

COMPARISON OF URINARY FREE BETA (hCG) AND BETA-CORE (hCG) IN PRENATAL SCREENING FOR CHROMOSOMAL ABNORMALITIES

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SUMMARY

To evaluate the potential utility of free beta (hCG) and beta-core (hCG) in a prenatal screening protocol for Down syndrome we analysed these markers in dried maternal urine specimens from 163 control, 13 Down syndrome and 5 trisomy 18 pregnancies from 8 to 25 weeks' gestation. All results are reported after normalization for urinary creatinine determined by modified Jaffe reagent assay. The correlation of urinary free beta (hCG) and urinary beta-core (hCG) was 0.61 in controls and 0.93 in Down syndrome. Median MoM values in Down syndrome were 2.42 for urinary free beta (hCG) and 2.40 for beta-core (hCG). In trisomy 18 the median MoM was 0.35 and 0.34 for free beta (hCG) and beta-core (hCG), respectively. The degree of elevation observed in DS cases with urinary free beta (hCG) is consistent with previous reports. Studies of beta-core (hCG) in Down syndrome have yielded discrepant results. In this study, beta-core (hCG) in Down syndrome is lower than values observed in early reports but consistent with more recent reports. © 1998 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome screening; maternal urine; free beta (hCG); beta-core (hCG)

INTRODUCTION

Maternal serum multiple marker screening for open neural tube defects and chromosomal abnormalities is conducted in the second trimester of pregnancy. Recently, prenatal screening for Down syndrome has begun in the first trimester using the biochemical serum markers free beta (hCG) and pregnancy associated plasma protein A (PAPP-A) combined with the ultrasound marker, nuchal

translucency (Orlandi *et al.*, 1996). Preliminary estimates of detection efficiency for this early combined screening protocol approximate at 80–90 per cent. In 1994, Cuckle *et al.* (1994) reported that maternal urinary beta-core (hCG) levels were extremely elevated in cases of Down syndrome and may represent an alternative to maternal serum based screening methods.

Since the initial report by Cuckle *et al.*, studies have sought to confirm these promising results. A similar degree of elevation was observed by Cuckle *et al.* (1995) in an expanded data set and subsequently by Canick *et al.* in two different data sets (Canick *et al.*, 1995, 1996) and in a separate

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independent data set by Isozaki *et al.* (1997). However, studies of three additional sample sets have shown a significantly lower degree of elevation for beta-core (hCG) in the second trimester (Hayashi and Kozu, 1995; Spencer *et al.*, 1996; Muller, 1996).

In addition to beta-core (hCG), investigators have analysed urinary free beta (hCG) and have observed levels two to three times higher than normal in Down syndrome cases, similar to that seen in maternal blood (Spencer *et al.*, 1996; Hayashi *et al.*, 1996; Muller, 1996; Cole *et al.*, 1997). We present data on an independent set of maternal urine samples directly evaluating the effectiveness of urinary free beta (hCG) versus beta-core (hCG) in screening for Down syndrome. These results are compared with previously published data sets.

MATERIALS AND METHODS

Dried maternal urine specimens were collected from 163 control, 13 Down syndrome and 5 trisomy 18 affected pregnancies between 8 and 25 gestational weeks. Dried specimens were produced by dipping a 1" × 5" strip of 903 filter paper (Schleicher and Schuell, Keene, NH) into freshly voided urine specimens and then allowing the paper to dry at room temperature for at least three hours. Dried specimens were shipped to the analytical laboratory (NTD Laboratories, Huntington Station) at ambient temperature. In a subset of 100 control cases, matched dried blood specimens were also collected.

Upon receipt in the laboratory dried urine specimens were punched into a 96-well microtitre plate and eluted in 200 μ l of elution buffer containing stabilizing protein, Tween-20 and preservative. Free beta (hCG) was analysed using an in-house ELISA modified to accept dried urine specimens. All assay calibrators and quality control samples used in the immunoassay procedure were prepared as dried specimens and eluted in a manner identical to patient samples. The correlation between dried urine and liquid urine free beta was 0.90 in a set of 45 matched unaffected specimens. Sample eluates were diluted 1 to 50. Cross-reactivity of our free beta (hCG) assay to beta-core (hCG) was evaluated with a purified preparation (Fitzgerald, 98 per cent purity) in a liquid non-competitive format and found to be 0.2 per cent.

Beta-core (hCG) was analysed with a commercially available ELISA (Urinary Gonadotropin Fragment (UGF), Toagosei Co., Ltd, Tokyo, Japan). Sample eluates were diluted 1 to 100. Cross-reactivity of the beta-core (hCG) assay to free beta (hCG) was 0.122 per cent as per the manufacturer.

Urinary creatinine was determined using the Jaffe reagent assay (#555-A, Sigma Diagnostics, St Louis, MO) modified for microtitre plate use and dried urine analysis. The correlation of dried creatinine and liquid creatinine values was 0.97 in 45 matched unaffected specimens. All analyte values for maternal urine free beta (hCG) and beta-core (hCG) were normalized for urinary creatinine. Multiples of the gestational week specific median (MoM) were then calculated for each sample. Detection efficiency and false-positive rates based on combining maternal urine analyte MoM values with maternal age were calculated by modelling the observed likelihood ratios from log-Gaussian distributions of the analytes with the maternal age distribution of live births in the U.S.A.

RESULTS

Urinary free beta (hCG) and urinary beta-core (hCG) in individual cases of trisomy 21 and trisomy 18 are plotted versus gestational age in Figs 1 and 2. Free beta (hCG) was significantly raised in the 13 cases of trisomy 21 (2.42 median MoM; range: 0.31 to 20.48) and significantly lowered in the 5 cases of trisomy 18 (0.35 median MoM; range: 0.04 to 0.81). The 10th, 50th and 90th percentiles of the unaffected free beta MoM distribution were 0.37, 1.03 and 2.21, respectively. The geometric mean and standard deviation (ln) in unaffected cases was 0.946 and 0.741, respectively. In Down syndrome cases the geometric mean was 2.15 and the SD (ln) was 1.274. Correlation between dried urine and dried blood free beta (hCG) was 0.6 in a subset of 100 unaffected cases.

Similarly, beta-core (hCG) was also raised in cases of trisomy 21 (2.40 median MoM; range: 0.25 to 11.05) and lowered in cases of trisomy 18 (0.34 median MoM; range: 0.01 to 1.14). The 10th, 50th and 90th percentiles of the unaffected beta-core (hCG) MoM distribution were 0.34, 0.96 and 2.67, respectively. The geometric mean was 1.00 and the standard deviation (ln) 0.787 in unaffected cases. In Down syndrome cases the geometric mean was 2.12 and the SD (ln) was 1.238.

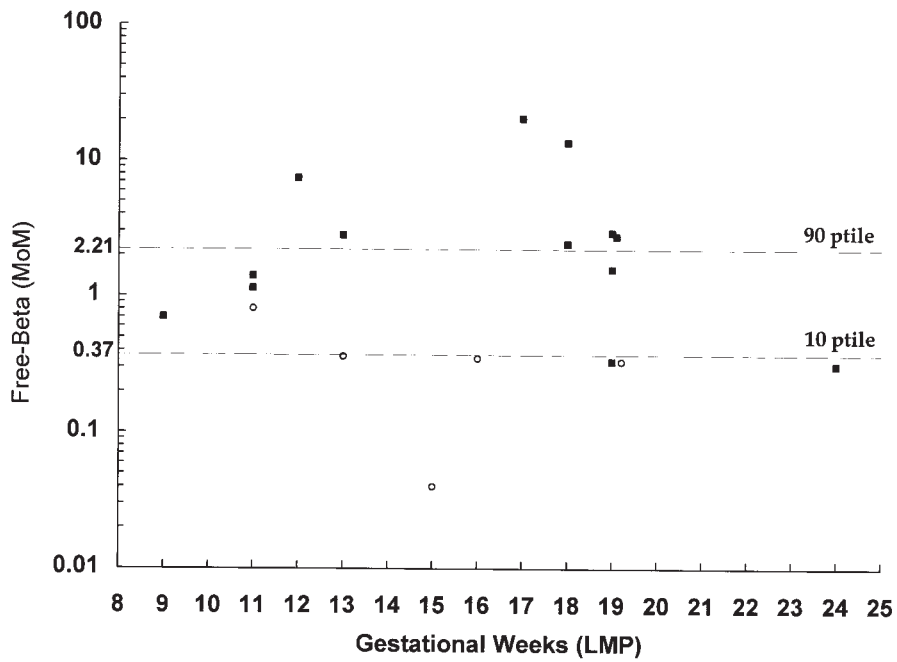


Fig. 1—Distribution of free beta (hCG) MoM values in affected cases; ■ Down syndrome, ○ trisomy 18

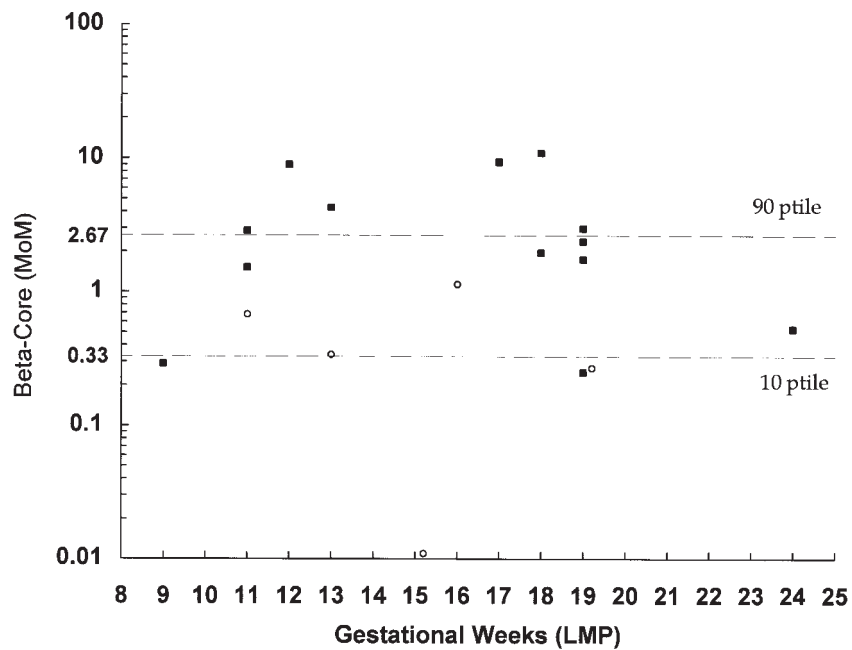


Fig. 2—Distribution of beta-core (hCG) MoM values in affected cases; ■ Down syndrome, ○ trisomy 18

Table I—Distribution of urinary free beta (hCG) and beta-core (hCG) in unaffected and Down syndrome (DS)

MoM	Free beta (hCG)		Beta-core (hCG)	
	Unaffected (n=163)	DS (n=13)	Unaffected (n=163)	DS (n=13)
>0.5	130 (79.8%)	11 (84.6%)	133 (81.6%)	11 (84.6%)
>1.0	86 (52.8%)	10 (76.9%)	80 (49.1%)	10 (76.9%)
>1.50	43 (26.4%)	8 (61.5%)	46 (28.2%)	10 (76.9%)
>2.00	22 (13.5%)	7 (53.8%)	27 (16.6%)	7 (53.8%)
>2.50	13 (8.0%)	6 (46.2%)	19 (11.7%)	6 (46.2%)
>3.00	9 (5.5%)	3 (23.1%)	12 (7.4%)	5 (38.5%)
>3.50	4 (2.5%)	3 (23.1%)	12 (7.4%)	5 (38.5%)
>4.00	2 (1.2%)	3 (23.1%)	6 (3.7%)	4 (30.8%)
>4.50	2 (1.2%)	3 (23.1%)	4 (2.5%)	3 (23.1%)

Table II—Down syndrome detection efficiency at a five per cent false-positive rate for urinary free beta (hCG) and beta-core (hCG)

Analyte	Under 35 years		All ages	
	Cut-off risk	Detection efficiency	Cut-off risk	Detection efficiency
Free beta (hCG)	1/595	32.7%	1/335	44.4%
Beta-core (hCG)	1/505	32.7%	1/295	44.3%

Note. MoM values below 0.62 for free beta (hCG) and 0.60 for beta-core (hCG) were set equal to 0.62 or 0.60 prior to calculating likelihood ratios since likelihood ratios for MoM values below 0.62 or 0.60 were unexpectedly higher than the likelihood ratios calculated at 0.62 or 0.60.

A significant correlation was found between urinary free beta (hCG) and beta-core (hCG) values in both Down syndrome cases ($r=0.93$) and controls ($r=0.61$). Table I shows the number of Down syndrome and unaffected cases above specific MoM cut-offs. For urinary free beta (hCG), 3/13 Down syndrome cases (23 per cent) were above the 95th percentile of unaffected pregnancies (i.e., 5 per cent false-positive rate) and 7/13 cases (54 per cent) were above the 90th percentile (i.e., 10 per cent false-positive rate). For beta-core (hCG), 4/13 (31 per cent) and 6/13 (46 per cent) Down syndrome cases were above the 95th and 90th percentiles of unaffected pregnancies, respectively.

Table II shows the modelled detection efficiency for each marker at a 5 per cent false-positive rate after including maternal age. The detection efficiency of free beta (hCG) and age (44.4 per cent)

was equal to that of beta-core (hCG) and age (44.3 per cent).

DISCUSSION

The median urinary free beta (hCG) level in the 13 Down syndrome cases was 2.42 MoM (2.84 in second trimester and 1.40 in first trimester), consistent with previously published data sets (Table III) and similar in degree of elevation to the 2.64 median Down syndrome MoM in liquid serum samples (Macri *et al.*, 1994) and 2.4 median Down syndrome MoM in dried blood samples (Macri *et al.*, 1996). Further, in our small series of five trisomy 18 cases the median urinary free beta (hCG) MoM of 0.35 is similar to the 0.2 MoM observed in maternal serum (Krantz *et al.*, 1997). These similar median affected MoMs are not

Table III—Urinary free-beta sample sets

Study	Assay	First trimester (9–13 weeks)		Second trimester (14–22 weeks)	
		<i>n</i>	Median MoM	<i>n</i>	Median MoM
Spencer <i>et al.</i> (1997)	CIS	22	1.87	—	—
Spencer <i>et al.</i> (1996)	CIS	—	—	29	2.47
Cole <i>et al.</i> (1997)	Bioclone	—	—	14	2.61
Current study	In-house	5	1.40	8	2.84
Muller (1996)	*	—	—	30	2.86
Hayashi <i>et al.</i> (1996)	CIS	—	—	3	3.52

*Information not available.

surprising in light of the significant correlation ($r=0.6$) observed between dried urine free beta (hCG) and dried blood free beta (hCG) in this study in specimens from 100 unaffected patients.

Overall, Down syndrome detection efficiency was similar for both urinary free beta (hCG) and beta-core (hCG) (Table II). Among the second-trimester samples 5/8 (62.5 per cent) had a free beta (hCG) value above the 90th percentile while 3/8 (37.5 per cent) had a beta-core (hCG) value above the 90th percentile. This is consistent with Spencer *et al.* (1996) who found that the detection efficiency of urinary free beta (hCG) was greater than that of beta-core (hCG), and Hayashi *et al.* (1996) who found a higher MoM with urinary free beta (hCG) than urinary beta-core (hCG) in two out of three Down syndrome cases. The result, however, contrasts with Cole *et al.* (1997), who found that detection efficiency was greater with beta-core (hCG) than free beta (hCG) in a set of 14 Down syndrome cases. The Cole *et al.* study may be biased, however, since this data set was previously known to have an extremely high beta-core (hCG) Down syndrome MoM prior to evaluating free beta (hCG).

Previous studies on urinary beta-core (hCG) demonstrate discrepant results in terms of gestational week specific medians (Spencer *et al.*, 1996). Beta-core (hCG) gestational week specific median values in the present study (18.56, 13.94 and 10.48 nmol/nmol at 14, 16 and 18 weeks, respectively) are closest to those observed by Cuckle *et al.* (1995) (24.89, 15.32 and 9.43 nmol/nmol, respectively).

Large discrepancies have also been seen among studies with median MoM values in Down syndrome cases varying between 1.33 and 7.53 MoM

in the second trimester and between 1.06 and 2.91 MoM in first trimester (Table IV). The second-trimester beta-core (hCG) Down syndrome median MoM value of 2.18 in this study is significantly lower than that seen in four sample sets (Isozaki *et al.*, 1997; Canick *et al.*, 1995, 1996; Cuckle *et al.*, 1994, 1995), but similar to the two largest sample sets (Muller, 1996; Spencer *et al.*, 1996) and significantly higher than one other sample set (Hayashi and Koza, 1995).

Attempts to explain observed discrepancies among beta-core (hCG) studies are ongoing. Several studies were conducted in the first trimester of pregnancy. In fact, the two studies with the lowest median Down syndrome MoM contained only first-trimester specimens (Macintosh *et al.*, 1997; Kornman *et al.*, 1997). In addition, the only first-trimester sample in the Cuckle *et al.* (1995) study had a MoM of 1.01. Spencer *et al.* (1997), however, demonstrated a median Down syndrome MoM of 2.91 in 22 first-trimester samples. The median MoM in five first-trimester Down syndrome samples in the present study was 2.89, similar to results observed by Spencer *et al.* (1997). Larger data sets are needed to better assess the degree of elevation of beta-core (hCG) in the first trimester.

Isozaki *et al.* (1997) noted that studies containing the three sample sets with the highest median beta-core (hCG) Down syndrome MoM values (see Table IV) had limitations because they contained a disproportionate number of Down syndrome cases and the Down syndrome cases were collected either at multiple sites or at different locations from unaffected samples. Isozaki *et al.* (1997) attempted to avoid this problem by conducting a prospective study in which all

Table IV—Urinary beta core sample sets

Study	Assay	First trimester (9–13 weeks)		Second trimester (14–22 weeks)	
		<i>n</i>	MoM	<i>n</i>	MoM
Cuckle <i>et al.</i> (1996)	Triton UGP	9	1.06	—	—
Kornman <i>et al.</i> (1997)	Triton UGP	5	1.13	—	—
MacIntosh <i>et al.</i> (1997)	S504	9	1.16	—	—
Spencer (1997)	Triton UGP	22	2.91	—	—
Hayashi and Kozu (1995)	Wako	—	—	5	1.33
Muller (1996)	Triton UGP	—	—	30	1.99
Current study	Toagosie UGF	5	2.89	8	2.18
Spencer <i>et al.</i> (1996)	Triton UGP	—	—	29	2.35
Isozaki <i>et al.</i> (1997)	B210*	1	4.7	12	4.05
Canick <i>et al.</i> (1996)	Triton UGP	—	—	18	5.02
Canick <i>et al.</i> (1995)	Triton UGP	—	—	14	5.34
Cole (1997)	Various	—	—	—	4.73–7.53
Cuckle <i>et al.</i> (1994, 1995)	S504	1	1.01	23	6.10

*The B210 assay is considered equivalent to the triton UGP assay (Isozaki *et al.*, 1997).

samples were collected at a single location. However, there was also a disproportionate number of Down syndrome cases in this prospective study group (13/726) for which the authors had no explanation. Further, the authors reported a Down syndrome detection of 8/13 (62 per cent) cases above the 95th percentile. However, using the distributional parameters for beta-core (hCG) presented in their study (median: 0.0; SD (Log_{10}): 0.368), the 95th percentile would be 4.03 MoM, indicating that only 7/13 (54 per cent) cases were above the 95th percentile. Regardless, reported results (median Down syndrome MoM 4.1, detection efficiency 62 per cent) from this prospective study were less favourable than those seen with the three earlier data sets (Canick *et al.*, 1995, 1996; Cole *et al.*, 1997; Cuckle *et al.*, 1994, 1995).

Cole and colleagues have focused on assay methods to explain discrepancies in median Down syndrome MoM results among studies (Cole, 1995, 1997). Specifically, Cole has argued that cross-reactivity with free beta (hCG) is a significant problem in beta-core (hCG) assays and should be avoided. While it is desirable to have highly specific assays for analytes being tested, it is unlikely that cross-reactivity to minor components will have a significant impact on results. Since there is a 200-fold molar excess of urinary beta-core (hCG) compared with free beta (hCG) during pregnancy, a beta-core (hCG) assay which cross-

reacts 100 per cent with free beta (hCG) will be subject to free beta (hCG) interference of no more than approximately 0.5 per cent. Furthermore, in comparing studies using the same commercial beta-core (hCG) assay, vast discrepancies in Down syndrome median MoMs have been observed. For example, using the Ciba Corning Triton UGP assay, Spencer *et al.* (1996) observed a median Down syndrome MoM value of 2.35 MoM, while Canick *et al.* (1995) observed a value of 5.34 MoM. In this study we observed a median Down syndrome MoM of 2.40 with the Toagosie UGF EIA assay, while Cole *et al.* (1997) observed a 7.53 median Down syndrome MoM value with the same assay kit.

The most likely explanation for discrepancies among various beta-core (hCG) studies relates to the impact of the large sample dilution required for maternal urinary beta-core (hCG) assays which range as high as 2500 to 25 000-fold (Canick *et al.*, 1995). These large dilutions can lead to unacceptably high inter-assay variation. The impact of these dilutions is apparent not only when comparing results in Down syndrome cases but also in comparing unaffected gestational week specific medians reported in these studies (Spencer *et al.*, 1996). Median Down syndrome MoM values with urinary free beta (hCG) have been more consistent between studies (Table IV). This consistency may be due to a lower level of free beta (hCG) in urine,

which in turn requires less dilution for assay analysis. Until beta-core (hCG) assays are designed specifically for use in pregnancy and do not require extreme dilution it will be difficult to make a robust estimate of the degree of elevation of beta-core (hCG) in Down syndrome cases.

Recently, Cole *et al.* (1997) have raised the issue of free beta (hCG) stability in maternal urine. This issue was previously raised when maternal serum free beta (hCG) was first introduced as a screening test for Down syndrome (Knight and Cole, 1991). Despite claims, free beta (hCG) stability has not been an issue in clinical practice and the free beta (hCG) biochemical marker consistently outperforms other second-trimester maternal serum markers (Macri and Spencer, 1996; Wenstrom *et al.*, 1997a, b). Cole *et al.* (1997) have recently analysed various hCG metabolites in maternal urine samples using 12 distinct immunoassays. The assay demonstrating the lowest standard deviation in the unaffected population was that for free beta (hCG). Such a distribution would not be expected of an analyte considered to be as unstable as claimed by Cole *et al.* Indeed, Spencer *et al.* (1996) have found urinary free beta (hCG) to be extremely stable. Finally, as demonstrated in maternal serum (Spencer *et al.*, 1993), the dried sample format employed in the present study should add additional analyte stability.

In conclusion, prenatal screening with urinary markers requires further investigation to sort out the relative benefits of either urinary free beta (hCG) or beta-core (hCG) and to explain observed discrepancies in results obtained with beta-core (hCG) assays. As prenatal screening for Down syndrome moves rapidly towards the first trimester with biochemical markers as well as nuchal translucency measurement (Nicolaidis *et al.*, 1994), the effectiveness of urinary metabolites versus serum analytes (free beta (hCG) and PAPP-A) in early pregnancy will require particular scrutiny.

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