

First trimester biochemical screening for Down syndrome: free beta hCG versus intact hCG

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To compare free beta hCG versus intact hCG in first trimester Down syndrome screening we analysed 63 cases of Down syndrome and 400 unaffected control pregnancies between 10 and 13 weeks' gestation. The Down syndrome median multiple of the median (MoM) was significantly higher ($p=0.001$) for free beta hCG (1.89 MoM) than for intact hCG (1.37 MoM). Although distributions for free beta hCG (unaffected, 0.2157; DS, 0.2322) are wider than for intact hCG (unaffected, 0.1697; DS, 0.2158), overall 27% of Down syndrome cases were above the 95th percentile for free beta hCG compared to 19% for intact hCG. Combined with maternal age, free beta hCG detected 45% of Down syndrome pregnancies at a 5% false positive rate. Intact hCG combined with maternal age demonstrated a detection efficiency comparable to maternal age alone (35% versus 32%). In contrast, a recent study (Haddow *et al.*, 1998—NEJM 338: 955–961) indicated that intact hCG yielded a higher first trimester Down syndrome detection efficiency than free beta hCG (29% versus 25% respectively). Re-analysis of distribution parameters in the Haddow *et al.* study, however, show that free beta hCG was actually the better marker (23% detection for intact hCG versus 29% for free beta hCG). Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome; free beta hCG; hCG; first trimester screening

INTRODUCTION

Recent studies suggest high detection efficiency in Down syndrome (DS) screening prior to 14 weeks of pregnancy, when ultrasound evaluation of nuchal translucency is combined with specific maternal serum biochemical markers (Orlandi *et al.*, 1997, De Biasio *et al.*, 1999, de Graff *et al.*, 1999). Of the commonly used second trimester maternal serum markers (AFP, intact hCG, free beta hCG and unconjugated estriol) only free beta hCG appears productive prior to 14 weeks' gestation.

A recent prospective study suggests that although free beta hCG multiple of the median (MoM) values were higher than intact hCG in first trimester DS cases, the distribution in free beta hCG levels was greater and thus overall detection was better using intact hCG (Haddow *et al.*, 1998). This conclusion is in contrast to numerous previously published retrospective studies in which intact hCG is shown to give poor discrimination between DS and unaffected cases (Spencer, 1997). In anticipation of more widespread first trimester screening, we undertook the present case-control study to address this issue and attempt to clarify the potential roles of these biochemical markers.

MATERIALS AND METHODS

Free beta hCG and intact hCG were analysed in a total of 63 DS (35 liquid serum and 28 dried blood) and 400 unaffected (200 liquid serum and 200 dried blood) specimens between 10 and 13 weeks' gestation. One hundred unaffected control specimens were selected for each gestational week (50 liquid serum and 50 dried blood) from ultrasound dated, white, non-diabetic, singleton pregnancies. The mean (SD) maternal age was 38.0 (5.09) years in the DS cases and 35.8 (5.71) years in the unaffected controls.

Free beta hCG was measured using in-house developed ELISAs, previously described for liquid (Krantz *et al.*, 1996) and dried (Macri *et al.*, 1996) specimens. The liquid serum free beta values were available from previous retrospective studies (Brambati *et al.*, 1997; Morssink *et al.*, 1998) and dried blood free beta values were available from ongoing prospective analyses (Orlandi *et al.*, 1997; Krantz *et al.*, in press). Intact hCG was analysed with a solid phase, two site chemiluminescent procedure (Immulite system: DPC, Los Angeles, CA, USA). Dried blood samples were eluted from a 0.209 inch punch into 225 µl of buffer solution. A dilution factor of 53 was calculated based on a serum absorbency value of 1.51 µl/0.125 inch punch for the #903 blood collection cards (Schleicher and Schuell, Keene, NH, USA). Intact hCG was then analysed in the dried blood elutes along with the liquid serum samples following the manufacturer's protocol.

Regressed gestational day-specific unaffected med-

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ians were calculated for liquid and dried samples separately. MoMs were then calculated for each liquid and dried sample and the overall MoM distribution of all samples was calculated. The detection efficiency for each marker was determined based on the observed percentage of DS cases with a MoM value above the observed 95th percentile of the unaffected MoMs as well as based on the Gaussian distribution parameters. Detection efficiency and false positive rates based on combining maternal serum analyte MoM values with maternal age were determined by modelling observed likelihood ratios with the maternal age distribution of live births in the USA.

A meta-analysis of the distribution parameters of intact hCG versus free beta hCG was performed on seven case-control studies in which both analytes were measured in the same sample set. The seven studies include the current study and six others identified from a Medline database search.

RESULTS

MoM levels of maternal serum free beta hCG and intact hCG in individual cases of DS are plotted by gestational age in Figures 1a and b respectively. The

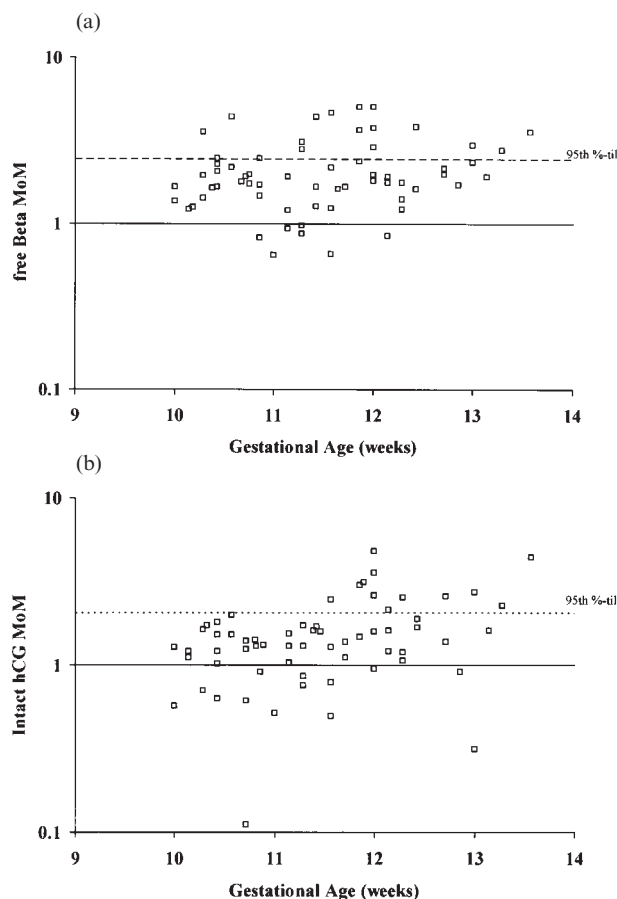


Figure 1—(a) Free beta hCG MoM values of DS cases plotted by gestational age. (95th percentile of the unaffected distribution = - - -) (b) Intact hCG MoM values of DS cases plotted by gestational age. (95th percentile of the unaffected distribution = - - -)

median MoM in 63 DS cases was significantly higher (Wilcoxon signed rank test, $p=0.001$) with free beta hCG (1.89 MoM) than intact hCG (1.37 MoM). The observed 95th percentile of the unaffected distribution was 2.43 MoM for free beta hCG and 2.05 MoM for intact hCG. Standard deviation (\log_{10}) of free beta hCG and intact hCG was 0.2157 and 0.1697 in unaffected and 0.2322 and 0.2158 in DS affected distributions, respectively. The correlations between free beta hCG and intact hCG were 0.626 in unaffected controls and 0.452 in DS cases.

Figure 2 illustrates detection efficiencies at a fixed 5% false positive rate for both intact hCG and free beta hCG, alone and in combination with maternal age. Nineteen per cent of DS cases were above the observed 95th percentile for intact hCG. When combined with maternal age, intact hCG detection showed a marginal and statistically insignificant increase ($\chi^2=2.25; p=0.134$) over that achievable by maternal age assessment alone (35% versus 32%). Twenty seven per cent of DS cases were above the observed 95th percentile for free beta hCG and when combined with maternal age this marker increased detection to 45%.

Figures 1a and b show that the greatest difference in detection efficiency occurs prior to 12 weeks' gestation where 10 of 42 DS cases (24%) lie above the 95th percentile for free beta hCG while only 3 of 42 lie above the 95th percentile for intact hCG (7.1%).

At a fixed 60% detection efficiency, intact hCG resulted in an approximate doubling of the false positive rate either alone (14.9% versus 30%) or in combination with maternal age (10.5% versus 17.7%) when compared to free beta hCG (Figure 3).

The meta-analysis in Table 1 shows that free beta hCG is significantly higher in the DS cases by an average of 49% (1.89 versus 1.27 MoM). Although the distribution of free beta hCG is wider than that of intact hCG, calculation of the percentage of DS cases above the 95th percentile of the unaffected distribution indicates that, on average, free beta hCG will detect approximately twice as many cases as intact hCG (30.4% versus 16.2%). Using the overall distribution parameters and factoring in the US maternal age distribution, the detection efficiency at a 5% false positive rate was 32%, 34% and 45% for maternal age alone, intact hCG with age and free beta hCG with age, respectively.

DISCUSSION

Intact hCG is known to be significantly raised in maternal serum during the second trimester of pregnancy and was first reported as a biochemical marker for DS in 1987 (Bogart *et al.*, 1987). Subsequently, free beta hCG, an independent maternal serum analyte, was observed to be proportionally higher than intact hCG in second trimester DS cases and to provide enhanced detection (Macri *et al.*, 1990, 1994; Spencer *et al.*, 1992). The current study and meta-analysis of seven first trimester case-control

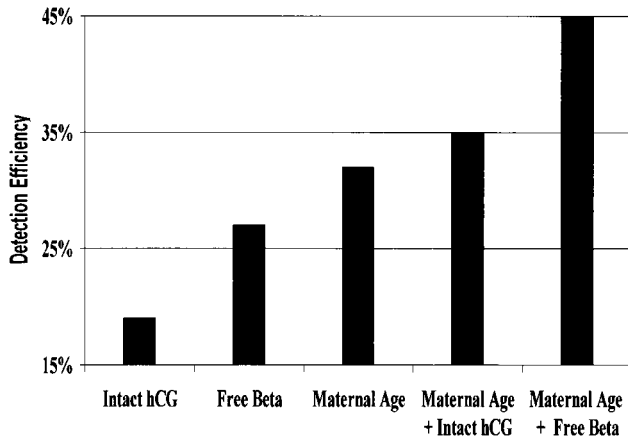


Figure 2—Detection efficiency at a fixed 5% false positive rate

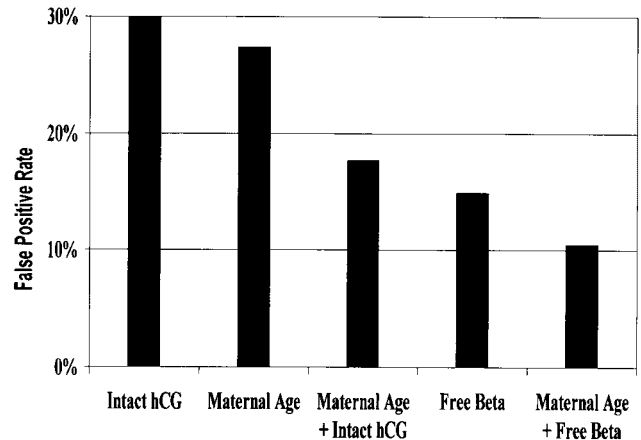


Figure 3—False positive rate at a fixed 60% detection efficiency

studies (Table 1) in which free beta hCG and intact hCG were directly compared in the same sample set reveals that despite wider distribution approximately twice the number of DS cases fall above the 95th percentile of the unaffected population for free beta hCG relative to intact hCG.

A recent first trimester prospective study (Haddow *et al.*, 1998) claimed that when combined with maternal age, intact hCG provided higher detection efficiency than free beta hCG (29% versus 25%) even though free beta hCG levels were significantly higher. The authors attributed this observation to the tighter distribution of intact hCG. However, this calculation was made by setting an unrealistic observed false positive rate of 5% in a population of patients in which 82% were greater than 35 years of age. Reanalysis of the distribution parameters provided in that study indicates free beta hCG actually resulted in more cases falling above the 95th percentile compared to intact hCG (Table 2). This result is consistent with our study and the meta-analysis of retrospective case-control studies in which free beta hCG and intact hCG were

measured in the same sample set (Table 1). Factoring in the maternal age distribution of live births in the US to correct for maternal age bias in the Haddow *et al.* (1998) study, free beta hCG demonstrated a higher detection efficiency compared to intact hCG (45% versus 41% at a 5% false positive rate).

The physiological mechanisms underlying the increase of either maternal serum hCG or free beta hCG in cases of fetal DS remain unclear. Differential expression of the genes for hCG alpha and hCG beta may account for the higher maternal serum levels of free beta hCG subunit relative to intact hCG observed in cases of DS (Eldar-Geva *et al.*, 1995). These authors further suggested that the overall increase in all molecular forms of hCG (free alpha, free beta hCG and intact hCG) observed in the second trimester in trisomy 21 pregnancies may be brought about by relative immaturity of the DS placenta.

In the first trimester, when the maturity differential between the DS and normal placenta is less, the relatively large overall increase in hCG is no longer observed. However, the differential expression of the

Table 1—Meta-analysis of intact hCG versus free beta hCG in case-control studies in which both analytes were measured in the same sample set

Study	n	Intact hCG						Free beta hCG					
		Unaffected		DS cases		%DS ^a >95th percentile	Unaffected		DS cases		%DS ^a >95th percentile		
		Median	SD	Median	SD		Median	SD	Median	SD			
Aitken <i>et al.</i> , 1993	320	16	1.0	0.2190	0.97	0.3150	11.8%	1.0	0.2580	1.96	0.2560	30.3%	
Macintosh <i>et al.</i> , 1994	258	21	1.0	–	1.45	0.4512	–	1.0	–	2.10	0.4280	–	
Brizot <i>et al.</i> , 1995	394	41	1.0	0.2054	1.50	0.1939	20.2%	1.0	0.2911	2.00	0.3168	28.7%	
Biagiotti <i>et al.</i> , 1995	246	41	1.01	0.2458	1.12	0.2555	8.2%	1.0	0.2208	2.00	0.2294	39.3%	
Wald <i>et al.</i> , 1996 ^b	383	77	1.0	–	1.23	0.1957	13.0%	1.0	0.2833	1.79	0.2870	22.9%	
Jauniaux <i>et al.</i> , 1996	51	17	1.0	0.2375	0.96	0.1347	0.0%	1.0	0.3474	1.46	0.1673	0.1%	
Current study	400	63	1.0	0.1697	1.37	0.2158	25.5%	1.0	0.2157	1.89	0.2322	36.8%	
Overall	2052	276	1.0	0.2083	1.27	0.2421	16.2%	1.0	0.2545	1.89	0.2782	30.4%	

^aBased on normal distribution parameters.

^bAuthor provided observed percentage of DS cases >95th percentile for intact hCG.

– indicates data was not available; Unaff. = unaffected.

Table 2—Reanalysis of Haddow *et al.* (1998)

Analyte	Published parameters				Reported detection efficiency	Reanalysis of published parameters	
	Unaff. median	Unaff. SD	DS median	DS SD		95th percentile	DS cases >95th percentile
Free beta hCG	1.0	0.275	2.05	.257	25%	2.83	29%
Intact hCG	1.0	0.188	1.51	.175	29%	2.04	23%

beta subunit versus the alpha subunit in DS cases apparently remains in the first trimester, as observed in the present study at the protein level.

Second trimester biochemical screening for DS has been controversial in terms of the identification of specific markers and the marker combinations that are most productive. As we investigate and introduce first trimester screening, it is important to reflect on lessons learned during the evolution of second trimester screening. One significant lesson relates to the importance of rigorously evaluating the individual contribution of each biochemical marker prior to including it in a multi-marker protocol. For example, Hook (1995) has noted that in the initial evaluation of unconjugated oestriol within a triple screen protocol there was an overestimate of the standard deviation of AFP. This resulted in the conclusion that unconjugated oestriol contributes to overall DS detection efficiency, an issue that remains controversial today. In the first trimester study of Haddow *et al.* (1998) we again observe a situation in which faulty analysis of data may lead to erroneous conclusions on the utility of specific biochemical markers.

The data in the current study and others indicate that intact hCG is not sufficiently raised in DS to be an effective first trimester marker. When combined with maternal age, intact hCG increases detection only 2–3% more than maternal age alone. Free beta hCG, however, is approximately 40% higher than intact hCG and, despite a wider distribution, is productive in the first trimester, detecting 45% of cases when combined with maternal age. Recent reports demonstrate that including free beta hCG in a combined first trimester biochemical and ultrasound protocol may result in up to 90% detection of DS at very early stages of gestation (Orlandi *et al.*, 1997; De Biasio *et al.*, 1999; de Graff *et al.*, 1999; Spencer *et al.*, 1999). This exciting development in improved prenatal screening is a welcomed advantage to clinicians and patients.

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