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Second-trimester maternal serum screening for Down's syndrome: free β-human chorionic gonadotrophin (HCG) and α-fetoprotein, with or without unconjugated oestriol, compared with total HCG, α-fetoprotein and unconjugated oestriol

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The aim of our study was to compare three protocols for second-trimester maternal serum screening for Down's syndrome in the same serum samples, using two triple tests [total human chorionic gonadotrophin (HCG), α-fetoprotein, unconjugated oestriol; and free β-HCG, α-fetoprotein, unconjugated oestriol] and a double test (free β-HCG and α-fetoprotein). The three protocols were compared in a series of 23 serum samples from Down's syndrome pregnancies and in a cohort of 2516 pregnant women receiving routine antenatal care between June 1992 and June 1993. Among the 23 affected cases, at a cut-off risk of 1:380, the detection rate of Down's syndrome was comparable with the double test (74%; 17/23) and the triple tests (65%; 15/23) (not significantly different). At the same cut-off risk, in the cohort of 2516 pregnant women screened between 15 and 18 weeks gestation, both protocols using free β-HCG achieved a significant reduction of the number of false positive cases (P = 0.013 and 0.004 for double and triple tests respectively). We conclude that, compared to total HCG, α-fetoprotein and unconjugated oestriol, use of free β-HCG and α-fetoprotein represents a better second-trimester screening test for Down's syndrome, because it significantly decreases the false positive rate at a lower running cost. The addition of unconjugated oestriol to the double test adds no further advantage.

Key words: Down's syndrome/α-fetoprotein/free β-HCG/maternal serum screening/unconjugated oestriol

Introduction

Second-trimester maternal serum screening for Down's syndrome (DS) has become common practice in several Western countries. Despite the large number of pregnant women screened to date, the relative merit of total human chorionic gonadotrophin (HCG) versus its free β-subunit, as well as the adjunctive role of unconjugated oestriol in such programmes, remain controversial issues. The improved screening performances reported by several investigators when using free β-HCG instead of total HCG (Macri *et al.*, 1990b, 1994; Cuckle *et al.*, 1992; Ryall *et al.*, 1992; Spencer *et al.*, 1992, 1993) have not been confirmed by others (Milunski *et al.*, 1993; Stone *et al.*, 1993; Wald *et al.*, 1993). The effectiveness of unconjugated oestriol as a screening variable is equally debated (Wald *et al.*, 1988; Macri *et al.*, 1990a; Spencer *et al.*, 1992; Crossley *et al.*, 1993). These conflicting results have prompted us to perform a direct comparison of three protocols for DS screening in the same serum samples, using two 'triple tests' [total HCG, α-fetoprotein, unconjugated oestriol ('TT'); and free β-HCG, α-fetoprotein, unconjugated oestriol ('TTFB')] and a 'double test' [free β-HCG and α-fetoprotein ('DT')].

Materials and methods

To achieve this comparison, the three protocols were compared in a series of 23 serum samples from DS pregnancies (18 frozen samples and five samples collected during the prospective study) and in a cohort of 2516 pregnant women with normal pregnancy outcome receiving routine antenatal care in Geneva between June 1992 and June 1993.

Hormone measurements were performed in fresh serum samples in three different laboratories, using the following methods: laboratories 1 and 2 measured α-fetoprotein (αFP) and total HCG by IMX (Abbott AG, Cham, Switzerland); laboratory 3 measured αFP with ES600 (Boehringer, Mannheim, Germany) and total HCG by Stratus (Baxter AG, Zürich, Switzerland). Unconjugated oestriol and free β-HCG were measured by radioimmunoassay in all samples by laboratory 1, using Kodak-Amerlex-M Estriol kit (Polymed SA, Geneva, Switzerland) and FBHCG (CIS-Bio-International, Gif-sur-Yvette, France) respectively.

Median values for weeks 15–18 were previously established in the three laboratories for αFP and total HCG and in laboratory 1 for unconjugated oestriol and free β-HCG, using the same sera (50 per week) obtained from pregnant women with a normal pregnancy outcome. Median values were monitored throughout the study and updated as necessary. Concentrations of the serum markers were expressed in multiples of the medians (MOM) for pregnancies of the same gestational age.

Gestational age was determined from last menstrual period (LMP) or ultrasound examination. Almost all our patients had an ultrasound dating scan prior to or at the time of serum screening. When LMP and ultrasound estimates of gestational age were in agreement, LMP estimate was considered. When LMP and ultrasound estimates were divergent, ultrasound estimate was selected if the difference between LMP and ultrasound derived gestational age was $>$12 days. This was the case in 10% of our patients.

Serum samples were obtained between 15 and 18 weeks gestation. Of the 23 patients with fetal DS (median age 32 years), six (26%) were $\geqslant$35 years old. Of the 2516 patients screened, 133 (5.3%) were $\geqslant$35 years old (median age 36 years); 2383 (94.7%) were $<$35 years (median age 29 years).

The patient-specific risk for term DS was computed from her age

Table I. Down's syndrome (DS) detection rate depending on serum markers used (total number of DS cases = 23). Cut-off risk 1:380

	DS cases detected					
	TT		DT		TTFB	
	n	%	*n*	%	*n*	%
Stored samples (*n* = 18)	12	67	15	83	14	78
Per-study samples (*n* = 5)	3	60	2	40	1	20
Total (*n* = 23)	15	65	17	74	15	65

TT = triple test [total human chorionic gonadotrophin (HCG), α-fetoprotein, unconjugated oestriol], DT = double test (free β-HCG, α-fetoprotein), TTFB = triple test (free βHCG, α-fetoprotein, unconjugated oestriol).

Table II. Down's syndrome (DS) detection rate depending on serum markers used (total number of DS cases = 23). Cut-off risk 1:270

	DS cases detected					
	TT		DT		TTFB	
	n	%	*n*	%	*n*	%
Stored samples (*n* = 18)	12	67	14	78	14	78
Per-study samples (*n* = 5)	3	60	0	0	1	20
Total (*n* = 23)	15	65	14	61	15	65

TT = triple test [total human chorionic gonadotrophin (HCG), α-fetoprotein, unconjugated oestriol], DT = double test (free β-HCG, α-fetoprotein), TTFB = triple test (free βHCG, α-fetoprotein, unconjugated oestriol).

and the trivariate or bivariate Gaussian frequency distribution of the serum markers, using commercially available software programs (ALPHA, Logical Medical Systems Ltd., London, UK; and CIS-Bio-International). Later comparisons were computed with hospital-developed software, using the data from Wald *et al.* (1988) and Spencer *et al.* (1992). Cut-off risk indicating further investigation was set at 1:380.

Clinical management was based on the triple test (TT) results. Free β-HCG was concurrently assayed on the serum samples for later evaluation and comparison. This study received ethical approval from the Ethics Committee of our institution. All patients participating in the study gave their informed consent.

Statistical comparisons were done using the McNemar test, or its exact version, when appropriate.

Results

Table I shows the results obtained with the three protocols in the series of 23 serum samples from DS pregnancies. Overall, at a cut-off risk of 1:380, two more DS cases were detected with the protocol using free β-HCG and α-fetoprotein [17/23 (74%; 95% confidence interval 52–90%); versus 15/23 (65%; 43–84%)]. This difference in detection rate did not reach statistical significance ($P = 0.69$). The median MOM values for free β-HCG, total HCG, α-fetoprotein and unconjugated oestriol in the DS cases were 2.68, 1.94, 0.77 and 0.81 respectively. From the five DS cases ascertained during the prospective study, at a cut-off risk of 1:380, three were detected with the triple test using total HCG, two with the double test and one with the triple test using free β-HCG (Table I). At a cut-off risk of 1:270, the respective numbers were three, none and one (Table II).

Results of the screening study are presented in Table III. Overall, at a cut-off risk of 1:380, we observed a significant reduction of the number of positive cases with both protocols using free β-HCG instead of total HCG. This reduction of positive rate was only observed at 15 weeks gestation; this effect at 15 weeks persisted at a cut-off of 1:270 with use of free β-HCG (Table IV).

Discussion

Our results in DS pregnancies are in agreement with those reported by Spencer *et al.* (1992) and Macri *et al.* (1994). The

Table III. Screening study: number of positive cases depending on serum markers used; cut-off risk 1:380

Gestational age (weeks)	Number of patients	DS cases detected					
		TT		DT		TTFB	
		n	%	*n*	%	*n*	%
15	442	52	11.8[a]	29	6.6[a]	36	8.1[a]
16	1368	95	6.9	90	6.6	82	6.0
17	558	39	7.0	36	6.5	34	6.1
18	148	16	10.8	12	8.1	13	8.8
Total	2516	202	8.0[b]	167	6.6[b]	165	6.6[b]

TT = triple test [total human chorionic gonadotrophin (HCG), α-fetoprotein, unconjugated oestriol], DT = double test (free β-HCG, α-fetoprotein), TTFB = triple test (free βHCG, α-fetoprotein, unconjugated oestriol).
[a]*P*-values: DT versus TT: 0.0002; TTFB versus TT: 0.0052; DT versus TTFB: 0.14.
[b]*P*-values: DT versus TT: 0.013; TTFB versus TT: 0.0037; DT versus TTFB: 0.92.

Table IV. Screening study: number of positive cases depending on serum markers used; cut-off risk 1:270

Gestational age (weeks)	Number of patients	DS cases detected					
		TT		DT		TTFB	
		n	%	*n*	%	*n*	%
15	442	41	9.3[a]	20	4.5[a]	28	6.3[a]
16	1368	63	4.6	62	4.5	62	4.5
17	558	22	3.9	27	4.8	23	4.1
18	148	11	7.4	11	7.4	10	6.8
Total	2516	137	5.4[b]	120	4.8	123	4.9[b]

TT = triple test [total human chorionic gonadotrophin (HCG), α-fetoprotein, unconjugated oestriol], DT = double test (free β-HCG, α-fetoprotein), TTFB = triple test (free βHCG, α-fetoprotein, unconjugated oestriol).
[a]*P*-values: DT versus TT: 0.0008; TTFB versus TT: 0.015; DT versus TTFB: 0.096.
[b]*P*-values: DT versus TT: 0.18; TTFB versus TT: 0.22; DT versus TTFB: 0.82.

higher detection rate of DS pregnancies reported with free β-HCG as compared with total HCG is explained by the wider separation between the median concentrations in affected and unaffected pregnancies [2.07 MOM and 2.64 MOM for total HCG and free β-HCG respectively, as reported by Macri *et al.* (1994)]. For a fixed false positive rate, an 8–10% higher detection rate can be predicted (Cuckle *et al.*, 1992). In our series of 23 affected cases, the median concentrations measured were 1.94 MOM for total HCG and 2.68 MOM for free β-HCG.

This improvement of the detection rate was, however, not confirmed in our screening study. Given the small number of affected cases ($n = 5$), an estimate of the detection rate is subject to considerable random error, and no valid conclusion can be drawn from this observation. Moreover, the unusually low median concentration of free β-HCG (1.77 MOM) in these five affected cases may explain the lower detection rate. Thus, our results are not in conflict with those reported in larger prospective studies using the same serum markers (Spencer *et al.*, 1993; Macri *et al.*, 1994).

Furthermore, it should be pointed out that among the prospective studies comparing different DS screening protocols published to date, including ours, none has the statistical power to document a real difference in detection rates (to be able to detect a 15% difference (for example 75 versus 60%), with a two-sided test at $\alpha = 5\%$ and a power of 80% would require about 290 DS cases). Thus, lack of difference in detection rates cannot be considered as equivalence before a sufficient number of affected cases has been ascertained.

The design of our study allowed us, by comparing several combinations of four screening parameters, to evaluate the relative contribution of a given variable to the final screening results. The relative roles of unconjugated oestriol and free β-HCG can be estimated from the comparisons of DT with TTFB and of TT with TTFB respectively. These comparisons allow us to conclude that use of free β-HCG in a screening protocol is associated with a lower false-positive rate and that the addition of unconjugated oestriol to the double test adds no further advantage (Table III).

Kellner *et al.* (1995), in a similar study comparing TT with DT in the same serum samples, reached opposite conclusions. The only apparent difference between that study and ours is the lower cut-off risk selected (1:270). However, in our prospective cohort, at the same cut-off risk, the results obtained with all three protocols are comparable and we still observe a significant reduction of the false-positive rate at 15 weeks with use of free β-HCG (Table IV). Considering the limited number of affected cases included, it is impossible to reach definitive conclusions concerning the detection rate of fetal DS. Additional comparative studies are needed to clarify these points further.

The significant reduction of the number of false positive cases observed in our prospective study is an important advantage of the screening protocols using free β-HCG. Indeed, lowering the false positive rate is a critical issue in patient care, as it means less parental anxiety, fewer unnecessary invasive tests and reduced costs. In practice, by using the double test instead of the triple test with total HCG, we would have reduced our potential number of amniocenteses by 35 (17%) (Table III).

Finally, a further advantage of using free β-HCG as a serum marker for DS is that it has its highest detection efficiency between 14 and 16 weeks gestation (Spencer *et al.*, 1993; Macri *et al.*, 1994). This feature is reflected in our prospective study by the significant reduction of the false positive rate observed at 15 weeks. Free β-HCG can thus be used for screening before 15 weeks, and has been advocated to be a promising marker, together with PAPP-A, during the first trimester (MacIntosh *et al.*, 1994).

We thus conclude from the comparison of the triple and double tests for the detection of DS pregnancies that, in our hands, the combined use of free β-HCG and α-fetoprotein instead of total HCG, α-fetoprotein and unconjugated oestriol is a better screening test, because it significantly decreases the number of false positive cases at a lower running cost. The addition of unconjugated oestriol to free β-HCG and α-fetoprotein adds no further advantage. Additional comparative studies are still needed to confirm our results and to allow a better estimation of DS detection rates.

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