PRENATAL DIAGNOSIS, VOL. **17:8:** 707–716 (1997)

COMPARISON OF DOWN'S SYNDROME SCREENING STRATEGIES IN ASIANS COMBINING SERUM FREE BETA-hCG AND ALPHA-FETOPROTEIN WITH MATERNAL AGE

J. J. HSU[1]* **, T. T. HSIEH[1]**, Y. K. SOONG[2]** AND K. SPENCER[3]

[1]*Department of Obstetrics and Gynecology, Taipei Chang Gung Memorial Hospital, Taipei, Taiwan, Republic of China*
[2]*Lin-Kou Chang Gung Memorial Hospital, Tau-Young, Taiwan, Republic of China*
[3]*Endocrine Unit, Clinical Biochemistry Department, Oldchurch Hospital, Romford, Essex RM7 0BE, U.K.*

Received 16 August 1996
Revised 3 February 1997

SUMMARY

High free beta human chorionic gonadotropin (β-hCG) and low alpha-fetoprotein (AFP) levels were found in 47 Asian Down's syndrome pregnancies (median values 2·79 and 0·77 MOM, respectively). At a 5 per cent false-positive rate, free β-hCG alone would identify 46·8 per cent of Down's syndrome pregnancies, age alone detected 34·5 per cent of affected cases, whilst AFP alone detected 17 per cent and free β-hCG/AFP MOM ratios detected 48·9 per cent of Down's syndrome cases. When combined with maternal age-specific risk, free β-hCG could achieve a 59·6 per cent detection rate, with AFP achieving 42·6 per cent, free β-hCG/AFP MOM ratios 61·7 per cent, and combined free β-hCG and AFP a detection rate of 63·8 per cent for a 5 per cent false-positive rate. Down's syndrome screening at an early gestational age (before 18 weeks) could achieve a 68 per cent detection rate with a 5 per cent false-positive rate, compared with a 59·1 per cent detection rate for a 5·2 per cent false-positive rate when screening at a late gestational age. The use of free β-hCG in Down's syndrome screening programmes can yield an improved efficacy in the detection of Down's syndrome in an Asian population. © 1997 by John Wiley & Sons, Ltd.

Prenat. Diagn. 17: 707–716, 1997
No. of Figures: 2. No. of Tables: 3. No. of References: 29.

KEY WORDS: Down's syndrome; screening; free β-hCG; Asian

INTRODUCTION

The overall birth prevalence of Down's syndrome is approximately 1 per 848 births in Taiwan (Lin, 1994). A screening policy for Down's syndrome in Taiwan was initially based on the offer of amniocentesis to pregnant women older than 35 years of age at delivery. In our population, only 25–30 per cent of all cases of fetal Down's syndrome could be identified in this old age population. The remaining 70–75 per cent of Down's syndrome cases occur in pregnant women younger than 35. In order to select young age pregnancies with an increased risk of Down's syndrome, maternal serum screening for Down's syndrome has become an essential prenatal examination in Taiwan and in Western countries.

Such screening was initiated from the observation of an association between low maternal serum alpha-fetoprotein (AFP) and Down's syndrome pregnancies in either whites or Asians (Merkatz *et al.*, 1984; Hsu *et al.*, 1993). In order to increase the detection rate and reduce the false-positive rate, screening strategies incorporating

*Correspondence to: Dr Jenn-Jeih Hsu, Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, 199 Tung-Hwa North Road, Taipei, Taiwan, Republic of China.

**Taiwan Down Syndrome Screening Group.

Contract grant sponsor: Department of Health, the Executive Yuan, R.O.C.

CCC 0197–3851/97/080707–10 $17.50

combinations of various additional biochemical markers were proposed. Human chorionic gonadotropin (hCG) is a placenta-derived glycoprotein consisting of two subunits, the α-subunit and β-subunit. Free β-hCG has been reported as a significantly elevated marker in Down's syndrome pregnancies (Macri *et al.*, 1990; Spencer, 1991). Since the β-subunit is specific to hCG, some authors advocate incorporating free β-hCG in serum screening for Down's syndrome. Although a few authors are still uncertain of the effectiveness of using free β-hCG in prenatal screening for Down's syndrome (Stone *et al.*, 1993; Wald *et al.*, 1994), measurement of free β-hCG has been reported to improve the performance of Down's syndrome screening (Ryall *et al.*, 1992; Spencer *et al.*, 1992a; Wald *et al.*, 1993; Macri *et al.*, 1994).

Since Asians account for a significant proportion of the world population, it is mandatory to establish that Down's syndrome screening programmes developed essentially with European or American populations can be effective in Asian countries and achieve a high degree of efficacy. Because of the debate over the additional benefit of unconjugated oestriol (Spencer, 1994), it was not incorporated into our screening programmes. To ascertain the potential value of free β-hCG in Down's syndrome screening, we collected sizable Asian data to evaluate the two-analyte screening strategy combining serum free β-hCG and AFP with maternal age in an Asian population.

MATERIALS AND METHODS

Between 1992 and 1996, AFP and free β-hCG data were available from 47 singleton pregnancies affected with Down's syndrome in our four centres. Eleven sets of data were available from a previous study (Hsu *et al.*, 1996a) with sample storage at -70°C for $9{\cdot}1 \pm 5{\cdot}7$ months (range 3·1–20·9 months). Thirty-six sets of data were collected from routine serum screening for Down's syndrome, including 25 cases with screening positive after cytogenetic confirmation by midtrimester amniocentesis and 11 cases with screening negative, collated on the basis of abnormal birth outcome (cytogenetically confirmed). As a suitable population for comparison, we used the results from 8218 unaffected singleton pregnancies from routine serum screening in Taipei centre as controls. Information on the outcome of the controls was obtained partly from karyotyping and partly from information collected at delivery. None of the affected and unaffected pregnancies was associated with neural tube defects or other congenital anomalies. Gestational age was determined by ultrasound in 84 per cent of cases and last menstrual period in 16 per cent of cases. It ranged from 14 to 23 weeks (mean 18·0 weeks in Down's syndrome pregnancies and 16·9 weeks in unaffected pregnancies).

Free β-hCG was measured by a solid phase, two-site immunoradiometric assay (ELSA F-βHCG; CIS Ltd, Gif-sur-Yvette Cedex, France). The analytical performance and specificity of this assay have been previously described (Spencer, 1991; Macri *et al.*, 1993a). AFP was measured by a commercially available enzyme immunoassay kit (Abbott EIA-AFP kit, North Chicago, IL, U.S.A.) with a between-assay coefficient of variation (CV) and within-assay CV of 4 and 4·3 per cent, respectively. To correct for gestational variation of the two measured analytes, results were expressed as multiples of the median (MOM) for unaffected pregnancies at the relevant gestational age. For this, we used our own normative median values of AFP and free β-hCG at each week of gestation (Hsieh *et al.*, 1995). The AFP and free β-hCG MOM levels were adjusted by our Taiwanese weight-correction formula [adjusted AFP MOM = measured AFP MOM/$10^{(0{\cdot}000195\,.\,\text{weight (kg)}^2 - 0{\cdot}036993\,.\,\text{weight (kg)} + 2{\cdot}426925)}$ and adjusted free β-hCG MOM = measured free β-hCG MOM/$10^{(0{\cdot}000174\,.\,\text{weight (kg)}^2 - 0{\cdot}033334\,.\,\text{weight (kg)} + 2{\cdot}308748)}$]. The maternal age-specific risk was calculated from the formulae compiled by a previous study (Cuckle *et al.*, 1987). The likelihood ratio of Down's syndrome pregnancies was calculated with the single analyte using a univariate algorithm and with the two analytes using a bivariate algorithm from the overlapping log Gaussian distribution curves of the analytes. Calculation of the risk of Down's syndrome using individual or combinations of biochemical marker(s), and the maternal age-specific risk, was performed according to the procedure outlined by Reynolds and Penney (1990).

Data analyses were accomplished using the statistical package SPSS for Windows Version 6 (SPSS Inc., Chicago, IL, U.S.A.). We used the Kolmogorov–Smirnov test to assess the normal Gaussian distributions of the analytes and Spearman or Pearson correlation coefficients to assess the correlation among these various indices. The Mann-Whitney *U*-test was used to assess the

difference of the detection rate in both maternal age and gestational age. $P<0{\cdot}05$ was considered statistically significant. To compare the effectiveness of various screening strategies, receiver operating characteristic (ROC) curves for maternal age, serum AFP or free β-hCG, and various combined methods were established by plotting the detection rate against the respective false-positive rate.

RESULTS

Demographic data

The Taiwanese are Asians, with 98·5 per cent of the population being ethnically Chinese and 1·5 per cent Polynesian in origin. Although our maternal serum screening programme was used mainly for women under 35 years of age, 5·9 per cent (485/8218) of the control population was 35 years or older versus 38·3 per cent (18/47) in the Down's syndrome pregnancies. The mean and standard deviation (SD) of maternal age and weight were $31{\cdot}4 \pm 4{\cdot}5$ years (median 30·8 years, range 23·2–43·5 years) and $55{\cdot}8 \pm 5{\cdot}3$ kg (median 55 kg, range 45–65 kg) in Down's syndrome pregnancies, respectively; and $29{\cdot}4 \pm 3{\cdot}5$ years (median 29·4 years, range 17·0–44·4 years) and $55{\cdot}2 \pm 9{\cdot}1$ kg (median 54·8 kg, range 41·1–85·0 kg) in unaffected pregnancies, respectively.

Measured analyte

Figure 1 shows the serum free β-hCG and AFP MOM values for each Down's syndrome case at various weeks of gestation and indicates that high free β-hCG and low AFP levels are strongly associated with Asian Down's syndrome pregnancies. The median value of free β-hCG in Down's syndrome pregnancies was 2·79 MOM, significantly higher than in unaffected pregnancies [$P<0{\cdot}05$; 95 per cent confidence interval (CI) 2·50–3·61 MOM]. The tenth and 90th centiles for Down's syndrome pregnancies were 0·81 and 5·82 MOM, respectively, and for the unaffected pregnancies 0·50 and 2·21 MOM, respectively. The median MOM value of AFP in Down's syndrome pregnancies was 0·76, significantly lower than in unaffected pregnancies ($P<0{\cdot}05$; 95 per cent CI 0·74–0·95 MOM). The tenth and 90th centiles for Down's syndrome pregnancies were 0·45 and 1·30 MOM, and for the unaffected pregnancies 0·63 and 1·62 MOM, respectively. Free β-hCG and AFP were shown to have no correlation with maternal age in both affected and unaffected pregnancies [r (unaffected) $= -0{\cdot}003$, $P=0{\cdot}81$; r (affected) $=0{\cdot}039$, $P=0{\cdot}79$; and r (unaffected) $=0{\cdot}018$, $P=0{\cdot}11$; r (affected) $=0{\cdot}289$, $P=0{\cdot}12$].

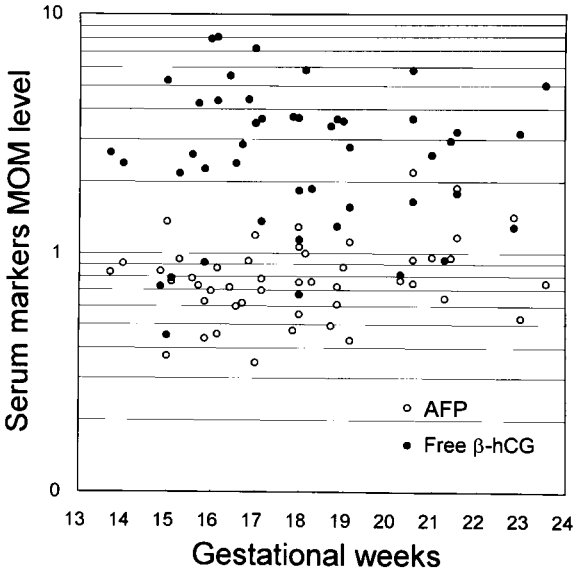

Fig. 1—Distribution of free β-hCG and AFP plotted as multiple of the median (MOM) levels at each week of gestation in 47 Down's syndrome pregnancies

Population statistics

The distributions of the AFP and free β-hCG MOM values on log transformation in both affected and unaffected pregnancies fit normal Gaussian distributions ($D=0{\cdot}085$, $P=0{\cdot}891$ and $D=0{\cdot}098$, $P=0{\cdot}753$ for Down's syndrome pregnancies, respectively; and $D=0{\cdot}014$, $P=0{\cdot}091$ and $D=0{\cdot}021$, $P=0{\cdot}078$ for unaffected pregnancies, respectively). Table I summarizes the statistics for the distribution of the two analytes in affected and unaffected pregnancies. There was no correlation between log free β-hCG and log AFP distributions in affected and unaffected pregnancies, with a correlation coefficient of $-0{\cdot}0535$ ($P=0{\cdot}721$) and $0{\cdot}0014$ ($P=0{\cdot}657$), respectively.

Free β-hCG (MOM)/AFP (MOM) ratios

The median ratio value in Down syndrome pregnancies was 3·31, significantly higher than that of the unaffected population ($P<0{\cdot}05$). The tenth

Table I—Statistical parameters describing the distribution of each analyte measured (log_{10} MOM) in Down's syndrome and unaffected pregnancies

Biochemical variables	Down's syndrome ($n=47$)	Unaffected ($n=8218$)
Median		
Free β-hCG	2·788	0·997
AFP	0·762	1·005
Free β-hCG/AFP	3·309	0·988
Mean		
Free β-hCG	0·3776	0·0110
AFP	− 0·1060	0·0027
Free β-hCG/AFP	0·4947	0·0089
Standard deviation		
Free β-hCG	0·3221	0·2605
AFP	0·1594	0·1668
Free β-hCG/AFP	0·3673	0·3080
Correlation coefficient		
Free β-hCG/AFP	− 0·0535 ($P=0·721$)	0·0014 ($P=0·657$)

and 90th centiles for Down's syndrome pregnancies were 1·01 and 9·33, and for the unaffected pregnancies 0·43 and 2·50, respectively. The ratio on log transformation in affected and unaffected pregnancies also showed a normal Gaussian distribution ($D=0·147$, $P=0·091$ and $D=0·024$, $P=0·065$, respectively) with a mean value (after log transformation) of 3·17 (2 SD range = 0·58–16·96) and 1·02 (2 SD range of 0·25–4·22) in affected and unaffected pregnancies, respectively.

Detection rate and false-positive rate

Figure 2 shows the ROC curves after smoothing for each analyte when used independently (without taking maternal age into account) or in combination (with maternal age-specific risk). It indicates that free β-hCG is more discriminating than age alone or AFP alone in the detection of Down's syndrome. At a 5 per cent false-positive rate, free β-hCG alone identified 46·8 per cent of Down's syndrome cases; age alone detected 34·5 per cent; AFP alone detected 17 per cent; and using the free β-hCG/AFP MOM ratio, a detection rate of 46·8 per cent was achieved. Significant improvements in the detection rates were observed when the *a priori* age-related risk of Down's syndrome was used in combination with the analyte concentration in a univariate risk calculation. After combining with the maternal age-specific risk, AFP achieved a 42·6 per cent detection rate, free β-hCG a 59·6 per cent detection rate, and the free β-hCG/AFP MOM ratio a 61·7 per cent detection rate for a 5 per cent false-positive rate. If a bivariate algorithm was used by incorporating *a priori* age-related risk combined with free β-hCG and AFP concentrations, 63·8 per cent of Down's syndrome cases would be detected at a 5 per cent false-positive rate.

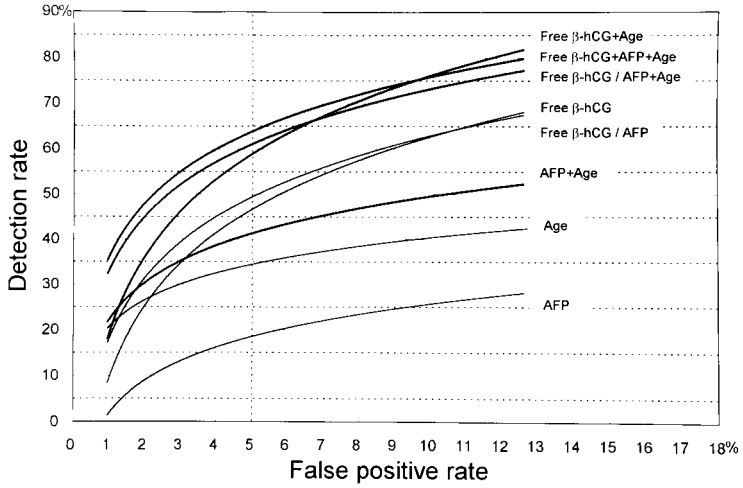

Fig. 2—Receiver operator characteristic (ROC) curves depicting the variation of the observed false-positive rate with the true positive rate for markers of Down's syndrome analysed independently and univariately or bivariately with maternal age. (The plotted line is a smoothed curve through the observed points)

Table II shows the observed detection rate and false-positive rate analysed by early gestational age (<18 weeks) and late gestational age (⩾18 weeks) in various Down's syndrome screening strategies with a fixed 5 per cent false-positive rate in the general population. In the screening strategy of combining free β-hCG and AFP with age, the observed detection efficiency (68·0 per cent) of early gestational age is slightly but not significantly better than that (59·1 per cent) of late gestational age ($P>0·05$). The mean age and false-positive rate in both subsets of Down's syndrome pregnancies were 32·5 years and 5·0 per cent in early gestational age and 34·5 years and 5·2 per cent in late gestational age. The median values of free β-hCG and AFP in early gestational age were 2·92 and 0·76 MOM, respectively, and for late gestational age 2·22 and 0·77 MOM, respectively.

Table III demonstrates the observed detection rate and false-positive rate analysed by younger women (<35 years of age) and elderly women (⩾aged 35) in various Down's syndrome screening strategies with a 5 per cent false-positive rate. The observed detection rate and false-positive rate of the screening strategy using serum free β-hCG and AFP combined with age in the younger women were 55·2 and 4·6 per cent, respectively, and for the elderly women 77·8 and 13·9 per cent, respectively ($P<0·05$). The median values of free β-hCG and AFP in the younger women were 2·65 and 0·74 MOM, respectively, and for the elderly women 2·72 and 0·77 MOM, respectively.

DISCUSSION

This study confirms the observation of previous studies that the serum AFP concentration is significantly lower in Asian Down's syndrome pregnancies (Hsu *et al.*, 1993). The median AFP value of Down's syndrome pregnancies was 0·76 MOM, which agrees closely with the data of Tabor *et al.* (1984) (0·75 MOM) in whites and Hsu *et al.* (1996b) (0·77 MOM) in Asians. Our data show poor discrimination between affected and unaffected pregnancies by the AFP alone (⩽0·5 MOM) screening strategy; 14·9 per cent of affected pregnancies would be detected with a 3·2 per cent false-positive rate, which is in agreement with the results of Spencer and Carpenter (1985) (14·8 per cent detection rate and 8·6 per cent false-positive rate) and those of Cuckle *et al.* (1984) (17 per cent detection rate and 5 per cent false-positive rate). Although combined maternal serum AFP and age would increase the detection rate (42·6 per cent), as also found by Wald *et al.* (1994) (36 per cent) and Cuckle *et al.* (1984) (40 per cent), its performance in serum screening for Down's syndrome remains poor. However, because of its widespread use in screening for neural tube defects, the role of AFP appears secure as a component of maternal serum screening for Down's syndrome.

The use of serum hCG in Down's syndrome screening is just a current complex matter. Although the use of total hCG is unequivocally established in serum screening for Down's syndrome (Crossley *et al.*, 1991; Haddow *et al.*, 1992; Cheng *et al.*, 1993), a superior detection rate was achieved in several studies by using free β-hCG (Spencer, 1991; Ryall *et al.*, 1992; Spencer *et al.*, 1992a; Wald *et al.*, 1993; Macri *et al.*, 1994). Free β-hCG has been suggested as the major contributor to the increased sensitivity of two-analyte screening. Our study also reveals the low contribution of AFP alone to the detection of Down's syndrome and that free β-hCG alone has more discriminating power than AFP. We found significantly elevated free β-hCG median values in Down's syndrome pregnancies of Asians, as also found in white women. The median values for affected pregnancies in whites were 2·36 MOM ($n=57$) (Ryall *et al.*, 1992), 2·41 MOM ($n=90$) (Spencer *et al.*, 1992a), 2·50 MOM ($n=21$) (Stone *et al.*, 1993), 2·22 MOM ($n=75$) (Wald *et al.*, 1993), 2·64 MOM ($n=480$) (Macri *et al.*, 1994), and 2·31 MOM ($n=72$) (Norgaard-Pedersen *et al.*, 1994), respectively, compared with 2·91 MOM ($n=23$) in our previous study (Hsu *et al.*, 1996b) and 2·79 MOM ($n=47$) in this study of Asians. Our data also show that second-trimester free β-hCG levels in affected and unaffected cases in Asians fit a log Gaussian distribution that was similar to the distributions of the whites. The standard deviations in affected and unaffected pregnancies in these studies were respectively 0·335 and 0·250 (Macri *et al.*, 1990), 0·301 and 0·294 (Ryall *et al.*, 1992), 0·332 and 0·254 (Spencer *et al.*, 1992a), 0·313 and 0·248 (Norgaard-Pedersen *et al.*, 1994), and 0·296 and 0·238 (Wald *et al.*, 1994) log MOM, compared with our previous estimates of 0·289 and 0·274 (Hsu *et al.*, 1996b) and the present estimates of 0·322 and 0·261.

Crossley *et al.* (1991) have shown some benefits in using the hCG/AFP MOM ratio with and without combination with maternal age. Spencer

Table II—Observed detection efficiency in gestational age before and after 18 weeks using free β-hCG and AFP when analysed independently and combined with maternal age at a cut-off value equivalent to a 5 per cent false-positive rate

Gestational age (weeks)	*n*	Screened positive at a 5 per cent false-positive rate with:													
		AFP ($\leq$0·54 MOM)*		Free β-hCG ($\geq$2·88 MOM)		Free β-hCG/AFP ($\geq$3·47)		AFP + age ($\geq$1:297)		Free β-hCG + age ($\geq$1:215)		Free β-hCG/AFP + age ($\geq$219)		Free β-hCG + AFP + age ($\geq$1:223)	
<18	6581	359	5·5%	346	5·3%	354	5·4%	351	5·3%	325	4·9%	326	5·0%	326	5·0%
$\geq$18	1601	53	3·3%	64	4·0%	55	3·4%	59	3·7%	84	5·2%	86	5·4%	84	5·2%
Total	8182	412	5·0%	410	5·0%	409	5·0%	410	5·0%	409	5·0%	412	5·0%	410	5·0%

Gestational age (weeks)	*n*	Observed detection rate for Down's syndrome pregnancies with:													
		AFP		Free β-hCG		Free β-hCG/AFP		AFP + age		Free β-hCG + age		Free β-hCG/AFP + age		Free β-hCG + AFP + age	
<18	25	5	20·0%	13	52·0%	15	60·0%	10	40·0%	15	60·0%	16	64·0%	17	68·0%
$\geq$18	22	3	13·6%	9	40·9%	8	36·4%	10	45·5%	13	59·1%	13	59·1%	13	59·1%
Total	47	8	17·0%	22	46·8%	23	48·9%	20	42·6%	28	59·6%	29	61·7%	30	63·8%

*Cut-off level at a 5 per cent positive rate.

Table III—Observed detection efficiency in maternal age before and after 35 years using free β-hCG and AFP when analysed independently and combined with maternal age at a cut-off value equivalent to a 5 per cent false-positive rate

Maternal age (years)	*n*	Screened positive at a 5 per cent false-positive rate with:													
		AFP (≤0·54 MOM)*		Free β-hCG (≥2·88 MOM)		Free β-hCG/AFP (≥3·47)		AFP + age (≥1:297)		Free β-hCG + age (≥1:215)		Free β-hCG/AFP + age (≥219)		Free β-hCG + AFP + age (≥1:223)	
<35	7816	401	5·1%	400	5·1%	397	5·1%	258	3·3%	349	4·5%	365	4·7%	359	4·6%
≥35	366	11	3·0%	10	2·7%	12	3·3%	152	41·5%	61	16·7%	47	12·8%	51	13·9%
Total	8182	412	5·0%	410	5·0%	409	5·0%	410	5·0%	410	5·0%	412	5·0%	410	5·0%
Maternal age (years)	*n*	Observed detection rate for Down's syndrome pregnancies with:													
		AFP		Free β-hCG		Free β-hCG/AFP		AFP + age		Free β-hCG + age		Free β-hCG/AFP + age		Free β-hCG + AFP + age	
<35	29	6	20·7%	13	44·8%	15	51·7%	4	13·8%	14	48·3%	15	51·7%	16	55·2%
≥35	18	2	11·1%	9	50·0%	8	44·4%	16	88·9%	14	77·8%	14	77·8%	14	77·8%
Total	47	8	17·0%	22	46·8%	23	48·9%	20	42·6%	28	59·6%	29	61·7%	30	63·8%

*Cut-off level at a 5 per cent positive rate.

et al. (1992a) found that for a free *β*-hCG/AFP MOM ratio cut-off of 3·0, 56 per cent of Down's syndrome cases would be identified for a 5·7 per cent false-positive rate. Our study revealed that a free *β*-hCG/AFP ratio cut-off of 4·0 could detect 43·5 per cent of the affected cases for a 5·9 per cent false-positive rate. Although using the free *β*-hCG/AFP MOM ratio could improve detection efficiency, there are no clear benefits to the use of the free *β*-hCG/AFP MOM ratio for Down's syndrome screening in this Asian population.

Our current performance is slightly but not significantly better than the results of Wald *et al.* (1993), but is similar to those of the others (Spencer *et al.*, 1992a; Macri *et al.*, 1994). At a 5 per cent false-positive rate, Wald *et al.* (1993) reported a 40 per cent detection rate with free *β*-hCG, 56 per cent with free *β*-hCG and age, and 58 per cent when combining free *β*-hCG, AFP, and age; Spencer *et al.* (1992a) reported a 46·7 per cent detection rate with free *β*-hCG, 57·1 per cent with free *β*-hCG and age, and 60 per cent when combining free *β*-hCG, AFP, and age, compared with our estimates of 46·8 per cent detection rate with free *β*-hCG, 59·6 per cent with free *β*-hCG and age, and 63·8 per cent when combining free *β*-hCG, AFP, and age. Using a screening strategy combining a serum free *β*-hCG, AFP, and age in a multivariate risk algorithm, Macri *et al.* (1994) reported a 69 per cent detection rate with a 5·6 per cent initial false-positive rate by a risk cut-off level of 1:300 and Spencer *et al.* (1992a) reported a 66 per cent detection rate with a 6 per cent false-positive rate by a risk cut-off of 1:280. At a cut-off level of 1:270 risk commonly used in Taiwan, the detection rate was 67·6 per cent for a 6·1 per cent false-positive rate in this study. Thus, the screening strategy of combining free *β*-hCG, AFP, and age can attain an effective performance in Asians which is comparable to that seen in whites.

The data in this study show that the observed detection efficiency is enhanced at early gestation. Combining free *β*-hCG and AFP with maternal age can identify 68 per cent of cases when samples are taken at early gestation, compared with a 59·1 per cent detection rate taken at late gestation for a 5 per cent false-positive rate. This finding has confirmed the observations of Macri *et al.* (1994) (82 per cent versus 70 per cent at a 5·6 per cent initial false-positive rate) and Spencer *et al.* (1992a) (77 per cent versus 54 per cent at a 6 per cent false-positive rate). There were no differences in the mean age and false-positive rate between two subsets of Down's syndrome pregnancies. Therefore, the causes of the observed difference in detection rate are neither age-specific risk nor false-positive rate. The increased detection rate is most likely due to the higher free *β*-hCG MOM found at early gestation. This study confirmed the higher free *β*-hCG MOM found at early gestation (2·92 versus 2·22) which was in agreement with the values of Spencer *et al.* (1993) (2·71 versus 2·30). Therefore, the optimal time to perform second-trimester Down's syndrome screening is before 18 weeks of gestation. If all second-trimester serum screening was peformed during this period, an additional 9 per cent of Down's syndrome cases would be detected, which is in agreemnet with the results of Macri *et al.* (1994) (12 per cent) and Spencer *et al.* (1992a) (23 per cent).

Of all the markers identified to date, free *β*-hCG is the marker of choice for use in Down's syndrome screening (Spencer *et al.*, 1992a). There are several good reasons for preferring free *β*-hCG. Firstly, the detection rate may increase by 4–9 per cent using free *β*-hCG instead of total hCG (Spencer *et al.*, 1992a; Wald *et al.*, 1993; Norgaard-Pedersen *et al.*, 1994). Secondly, a low analytical error in the estimate of risk may occur, due to the removal of any sample predilution step (Macri *et al.*, 1990). Thirdly, free *β*-hCG, but not total hCG, can be used for first-trimester serum screening programmes (Spencer *et al.*, 1992b; Macri *et al.*, 1993b; Wald *et al.*, 1996). A screening strategy combining free *β*-hCG and AFP with maternal age can be expected to achieve a screening efficiency in Asians comparable to those seen in whites. We feel that measurement of free *β*-hCG is justified for second-trimester Down's syndrome screening at present and promising for first-trimester screening in the future.

ACKNOWLEDGEMENTS

We thank Dr T. Y. Hsu (Kaohsiung Chang Gung Memorial Hospital, Kaohsiung) and Dr S. D. Chang (Keelung Chang Gung Memorial Hospital, Keelung) for their help with collecting affected data from their hospitals. This study was supported in part by a grant from the Department of Health, the Executive Yaun, R.O.C.

REFERENCES

Cheng, E.Y., Luthy, D.A., Zebelman, A.M., Williams, M.A., Lieppman, R.E., Hickok, D.E. (1993). A

prospective evaluation of a second-trimester screening test for fetal Down syndrome using maternal serum AFP, hCG, and unconjugated estriol, *Obstet. Gynecol.*, **81,** 72–77.

Crossley, J.A., Aitken, D.A., Connor, J.M. (1991). Prenatal screening for chromosome abnormalities using maternal serum chorionic gonadotropin, alpha-fetoprotein, and age, *Prenat. Diagn.*, **11,** 83–101.

Cuckle, H.S., Wald, N., Lindenbaum, R.H. (1984). Maternal serum alpha-fetoprotein measurement: screening test for Down's syndrome, *Lancet*, **i,** 926–929.

Cuckle, H.S., Wald, N.J., Thompson, S.G. (1987). 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.*, **94,** 387–402.

Haddow, J.E., Palomaki, G.E., Knight, G.J., Williams, J., Pulkkinen, A., Canick, J.A., Saller, D.N., Jr, Bowers, G.B. (1992). Prenatal screening for Down's syndrome with use of maternal serum markers, *N. Engl. J. Med.*, **327,** 588–593.

Hsieh, T.T., Hsu, J.J., Chen, C.P., Tsai, M.S., Hsieh, F.J., Hu, A., Spencer, K. (1995). 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.*, **57,** 1632.

Hsu, J.J., Hsieh, T.T., Liou, J.D., Soong, Y.K. (1993). Maternal serum and amniotic fluid alpha-fetoprotein in pregnancies with trisomy and other chromosomal abnormalities, *J. Matern. Fetal. Invest.*, **3,** 255–258.

Hsu, J.J., Ou, Y.C., Chen, K.C., Hsieh, T.T., Soong, Y.K. (1996a). High maternal serum free beta-hCG levels in Down syndrome pregnancies: a preliminary report, *Chang Gung Med. J.*, **19,** 36–41.

Hsu, J.J., Hsieh, T.T., Hsieh, F.J. (1996b). 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.*, **87,** 943–947.

Lin, S.J. (1994). Down syndrome in Taiwan, *Acta Paediatr. Sin.*, **35,** 28–31.

Macri, J.N., Kasturi, R.V., Krantz, D.A., Cook, E.J., Moore, N.D., Young, J.A., Romero, K., Larsen, J.W. (1990). Maternal serum Down's syndrome screening: free β-protein is a more effective marker than human chorionic gonadotropin, *Am. J. Obstet. Gynecol.*, **163,** 1248–1253.

Macri, J.N., Spencer, K., Anderson, R.W., Cook, E.J. (1993a). Free beta-chorionic gonadotropin: a cross-reactivity study of two immunometric assays used in prenatal maternal serum screening for Down's syndrome, *Ann. Clin. Biochem.*, **30,** 94–98.

Macri, J.N., Spencer, K., Aitken, D., Garver, K., Buchanan, P.D., Muller, F., Boue, A. (1993b). First-trimester free beta (hCG) screening for Down syndrome, *Prenat. Diagn.*, **13,** 557–562.

Macri, J.N., Spencer, K., Garver, K., Buchanan, P.D., Say, B., Carpenter, N.J., Muller, F., Boue, A. (1994). Maternal serum free beta hCG screening: results of studies including 480 cases of Down's syndrome, *Prenat. Diagn.*, **14,** 97–103.

Merkatz, I.R., Nitowsky, H.M., Macri, J.N., Johnson, W.E. (1984). An association between low maternal serum α-fetoprotein and fetal chromosomal abnormalities, *Am. J. Obstet. Gynecol.*, **148,** 886–891.

Norgaard-Pedersen, B., Alfthan, H., Arends, J., Hogdall, C.K., Larsen, S.O., Pettersson, K., Stenman, U.H., Salonen, R. (1994). A new simple and rapid dual assay for AFP and free beta hCG in screening for Down's syndrome, *Clin. Genet.*, **45,** 1–4.

Reynolds, T.M., Penney, M.D. (1990). The mathematical basis of multivariate risk screening: with special reference to screening for Down's syndrome associated pregnancy, *Ann. Clin. Biochem.*, **27,** 452–458.

Ryall, R.G., Staples, A.J., Robertson, E.F., Pollard, A.C. (1992). Improved performance in a prenatal screening program for Down's syndrome incorporating serum free hCG subunit analyses, *Prenat. Diagn.*, **12,** 251–261.

Spencer, K. (1991). Evaluation of an assay of the free β-subunit of choriogonadotropin and its potential value in screening for Down's syndrome, *Clin. Chem.*, **37,** 809–814.

Spencer, K. (1994). Is the measurement of unconjugated oestriol of value in screening for Down's syndrome? In: Grudzinskas, J.G., Chard, T., Chapman, M., Cuckle, H. (Eds). *Screening for Down's syndrome*, Cambridge: Cambridge University Press, 141–161.

Spencer, K., Carpenter, P. (1985). Screening for Down's syndrome using serum α-fetoprotein: a retrospective study indicating caution, *Br. Med. J.*, **290,** 1940–1943.

Spencer, K., Coombes, E.J., Mallard, A.S., Ward, A.M. (1992a). Free beta human choriogonadotropin in Down's syndrome screening: a multicentre study of its role compared with other biochemical markers, *Ann. Clin. Biochem.*, **29,** 506–518.

Spencer, K., Macri, J.N., Aitken, D.A., Connor, J.M. (1992b). Free β-hCG as first-trimester marker for fetal trisomy, *Lancet*, **339,** 1480–1481.

Spencer, K., Macri, J.N., Anderson, R.W., Aitken, D.A., Berry, E., Crossley, J.A., Wood, P.J., Coombes, E.J., Stroud, M., Worthington, D.J., Doran, J., Barbour, H., Wilmot, R. (1993). Dual analyte immunoassay in neural tube defect and Down's syndrome screening: results of a multicentre clinical trial, *Ann. Clin. Biochem.*, **30,** 394–401.

Stone, S., Henley, R., Reynolds, T., John, R. (1993). A comparison of total and free β-hCG assays in Down syndrome screening, *Prenat. Diagn.*, **13,** 535–537.

Tabor, A., Norgaard-Pedersen, B., Jacobsen, J.C. (1984). Low maternal serum AFP and Down's syndrome, *Lancet*, **ii,** 161.

Wald, N., Densem, J., Stone, R., Cheng, R. (1993). The use of free β-hCG in antenatal screening for Down's syndrome, *Br. J. Obstet. Gynaecol.*, **100,** 550–557.

Wald, N.J., Densem, J.W., Smith, D., Klee, G.G. (1994). Four-marker serum screening for Down's syndrome, *Prenat. Diagn.*, **14,** 707–716.

Wald, N.J., George, L., Smith, D., Densem, J.W., Petterson, K. (1996). Serum screening for Down's syndrome between 8 and 14 weeks of pregnancy, *Br. J. Obstet. Gynaecol.*, **103,** 407–412.