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ORIGINAL ARTICLE

Second trimester maternal serum screening using alpha fetoprotein, free beta human chorionic gonadotropin and maternal age specific risk: Result of chromosomal abnormalities detected in screen positive for Down syndrome in an Asian population

AN-SHINE CHAO, CHAO-LUNG CHUNG, CHIEN-DER WU, SHUENN DYH CHANG, PO-JEN CHENG, YU-TING LIN AND YUNG-KUEI SOONG

From the Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Kwei-Shan, Tao-Yuan, Taiwan

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Background. This study was to determine the incidence of chromosome abnormalitites in Taiwanese women undergoing prenatal chromosome analysis after a second trimester Down syndrome screening by using maternal age and serum dual-marker testing (α-fetoprotein and free-β unit human chorionic gonadotropin).
Methods. A total of 10,098 Taiwanese women with pregnancy between 15 and 23 weeks' gestation received second-trimester Down syndrome risk evaluation by dual-marker and maternal age specific risk testing in a single medical center. The study took 22 months. Ninety-seven percent of this study population was less than 34 years old. Ninety-six percent of our cases were screened between 15–20 weeks of gestation. This population was included only after a routine ultrasonography scan for correction of gestational age and exclusion of major structural anomalies. By using an algorithm to detect Down's syndrome, with a risk of 1:270 as a cut-off value, 816 patients were screen-positive for Down syndrome (screen-positive rate 8.0%). Karyotypes were reviewed for 670 (82.1%) mothers who received prenatal karyotype analysis.
Results. Twelve cases of Down syndrome were identified in the screen positive group with an estimated detection rate of 67% (false positive rate 8%). Three cases of Down syndrome were detected in late trimester among the screen-negative group. Seven other fetal chromosome abnormalities were also found among the screen-positive pregnancy. In addition, seven cases were screen-positive for trisomy 18; all of these patients received amniocentesis and only one case was confirmed.
Conclusion. These findings indicate that this screening program combining alpha-fetoprotein (AFP), free beta human chorionic gonadotropin (free–hCG) and maternal age-specific would achieve a screening efficiency in Taiwanese populations as comparable to those obtained in Caucasian populations. Our results also suggest that approximately 3% of pregnancies with a positive dual marker and maternal age-specific screen results will have a chromosome abnormality despite having a normal routine ultrasound scan. Mothers with positive screening results should be made aware of the implications of a positive result.

Key words: alpha-fetoprotein; Asian; chromosome study; free-β hCG; maternal serum screening

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Down syndrome screening using maternal age and various combinations of biochemical markers is a well-established obstetric practice in many developed countries (1–4). A number of prospective trials have reported that on Down syndrome screening with alpha fetoprotein (AFP), human chorionic gonadotropin (hCG) and unconjugated estriol (uE3) (triple-marker testing) can be used to identify cases of Down syndrome, trisomy 18, some cases of 45,X, and also increased risk for other fetal abnormalities (5–7). Adverse perinatal outcomes and pregnancy complications have also been discussed (8).

As more data were gathered (9–12), Spencer (13) and Norgaard-Pedersen et al. (14) refined the screening protocol to focus on two analytes, namely AFP and free β hCG. This combination has improved the detection rate of Down syndrome to 75%, with a false-positive rate of 5%. While a pregnant woman's race has been shown to influence the serum levels of AFP, hCG and uE3 (15, 16), there have been very few studies of maternal screening programs among Asian population (17–18). As whether Asians account for a significant proportion of the total population of the world, it is important to evaluate whether the bi-analyte screening program can be as effective in Asian populations as in Caucasian (19).

We herein describe a 22-month experience with maternal serum screening by combining the maternal age and the dual marker testing method and the report of all types of chromosome abnormalities detected in a Taiwanese population who were screened positive for Down syndrome.

Materials and methods

We retrospectively reviewed the clinical records of all pregnant women who underwent Down syndrome screening by the dual-marker testing method at our hospital between 1 July 1994 and 30 April 1996. Because an amniocentesis screening program was already in place for women over 35 years of age, more than 97% of the study population was 34 years of age or below. Patients with multiple fetuses, insulin-dependent diabetes, or known to have received prior chorionic villi sampling or amniocentesis were excluded from this analysis, as were those who presented after 23 weeks' gestational age. The gestational age was ascertained from the last menstrual period (LMP) and ultrasonographic measurement. Most (99%) of the women had a routine dating scan before 20 weeks of gestation. Fetuses with sonographic evidence of major structural anomalies (i.e. anencephaly, omphalocele) were also excluded. These 10,098 patients were young women carrying singleton pregnancies with no gross fetal anomalies. Serum samples were obtained between 14 and 23 weeks of gestation in all cases, and of these 96% of them were tested at less than 20 weeks of gestation. All the serum samples were separated within 24 h of collection and were analyzed in our medical center within 5 days.

Free β-hCG was measured by a solid phase, two-site immunoradiometric assay (ELSAF-β-hCG;CIS Ltd, Gif-sur-Yvette Cedex, France). AFP was measured with an enzyme immunoassay kit (Abbott EIA-AFP Kit, North Chicago, IL, USA). To correct for gestational variations of the two measured analytes, results were expressed as multiples of the median (MoM) for unaffected pregnancies at the relevant gestational age. We used our own normative median values of AFP and freeβ-hCG at each week of gestation (20). The maternal age-specific risk was calculated from the formula compiled by Cuckle et al. (21). The likelihood ratio of Down's syndrome pregnancies was calculated using a univariate algorithm for each analyte individually, and using a bivariate algorithm from the overlapping log Gaussian distribution curves for the two analytes together. We followed the procedures outlined by Reynolds and Penney (9) to calculate the risk of Down syndrome, by using combinations of biochemical markers and the maternal age-specific risk. The cut-off value for the population screen positive for Down syndrome was ≧1:270 and these pregnancies were recommended to undergo karyotype analysis. As for the estimated increased risk of trisomy 18, we followed the algorithm to calculate an individual women's risk for trisomy 18 which has been published by Palomaki et al. (22). The cut-off risk in our center was ≧1:200.

Follow-up data were collected from our own

Table I. Summary of Down syndrome screening

	Number	Percentage
Total patients screened	10,098	
Screen-positive patients	816	8.08%
Screen-positive with prenatal chromosome analysis	670	82.11%
Down syndrome, prenatally identified	12	1.79%
Other chromosome abnormalities prenatally identified	7	1.04%
Odds of Down syndrome given positive result		1:55
Odds of any chromosome abnormality given positive result		1:35

In most cases (667/670), chromosome analyses were based on amniotic fluid analysis; cord blood was used in two cases and chorionic villi samples in one.

Table II. Characteristics of cases with Down syndrome and positive or negative prenatal screening results

Pregnancy no.	Gestational age (week)	Maternal age (year)	Multiples of median		Down syndrome risk
			AFP	f-βhCG	
Cases with positive Down screen result					
1	16.6	30.9	0.44	3.42	1/54
2	20.4	36.2	0.62	1.67	1/164
3	18.1	30.8	0.73	6.02	1/20
4	15.4	31.2	0.68	2.86	1/147
5	16.1	36.3	0.38	4.65	1/7
6	17.0	33.2	0.72	7.41	1/7
7	15.4	28.3	0.53	2.68	1/167
8	17.5	33.6	0.79	7.39	1/7
9	18.0	39.1	0.46	2.15	1/30
10	15.6	30.8	0.34	2.46	1/78
11	18.4	30.6	0.88	4.28	1/90
12	18.5	29.4	0.78	3.96	1/87
(*n*=12)					
Cases with negative Down syndrome screen result (false negative)					
13	18.6	27.6	0.49	1.28	1/695
14	15.0	25.0	0.47	1.63	1/516
15	19.1	23.2	0.36	1.62	1/323
(*n*=3)					

Down syndrome risk was calculated according to the method of Reynolds and Penney (9). A risk of ≧1:270 was considered a positive result. AFP=alpha-fetoprotein, f-βhCG=free-Beta-human chorionic gonadotropin.

cytogenetic laboratory and perinatal unit. In addition, telephone interviews were conducted to determine the postnatal results in cases of high-risk pregnancies. At the time of preparation of this paper, all pregnancies had been completed and the overall follow-up information was available for 85% of the cases.

Results

The results of Down screening program are summarized in Table I. Of the 10,098 women who underwent dual-marker combining with maternal age-specific risk screening for Down's syndrome, about 8% were positive. Of those with positive results, 670 (82%) agreed to have chromosome study. Twelve cases of Down syndrome were detected as a result of amniotic fluid all karyotyping. Taking into consideration the number of patients screened at each age, and allowing for loss of affected fetuses between the time can be screening to term, the expected number of Down syndrome cases in the total screened population was computed from the live-born incidence of Down babies (5, 6, 21, 23). From these previous reports, the estimated prevalence of trisomy 21 in a population younger than 34 years old was between 1.6 and 1.75 per 1000. Therefore, the expected number in this study was

Table III. Other chromosome abnormalities in screened population

Case no.	Abnormalities	Gestational age (week)	Maternal age (year)	Multiples of median		Down syndrome risk
				AFP	f-βhCG	
Cases identified in Down syndrome screen positive group						
1	45,XX,t(13;22)	16.0	28.4	0.86	4.29	1/96
2	46,XX,t(1;14)	23.0	41.6	0.65	1.22	1/86
3	45,XX,t(13;22)	16.0	27.5	0.86	4.29	1/98
4	46,XX,t(1;14)	23.0	41.6	0.65	1.22	1/86
5	46,XX,t(2;11)	21.2	31.0	NA	NA	1/202
6[1]	47,XY, +18	16.5	30.4	0.36	0.18	1/626 (T18 risk 1/76)
7[1] (*n*=7)	46,XY,15p+	19.2	24.0	NA	NA	<1/270

[1]termination of pregnancy. NA=not available.

18. The detection rate can therefore be estimated as 12 in 18, or 67%.

Table II summarizes the results for Down syndrome cases identified by dual-marker and maternal age-specific risk screening. In addition, three cases in the screen-negative group were also identified prenatally. Fetuses 13 and 14 developed hydrops fetalis and polyhydromnios in the third trimester, while the mother of fetus 15 insisted on having a karyotype study after counseling.

Chromosomal abnormalities other than Down syndrome were identified in seven of the 670 patients who received prenatal chromosome analysis (Table III). Only one of the seven cases was associated with an abnormal sonographic finding. Fetus 6 (trisomy 18) had a facial cleft and congenital heart disease noted sonographically only after the result of the screening study was known. There were no supernumerary marker chromosomes. Pregnancies of familial balanced rearrangements (fetuses 1 through 5) were all associated with normal neonatal outcome. To date, there have been no additional chromosome abnormalities reported from the Department of Perinatology for about 80% of the babies from women who had a Down syndrome screen-positive result.

Dual-marker combining maternal age-specific risk screening program indicated an increased risk for trisomy 18 in seven pregnancies, all of which received prenatal chromosomal analysis. One pregnancy (in Table III) was identified and terminated, while the remaining six fetuses had normal karyotypes.

Discussion

This study confirms the findings of previous reports that prenatal screening using the maternal serum markers AFP and free β-hCG combined with maternal age is an effective method of identifying women at risk of having a Down syndrome pregnancy. The incidence of Down syndrome was 1.79% in those with positive dual-marker results and prenatal chromosome analysis.

The level of serum AFP in Asian women is generally higher than in white women, while the serum AFP concentration is significantly lower in Asian Down syndrome pregnancies (16). The median AFP value of Down syndrome pregnancy is around 0.7 MoM in whites. In our study, the AFP concentration was 0.65 MoM ($n=12$); however these AFP values alone were not significant ($\leqq 0.5$ MoM).

Several studies have reported a superior detection rate with free β-hCG as compared with total hCG. Free β-hCG has been suggested as the major contributor to the increased sensitivity of the two-analyte screening method (9–12). We found significantly elevated free β-hCG median values in Down syndrome pregnancies among Taiwanese women as is the case in white mothers. The median values for affected pregnancies in the white population are between 2.22 to 2.64 MoM. In this study, our median value was 3.5.

The Down syndrome detection rate in our series was at least 67% which indicates that the dual marker screening method is as effective as triple marker. The use of the 1:270 midtrimester risk as a cut off value yielded a false-positive rate of 8%. From Table II, using a cut-off value of 1:220 would reduce the false-positive rate to 6%, without affecting the detection rate. Using a 1:220 cut-off value also would not decrease the detection rate of other chromosome anomalies (Table III). However, because not all women with positive screening results had karyotype analysis and because the follow-up was not complete, the detection rate may underestimate the actual incidence of Down syndrome in this population. In addition, the study sample was selected after the exclusion of gross fetal abnormalities, so that some chromosomal abnormalities may have been excluded prior to the study rather than being missed by screening.

In our study, we observed some chromosomal abnormalities other than trisomy 21 in the Down syndrome screen-positive group. Among the 670 screen-positive patients, chromosome abnormalities other than trisomy 21 were found in only 1.04% (seven) cases, which is lower than the rate in previous reports (6, 24). No cases of sex chromosome abnormalities and supernumerary marker chromosomes were found in our study. This result might indicate that the routine use of early ultrasound scan to assess gestational age, fetal nuchal fold, and structural anomalies reduces the possibility of undetected chromosomal abnormalities in the general population. It is unknown at present whether the distribution of various karyotype abnormalities varies among ethnic groups. Large collaborative studies with dual-marker tests would be needed to address this issue (24–27).

Dual-marker combining maternal age-specific risk screening program, in Asian populations can be expected to achieve a screening efficiency similar to those in other races. The overall results indicate that among patients under 35 years of age with positive screening results, at least 3% of fetuses will have some type of chromosomal abnormality despite having normal findings on routine ultrasound examination. Doctors and genetic counsellors must advise mothers and family members of the implications of positive screening re-

sults, if informed decisions regarding screen-positive pregnancies are to be made.

References

1. Haddow JE, Palomaki GE, Knight GJ et al. Prenatal screening for Down's syndrome with use of maternal serum markers. N Engl J Med 1992; 327: 588–93.
2. Phillips OP, Elias S, Shulman LP, Anderson RN, Morgan D, Simpson JL. Maternal serum screening for fetal Down's syndrome in women older than 35 years of age using alpha-fetoprotein, hCG, and unconjugated estriol: a prospective 2 year study. Obstet Gynecol 1992; 80: 353–8.
3. Wald NJ, Kennard A, Densem JW, Cuckle HS, Chard T, Butler L. Antenatal maternal serum screening for Down's syndrome: results of a demonstration project. BMJ 1992; 305: 391–4.
4. Cheng EY, Luthy DA, Zebelman AM, Williams MA, Lieppman RE, Hickok DE. A prospective evaluation of a second-trimester screening test for fetal Down's syndrome using maternal serum alpha-fetoprotein, hCG and unconjugated estriol. Obstet Gynecol 1993; 81: 72–7.
5. Goodburn SF, Yates JRW, Raggatt PR et al. Second-trimester maternal serum screening using alpha-fetoprotein, human chorionic gonadotrophin, and unconjugated estriol: experience of a regional programme. Prenat Diagn 1994; 14: 391–402.
6. Benn PA, Horne D, Briganti S, Greenstein RM. Prenatal diagnosis of diverse chromosome abnormalities in a population of patients identified by triple-marker testing as screen positve for Down syndrome. Am J Obstet Gynecol 1995; 173: 496–501.
7. Suzumori K, Tanemura M, Murakami I et al. A retrospective evaluation of maternal serum screening for the detection of fetal aneuploidy. Prenat Diagn 1997; 17: 9: 861–6.
8. Burton BK. Unexplained elevated maternal serum alpha fetoprotein and adverse perinatal outcome. In: Elias S, Simpson JL, eds. Maternal serum screening for fetal genetic disorders. New York: Churchill Livingstone, 1992: 109–19.
9. Reynolds TM, Penney MD. The mathematical basis of multivariate risk screening with special reference to screening for Down's syndrome associated pregnancy. Clin Chem 1990; 27: 452–8.
10. Macri JN, Kasturi RV, Krantz DA et al. Maternal serum Down's syndrome screening: free β-protein is a more effective marker than human chorionic gonadotropin. Am J Obstet Gynecol 1990; 163: 1248–53.
11. Spencer K. Evaluation of an assay of the free β-submit of choriogonadotropin and its potential value in screening for Down's syndrome. Clin Chem 1991; 37: 809–14.
12. Ryall RG, Staples AJ, Robertson EF, Pollard AC. Improved performance in a prenatal screening program for Down's syndrome incorporating serum free hCG subunit analysis. Prenat Diagn 1992; 12: 251–61.
13. Spencer K. Is the measurement of unconjugated oestriol of value in screening for Down's syndrome? In: Grudzinskas JG, Chard T, Chapman M, Cuckle H, eds. Screening for Down's syndrome. Cambridge: Cambridge University Press, 1994: 141–61.
14. Norgaard-Pedersen B, Alfthan H, Arends J et al. A new simple and rapid dual assay for AFP and free beta hCG in screening for Down's syndrome. Clin Genet 1994; 45: 1–4.
15. Johnson AM, Palomaki GE, Haddow JE. Maternal serum α-fetoprotein levels in pregnancies among black and white women with fetal open spina bifida: a United States collaborative study. Am J Obstet Gynecol 1990; 162: 328–31.
16. O'Brien JE, Dvorin E, Drugna A, Johnson MP, Yaron Y, Evans MI. Race-ethnicity-specific variation in multiple-marker biochemical screening: alpha-fetoprotein, hCG and estriol. Obstet Gynecol 1997; 89: 355–8.
17. Onda T, Kitagawa M, Takeda O et al. Triple marker screening in native Japanese women. Prenat Diagn 1996; 16: 713–17.
18. Hsu JJ, Hsieh TT, Hsieh FJ. Down syndrome screening in an Asian population using alpha-fetoprotein and free β-hCG: a report of the Taiwan Down Syndrome Screening Group. Obstet Gynecol 1996; 87: 943–7.
19. Hsu JJ, Hsieh TT, Soong YK, Spencer K. Comparison of Down's syndrome screening strategies in Asians combining serum free beta-hCG and alpha-fetoprotein with maternal age. Prenat Diagn 1997; 17: 8: 707–16.
20. Hsieh TT, Hsu JJ, Chen CP et al. Down's syndrome screening with AFP and free β-hCG: an analysis of the influence of Chinese ethnic origin on screening parameters. Am J Hum Genet 1995; 57: 1632.
21. Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987; 94: 387–402.
22. Palomaki GE, Haddow JE, Knight GJ et al. Risk-based prenatal screening for trisomy 18 using alpha-fetoprotein, unconjugated oestriol and human chorionic gonadotropin. Prenat Diagn 1995; 15: 713–23.
23. Lin SJ. Down syndrome in Taiwan. Acta Paediatr Sin 1994; 35: 28–31.
24. Ko TM, Tseng LH, Hwa HL et. al. Cytogenetic analysis of amniotic fluid cells in 10,000 samples. Journal of Obstetrics and Gynecology Republic of China 1996; 35: 153–8.
25. Salonen R, Turpeinen U, Kurki L et al. Maternal serum screening for Down's syndrome on a population basis. Acta Obstet Gynecol Scand 1997; 76: 817–21.
26. Ferguson-Smith MA, Yates JRW. Maternal age specific rates for chromosome aberrations and factors influencing them: report of a collaborative study on 52,965 amniocenteses. Prenat Diagn 1984; (special issue): 5–44.
27. Hook EB, Cross PK. Extra structurally abnormal chromosomes (ESAC) detected at amniocentesis: frequency in approximately 75,000 prenatal cytogenetic diagnoses and associations with maternal and paternal age. Am J Hum Genet 1987; 40: 83–101.

Address for correspondence:

Dr. An-Shine Chao
Department of Obstetrics and Gynecology
Chang Gung Memorial Hospital
5, Fu-Hsing Street, Kwei-Shan, Tao-Yuan, Taiwan