormality.

In any event, the cost of maternal serum tests for clinical application need to be set in accordance with their accuracy. When administering these tests, sufficient counselling should be provided on their diagnostic limitations, so the patients may be better prepared to deal with the test results. The

understanding and consent of the pregnant woman and her family are a basic prerequisite, whatever the outcome.

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