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SPECIAL ARTICLE

Second-trimester double or triple screening for Down syndrome: A comparison of Chinese and Caucasian populations

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Abstract

Objectives: To compare the performance of double screening (measuring maternal serum levels of α-fetoprotein [AFP] and total β-human chorionic gonadotrophin [hCG] as markers for Down syndrome) with that of triple screening (also measuring levels of unconjugated estriol [uE3]) in the second trimester of pregnancy, and to compare ethnic variance between Chinese and Caucasian populations. *Methods:* The study investigated 15096 normal singleton pregnancies and 24 pregnancies affected with Down syndrome. Frequency distributions of AFP, hCG, and uE3 levels were analyzed. Likelihood ratios (LRs) were calculated using the multiple of median value (MoM) of AFP, hCG, and uE3 as variables. After multiplying maternal age risk by the LR values for the markers used in double and triple screening, the specific risks obtained with double and triple screening were estimated. The detection rate (DR) and false-positive rate (FPR) were calculated at different cut-off points. The serum markers' levels were also compared with those of Caucasian women. *Results:* The median MoM value of hCG was higher in women with affected pregnancies (1.40) than those with unaffected pregnancies (1.00). However, the median MoMs of AFP and uE3 (0.79 and 0.68) were lower in affected than in unaffected pregnancies. At a FPR of 5%, the detection rates reached with double and triple screening were 50%

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and 66.7%, respectively. Ratios of the 3 serum markers' medians to those in a study with Caucasian women were 1.06 (range = 1.04—1.09) for AFP, 1.14 (range = 1.10—1.17) for hCG, and 1.28 (range = 1.23—1.41) for uE3 for the relevant gestational weeks. *Conclusion:* Triple screening performed better than double screening in the second trimester. Ethnic variance should be taken into account in Down syndrome screening.

1. Introduction

Together with selective termination of pregnancy, maternal serum screening has been in clinical use for about 20 years as markers were discovered for Down syndrome. Screening has been performed in the second trimester and the marker first used was α-fetoprotein (AFP) [1], followed by human chorionic gonadotrophin (hCG) and unconjugated estriol (uE3). Measuring maternal serum levels of both AFP and hCG is known as double screening, and measuring levels of AFP, hCG, and uE3 is known as triple screening [2,3].

In the 1990s, researchers found an ultrasonographic marker, nuchal translucency thickness (NT), which, together with maternal age, could distinguish about 75% of fetuses with Down syndrome earlier in the pregnancy [4]. Then, it was found that NT testing in combination with measuring levels of pregnancy-associated plasma protein A and free β-hCG in the first trimester obtained a higher detection rate (DR) of 83%, with a false-positive rate (FPR) of 5% [5].

Maternal serum screening programs were introduced in China in the 1990s. Since the measurement of NT is very complex, only specifically trained physicians can perform the ultrasonographic evaluation [6,7]; first-trimester screening is not widely used in China. Meanwhile, double or triple screening in the second trimester has been gradually accepted by pregnant Chinese women, especially in large cities.

Because the rates of false-positive and false-negative results are high, the performance of the prevalent second-trimester screening methods should be examined in China. To assess performance, DRs and FPRs obtained with the two methods were compared. As several researchers have found that ethnic differences in serum markers levels may affect screening performance [8,9], the variance between Chinese women and women of Caucasian descent was also estimated.

2. Methods

From September 2001 to December 2004, 15 120 pregnant Chinese women were screened for fetal Down syndrome between 14 and 21 weeks pregnancy in 3 Shanghai hospitals. The records showed maternal age, gestational age (GA), and maternal serum concentrations of AFP, hCG, and uE3. Down

Table 1 Distribution of maternal age and gestation age in groups unaffected and affected by Down syndrome[a]

Shanghai hospital[b]	Age (years)			Gestational age (weeks)			No. of cases
	Mean	Median	S.D.	Mean	Median	S.D.	
CWI							
Unaffected	28.88	28.42	3.98	17.70	17.60	1.76	9149
Affected	36.02	35.77	3.88	18.16	18.05	1.27	12
Fudan							
Unaffected	28.44	27.93	3.96	17.64	17.60	1.92	2931
Affected	33.02	33.26	4.43	16.5	16.95	1.67	6
Putuo							
Unaffected	27.12	26.83	3.50	16.31	16.20	0.85	3016
Affected	28.87	29.59	4.56	16.12	16.35	0.61	6
Total							
Unaffected	28.44	27.95	3.94	17.42	17.3	1.75	15096
Affected	33.48[a]	33.59	5.70	17.23	17.25	1.78	24

[a] $P<0.05$ was considered significant.
[b] CWI, China Welfare Institute; Fudan, Fudan University Medical Center; Putuo, Maternal and Fetal Health Hospital, Putuo District.

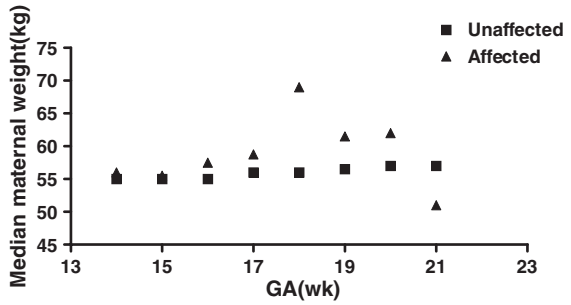

Figure 1 Comparison of maternal weight in unaffected and affected pregnancies.

syndrome was confirmed by karyotype analysis via amniocentesis or in the peripheral blood of the newborn. There were 9161 participants from International China Welfare Institute's Peace Hospital for the Protection of Mother and Child's Health, 2937 from Maternal and Fetal Health Hospital, Putuo District, and 3022 from Obstetrics and Gynecology Hospital of the Fudan University Medical Center.

Gestational age was estimated by the last menstruation if the woman's period had been regular, or by ultrasonographic scan. Maternal age was calculated for the expected delivery date. Maternal serum levels of AFP, hCG, and uE3 were determined using the Access Immunoassay System (Beckman Coulter, Inc Fullerton, CA, USA, who also provided the reagents). All data were supplied by Shanghai Teng Cheng Medical Tech-Info Co. Ltd., Shanghai, China. To correct gestational variations in the data, the serum marker levels were reported in multiples of median (MoM) for the relevant GAs. To minimize the systematic bias in different hospitals, the MoMs were calculated in different hospitals according to their own median values for the unaffected pregnancies. The MoMs were then converted to their log-equivalent to obtain the distribution parameters [10]. Down syndrome risk was assessed by a commonly used risk algorithm, using parameters in both Down syndrome and normal Gaussian distribution to calculate the likelihood ratio (LR) obtained with the different markers [10]. The LR was calculated as follows:

$$\mathrm{LR} = \frac{f_d(X)}{f_n(X)},$$

with

$f_d(X)$ height of the Gaussian distribution of Down syndrome in all pregnancies, and
$f_n(X)$ height of the Gaussian distribution in normal pregnancies.

Age-specific risk was calculated as follows [11]:

$$\text{Risk age} = \frac{0.000627 + e^{(-16.2395+0.286\times\text{age})}}{0.999373 - e^{(-16.2395+0.286\times\text{age})}}$$

The case-specific risk of Down syndrome in triple screening and double screening, respectively, was estimated using the following equations:

Risk with triple screening

$$= \text{age-specific risk} \times \mathrm{LR(AFP)} \times \mathrm{LR(hCG)} \times \mathrm{LR(uE3)}$$

Risk with double screening

$$= \text{Risk Age} \times \mathrm{LR(AFP)} \times \mathrm{LR(hCG)}.$$

3. Results

Among the 15120 pregnancies, 24 were affected with Down syndrome. The mean age was 33.48 years for the women in the affected group vs. 28.44 years in the nonaffected group ($t = 6.27$; $df = 15118$; $P < .05$) (Table 1). Maternal weight was higher in the affected than in the unaffected

Table 2 Comparison of median AFP, hCG and uE3 maternal serum concentrations in 3 hospitals[a]

GA (weeks)	AFP (ng/mL)			hCG (IU/mL)			uE3 (ng/mL)		
	CWI	Fudan	Putuo	CWI	Fudan	Putuo	CWI	Fudan	Putuo
14	30.70 (430)	29.44 (263)	31.70 (50)	59.70	67.71	73.72	0.80	0.72	0.61
15	35.30 (1060)	34.56 (295)	35.76 (795)	45.90	49.81	54.75	1.02	0.93	0.90
16	39.50 (1405)	38.42 (432)	39.18 (1365)	36.30	38.93	41.19	1.34	1.27	1.13
17	43.80 (1874)	45.17 (516)	45.51 (664)	30.30	31.48	33.43	1.69	1.50	1.45
18	51.35 (1824)	48.89 (532)	52.01 (123)	26.30	29.28	29.13	2.09	1.85	1.66
19	59.10 (1299)	59.83 (524)	65.29 (14)	23.30	26.60	23.77	2.50	2.27	1.77
20	66.70 (876)	61.37 (262)	63.08 (5)	21.90	22.76	30.36	2.85	2.49	2.27
21	77.00 (381)	71.16 (107)	–	22.80	24.49	–	3.13	2.75	–

Abbreviations: AFP, α-fetoprotein; hCG, human chorionic gonadotrophin; uE3, unconjuncted estriol.
[a] CWI, China Welfare Institute; Fudan, Fudan University Medical Center; Putuo, Maternal and Fetal Health Hospital, Putuo District.

Table 3 Comparison of median maternal serum concentrations of AFP, hCG, and uE3 between observed data for Chinese women and published data for Caucasian women with unaffected pregnancies

GA (weeks)	AFP (ng/mL)			hCG (IU/mL)			uE3 (ng/mL)		
	Calculated values for Chinese women	Previously published values for Caucasian women[a]	Ratio[b] (%)	Calculated values for Chinese women	Previously published values for Caucasian women[a]	Ratio[b] (%)	Calculated values for Chinese women	Previously published values for Caucasian women[a]	Ratio[b] (%)
14	30.2 (743)	—		62.40	—		0.76	—	
15	35.3 (2150)	33.3 (105)	106.0	49.40	42.4	116.5	0.97	0.73	132.9
16	39.29 (3202)	37.6 (199)	104.5	38.70	34.1	113.5	1.24	0.92	134.8
17	44.37 (3054)	42.5 (80)	104.4	31.15	28.3	110.1	1.61	1.15	140.0
18	50.9 (2479)	48.0 (72)	106.0	27.20	24.1	112.9	2.01	1.43	140.6
19	59.28 (1837)	54.3 (71)	109.2	24.07	21.2	113.5	2.43	1.79	135.8
20	65.4 (1143)	61.3 (41)	106.7	22.10	19.1	115.7	2.76	2.24	123.2
21	76.22 (488)	—		23.10	—		3.04	—	

Abbreviations: AFP, α-fetoprotein; hCG, human chorionic gonadotrophin; uE3, unconjuncted estriol.
[a] Data for Caucasian women were reported by MacRae et al. [12].
[b] Ratio of the serum markers' medians calculated in this study to those in a published study with Caucasian women [12] for the relevant gestational week.

group. Fig. 1 shows the median maternal weight for relevant GAs.

The median concentrations of AFP, hCG, and uE3 grouped by GA for the 3 hospitals are shown in Table 2. The difference among serum marker levels in the 3 hospitals for each week of gestation was found significant ($P<0.05$) by the Kruskal—Wallis test, except for AFP.

The median values of the serum markers were also compared with published values for Caucasian women [12]. The results show that maternal serum marker levels were higher in Chinese than in Caucasian women at each GA (Table 3). The serum marker levels were converted to MoMs, and MoM distributions for AFP, hCG, and uE3 levels are shown in Table 4. MoMs of AFP and uE3 levels were lower and MoMs of hCG levels were higher in the affected than in the unaffected group. For Chinese and Caucasian women with affected pregnancies [5], MoMs of serum marker levels were 0.79 vs. 0.74 for AFP, 1.40 vs. 2.05 for hCG, and 0.68 vs. 0.70 for uE3.

The DRs and FPRs were estimated at different cut-off points. Using a fixed FPR of 5%, DRs for double and triple screening were 50% and 66.7%, respectively. The triple-screening method showed a higher DR at every cut-off point compared with double screening (Table 5).

4. Discussion

Down syndrome is the most frequently identified chromosomal abnormality, occurring in approximately 1 per 800 to 1 per 1000 live births [13]. It is one of the most important causes of mental retardation.

In 1984, Cuckle and associates [14] determined that a low level of AFP in maternal serum was a high-risk marker for of Down syndrome. Thereafter, several maternal serum markers for Down syndrome were introduced in clinical practice.

There are 2 kinds of maternal serum screening methods, the first- and the second-trimester method. Although second-trimester screening has been widely used in China, but it is not known whether there is a difference in performance between double and triple screening in that country. Ethnic variance between Chinese and Caucasian women should also be estimated with large population samples.

Table 4 Comparison of median maternal serum concentrations of AFP, hCG, and uE3 between calculated data for Chinese women and previously published data for Caucasian women with affected pregnancies

	Unaffected pregnancy			Affected pregnancy		
	AFP MoM	hCG MoM	uE3 MoM	AFP MoM	hCG MoM	uE3 MoM
Mean	1.005	0.992	0.987	0.783	1.497	0.679
Median	1.000	1.000	1.000	0.790 (0.74)[a]	1.400 (2.05)[a]	0.679 (0.70)[a]
Log_{10} S.D.	0.1589	0.2297	0.1495	0.1925	0.3156	0.1744

Abbreviations: AFP, α-fetoprotein; hCG, human chorionic gonadotrophin; MoM, multiple of median; uE3, unconjuncted estriol.
[a] Values in parentheses were reported by Wald et al. [5].

Table 5 Comparison of detection rate (DR) and false-positive rate (FPR) in double and triple screening

Cut-off value	Double screening		Triple screening	
	DR	FPR	DR	FPR
207	—	—	66.7	5
270	50.0	4.7	75.0	6.4
276	50.0	5	—	—
350	62.5	6.9	79.2	8.2
400	62.5	8.2	83.3	9.4
500	70.8	11.0	87.5	11.5

In 1998, Wald and colleagues [15] reported that with a fixed FPR of 5%, the DRs of double and triple screening in the second trimester were 54% and 59%, respectively (with GA estimated by last menstrual period). In the present study, DRs were 50% and 66.7%, respectively, for a DR improvement of 16.7% at a fixed 5% FPR. These results suggest a higher performance for the triple-screening method. Since maternal serum concentration of uE3 increases greatly with GA, a slightly variance in GA may affect the results greatly [15] and an accurate GA estimation is very important. The difference in DRs between the 2 populations may be due to GA estimation. The present study's findings confirm that using uE3 as a third maternal marker in second-trimester screening for Down syndrome is a good choice.

It has been reported that serum levels of AFP, hCG, and uE3 were higher in Oriental than in Caucasian women [16—18]. In the present study, the medians of serum marker levels calculated at relevant GAs for the unaffected group were compared with published values for Caucasian women with unaffected pregnancies [12]. For the relevant gestational weeks, ratios of the 3 serum markers' medians to those of a study with Caucasian women were 1.06 (range = 1.04—1.09) for AFP, 1.14 (range = 1.10—1.17) for hCG, and 1.28 (range = 1.23—1.41) for uE3 (Table 3). The results confirmed the ethnic tendency already noted for higher AFP, hCG, and uE3 levels in Oriental than in Caucasian women.

In a study by Wald and colleagues [5] the median MoMs of serum markers were compared between Oriental and Caucasian women with affected pregnancies, and the MoMs for hCG levels were 1.40 for Chinese women vs. 2.05 for Caucasian women. Law and colleagues [19] reported that the total β-hCG MoM in 6 Chinese women with affected pregnancies varied from 0.115 to 3.779; the median β-hCG MoM was 1.598, which was closer to the 1.40 found in this study.

When pregnancies were unaffected in this study, serum hCG level was higher in Chinese women than in their Caucasian counterparts; but when pregnancies were affected, the MoM of hCG was lower in Chinese women. Wenstrom and associates [20] reported that maternal weight affected hCG concentration. In the present study, maternal weight was higher in affected pregnancies. May differences in maternal weight between the 2 populations have an effect on the difference in serum hCG levels? More data should be collected and further study carried out to compare the hCG serum levels of women from the 2 populations with affected pregnancies.

Muller and colleagues recommend correcting hCG values before calculating the risk of Down syndrome.

5. Conclusion

Triple screening measuring maternal serum levels of AFP, hCG and uE3 in the second trimester of pregnancy performed better than double screening (without measurement of uE3 level) among Chinese women. The economy permitting, triple screening should be seen as a priority. The parameters of maternal serum markers should be calculated using local data, and adjustments should be made for ethnic variance in Down syndrome prenatal screening.

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