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Maternal serum hyperglycosylated human chorionic gonadotrophin (HhCG) in the first trimester of pregnancies affected by Down syndrome, using a sialic acid-specific lectin immunoassay

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In a series of 54 cases of pregnancies complicated by Down syndrome and 224 unaffected pregnancies we examined maternal serum levels of hyperglycosylated human chorionic gonadotrophin (HhCG) in samples collected in the first trimester (11–13 weeks) using a sialic acid-specific lectin immunoassay. We compared these levels with those of other potential first trimester serum markers [free β-hCG, pregnancy-associated plasma protein A (PAPP-A) and total hCG (ThCG)] and modeled detection rates and false-positive rates of various biochemical markers in conjunction with fetal nuchal translucency (NT) and maternal age using an maternal age standardized population. Maternal serum HhCG in cases of Down syndrome were significantly elevated (median MoM 1.97) with 24/54 (44%) of cases above the 95th centile for unaffected pregnancies. Free β-hCG was also elevated (median MoM 2.09) with 33% of cases above the 95th centile. PAPP-A levels were reduced (median MoM 0.47) with 38% below the 5th centile. ThCG levels, whilst elevated (median MoM 1.34), had only 20% of cases above the 95th centile. Maternal serum HhCG levels were not correlated with fetal NT but showed significant correlation with ThCG and free β-hCG and with PAPP-A in the Down syndrome group ($r = 0.536$). Maternal serum HhCG levels in cases with Down syndrome had a significant correlation with gestational age, increasing as the gestation increased. When HhCG was combined together with fetal NT, PAPP-A and maternal age, at a 5% false-positive rate the modeled detection rate was 83%, some 6% lower than when free β-hCG was used and some 4% better than when ThCG was used. Maternal serum HhCG is unlikely to be of additional value when screening for Down syndrome in the first trimester. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: free β-hCG; total hCG; PAPP-A; nuchal translucency; trisomy 21; prenatal screening

INTRODUCTION

Human chorionic gonadotrophin (hCG) exists in maternal pregnancy serum in many different forms, varying from the intact hCG (alpha and beta dimer), the free hCG subunits [free alpha (hCGα) and free beta (hCGβ)], various nicked forms of intact (hCGn) or free β-hCG (hCGβn) and the more recently identified hyperglycosylated forms of intact hCG (HhCG) (and presumably free β-hCG) in which there are additional sialyl N-acetyllactosamine antennae (Cole, 1997; Elliott *et al*., 1997; Birken *et al*., 1999).

In the second trimester of pregnancy measurement of intact, total or free β-hCG, in conjunction with maternal age and maternal serum alpha fetoprotein (AFP), have found clinical utility when screening for pregnancies complicated by Down syndrome. Retrospective and prospective studies over the past decade have achieved detection rates of 65–75% for a 5% false-positive rate (Crossley *et al*., 1996; Macri and Spencer, 1996; Wald *et al*., 1997; Spencer, 1999).

In the first trimester of pregnancy measurement of intact or total hCG (ThCG) has been shown to have limited clinical utility (Spencer *et al*., 2000a). However, the free β-hCG subunit maintains the same clinical discrimination as in the second trimester, and in conjunction with maternal age and maternal serum pregnancy-associated plasma protein-A (PAPP-A), can be shown retrospectively (Spencer *et al*., 1999) and prospectively (Spencer *et al*., 2000b) to identify 60–65% of Down syndrome cases for a 5% false-positive rate. When fetal nuchal translucency (NT) thickness was added to this combination, retrospective and prospective detection rates of 90% have been achieved at a 5% false-positive rate (Spencer *et al*., 1999, 2000b).

Attempts to improve Down syndrome detection rates have been ongoing in recent years and one avenue has focused on the measurement of urine metabolites of hCG. Initial studies with urine β-core (hCGβcf) (Cuckle *et al*., 1994, 1995) showed considerable promise with a median in cases with Down syndrome of around 6 MoM. However, this initial optimism has not been sustained, with more extensive studies showing much poorer discrimination (Cuckle *et al*., 1999a; Hsu *et al*., 1999). Cole *et al*. (1997) initially reported increased levels of hyperglycosylated variants of hCG in urine from women carrying a fetus affected by Down syndrome. Subsequent studies (Cole *et al*., 1998, 1999a,b; Cuckle *et al*., 1999b) using a

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specific immunoassay to HhCG have shown potential detection rates of 80% at a 5% false-positive rate in urine with a median MoM of 9.5. However, concerns exist over both sample stability and the ability of urine creatinine to normalize for renal concentrating ability (Cole *et al*., 1999a). When examined in maternal serum, a pilot study (Shahabi *et al*., 1999) showed elevated levels in ten cases (median MoM 3.9) with 60% of cases above the 95th centile. However unpublished evidence (Cole *et al*., 1999b) suggests that gel separator tubes can interfere with HhCG detection in serum.

In an alternative immunoassay approach, Abushoufa *et al*. (2000) measured hCG glycoforms in serum from unaffected pregnancies and cases with Down syndrome using a sialic acid-specific lectin immunoassay. In this study in the second trimester the lectin immunoassay was shown to have greater discrimination than ThCG.

The present study extends the work of Abushoufa *et al*. (2000) by examining the value of HhCG measurement with a sialic acid-specific lectin immunoassay in the first trimester of pregnancy.

PATIENTS AND METHODS

Maternal serum samples from unaffected and pregnancies affected by Down syndrome have been collected from women attending Harold Wood Hospital as part of research studies into first trimester screening or since 1998 as part of a routine first trimester OSCAR screening program incorporating PAPP-A, free β-hCG and fetal NT (Spencer *et al*., 2000b). At the time of ultrasound examination crown–rump length (CRL) and NT were measured as previously described (Snijders *et al*., 1998) by sonographers certified by the Fetal Medicine Foundation. Blood samples were collected into plain vacutainers with no clotting activator and with no gel separators. After clotting the maternal serum was separated into aliquots and stored at –20°C. From this archive of samples from unaffected pregnancies and those affected by Down syndrome a series of 224 unaffected and 54 Down syndrome samples were retrieved for study. Table 1 summarises the two study populations.

Maternal serum free β-hCG, PAPP-A and ThCG were measured using the Kryptor analyser – a rapid random access immunoassay analyser using time-resolved amplified cryptate emission (TRACE) technology and the automated immunofluorescent assays [Brahms GmbH, Berlin, Germany (formerly CIS)]. The precision and performance of these assays has been previously reported (Spencer *et al*., 1999, 2000a).

Maternal serum HhCG was measured at two dilutions (1 : 500 and 1 : 1000) in singleton pregnancies using the lectin immunoassay described by Abushoufa *et al*. (2000). The mean of the two results after correction for dilution was used in further statistical analysis. Analysis of samples was performed with outcomes blinded to the assayer.

Table 1—Median and range for maternal age, gestational age, fetal crown–rump length, maternal weight and sample storage time in the Down syndrome group and the control group

	Down syndrome	Controls
n	54	224
Maternal age (years)	36.1 (20–44)	30.4 (16–41)
Gestational age (days)	87 (73–97)	84 (70–96)
Crown–rump length (mm)	62 (38–85)	55 (33–80)
Maternal weight (kg)	66 (44–109)	64 (41–113)
Sample storage time (days)	1187 (147–2354)	127 (116–1460)

Statistical analysis

To correct for marker variation with gestational age each value was converted to multiples of the median (MoM) for unaffected pregnancies at the same gestational age using either previously established relationships (Ong *et al*., 2000; Spencer *et al*., 2000a) or as established for HhCG in the present study. Regression analysis was carried out to derive the relationship between marker levels and gestational age. Correction of each MoM for maternal weight was also performed using the reciprocal-linear regression weight correction procedure of Neveux *et al*. (1996).

Statistical analysis of data was performed using Microsoft Excel 97 and Analyse-It (Smart Software, Leeds, UK) a statistical software add-in.

The performance of various marker combinations as potential screening procedures was examined using standard statistical modeling techniques (Royston and Thompson, 1992). Using the observed population parameters for HhCG , those for ThCG from Spencer *et al*. (2000a) and those for free β-hCG and PAPP-A and NT from Spencer *et al*. (1999), a series of 15 000 random MoM values were selected for each marker from within the Gaussian distributions of the $\log_{10}$ MoM of affected and unaffected pregnancies. These values were then used to calculate likelihood ratios for the combinations. The likelihood ratios were then used together with the age-related risk of trisomy 21 in the first trimester (Snijders *et al*., 1999) to calculate the expected detection rate of affected pregnancies at a fixed false-positive rate, in a population with the maternal age distribution of pregnancies in England and Wales (Office for National Statistics, 1997–1999).

RESULTS

HhCG levels decreased progressively with gestational age. Figure 1 shows the individual data and the best fit to a quadratic regression with the following form: median MoM = (6973.02*gestational day) + (−54.98* gestational day^2)–105324.66. Median levels of HhCG fell by an average 2154 IU/l/day between the 70th and 96th day of gestation.

HhCG in both unaffected and Down syndrome pregnancies followed a Gaussian distribution after

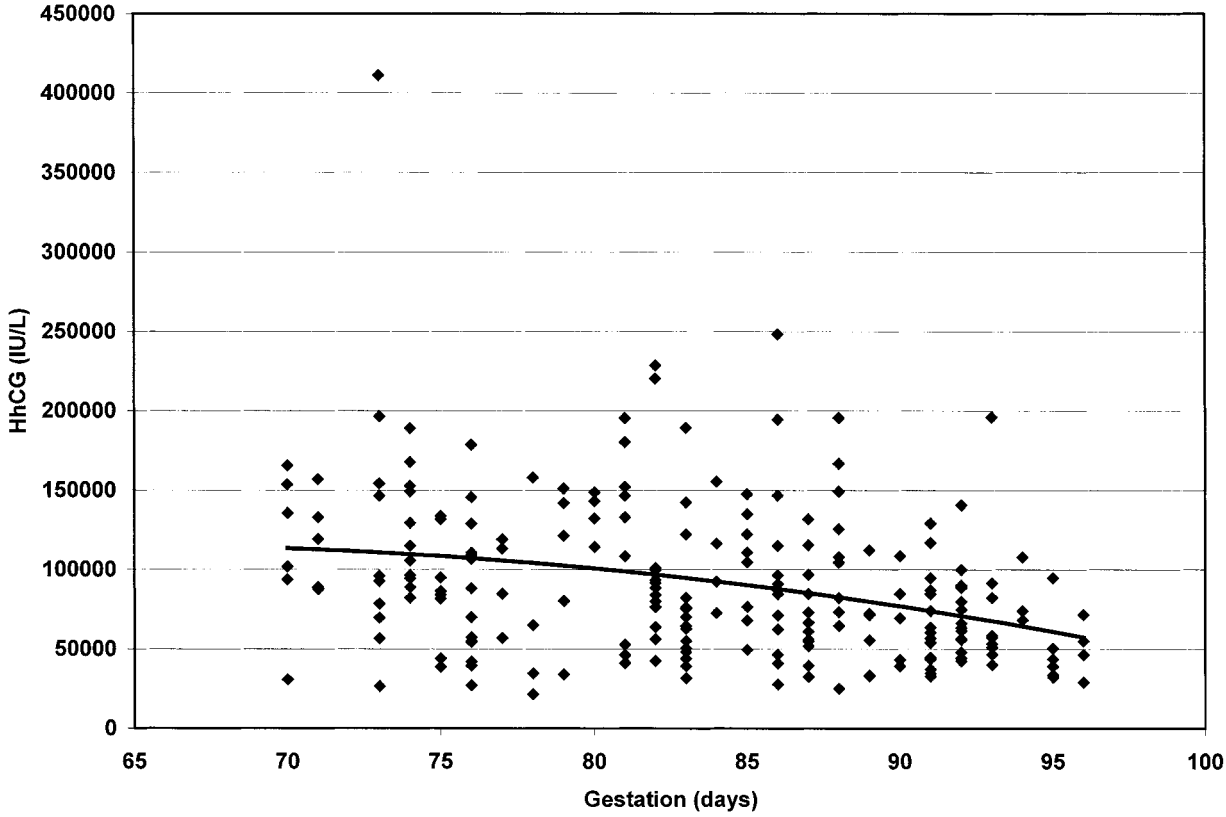

Figure 1—Hyperglycosylated human chorionic gonadotrophin (HhCG) variation with gestational age in unaffected pregnancies. The solid line is the median derived from quadratic regression

$\log_{10}$ transformation of MoM values, as determined by Kolmogorov and Anderson Darling tests with significance at the 0.01 level. The two distributions are shown in Figure 2.

Previous studies have shown that PAPP-A, free β-hCG, ThCG and fetal NT follow a Gaussian distribution after $\log_{10}$ transformation in both unaffected and affected pregnancies (Nicolaides *et al.*, 1998; Spencer *et al.*, 1999, 2000a).

The median MoM levels for the various markers in the study population are shown in Table 2. For HhCG the 10th to 90th centile of controls was 0.53–1.76 and for Down syndrome cases was 0.90–3.50. The respective 5th to 95th centiles were 0.43–2.07 and 0.82–4.43. HhCG levels were significantly elevated in cases of Down syndrome with 24/54 (44%) cases above the 95th centile and 30/54 (56%) cases above the 90th centile (see Figure 3). In comparison, 33% of cases of free β-hCG were above the 95th centile and 38% of PAPP-A were below the 5th centile (Spencer *et al.*, 1999). Similarly, ThCG was above the 95th centile in only 20% of cases (Spencer *et al.*, 2000a) and fetal NT was above the 95th centile in 71.8% of cases (Snijders *et al.*, 1998). The median MoMs obtained for the other markers were consistent with those obtained in larger series (Spencer *et al.*, 1999, 2000a)

Correlation of HhCG was investigated with other first trimester markers. HhCG was not correlated with fetal NT in the control group or the Down syndrome group ($r = 0.182$ and 0.148, respectively). However, HhCG was significantly correlated with ThCG and free β-hCG in both populations [r (downs) $= 0.905$ and r (controls) $= 0.712$ for ThCG; r (downs) $= 0.626$ and r (controls) $= 0.583$ for free β-hCG]. For PAPP-A a significant correlation was shown only in the Down syndrome population ($r = 0.536$). The statistical distribution of HhCG in the control and Down syndrome population is shown in Table 3.

When detection rates and false-positive rates for various marker combination were modeled against the most recent age distribution of pregnancies in England and Wales using previously derived population parameters for free β-hCG, NT and PAPP-A (Spencer *et al.*, 1999) and for those with ThCG (Spencer *et al.*, 2000a) and the parameters for HhCG from the present study, the results obtained are shown in Table 4.

Close examination of the individual HhCG MoMs in Figure 3 suggested an increase in MoM with gestational age. Correlation of HhCG MoM with gestational age showed a significant correlation coefficient of 0.3294 with a slope of 0.0703 and intercept of –3.9573. Examining the results by gestational age bands showed an increasing median MoM HhCG with gestation. At 80–84 days the median MoM HhCG was 1.82, increasing to 2.04 at 85–89 days and to 2.37 at 90–97 days. When the mean $\log_{10}$ MoM HhCG was compared between the 80–84-day period and the 90–97-day period, unpaired t-test with unequal variance showed these to be significantly different ($p = 0.0451$).

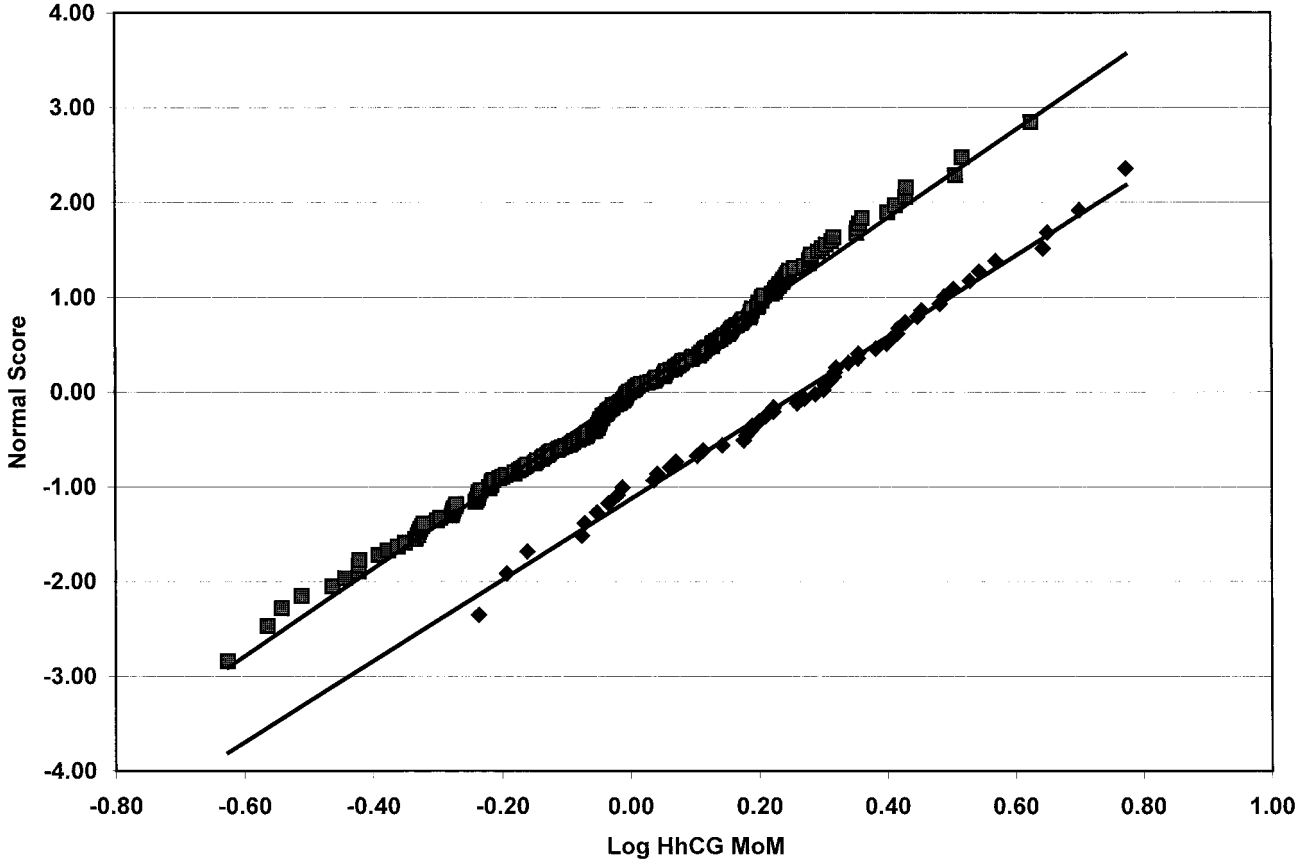

Figure 2—Probability distribution of $\log_{10}$ MoM hyperglycosylated human chorionic gonadotrophin (HhCG) in (shaded squares) unaffected pregnancies and in those with Down syndrome (solid triangles). The solid lines are the lines of best fit

DISCUSSION

In urine, Cole *et al.* (1999a,b) and Cuckle *et al.* (1999b) showed that HhCG levels decreased across the first and second trimester of pregnancy, although during the narrow 10–12-week period Weinans *et al.* (2000) found a flat profile. These results are consistent with the present authors' previous observations (Abushoufa *et al.*, 2000) and the observation in the present study where levels progressively fall from 10 to 14 weeks and mirror the falls in intact hCG and free β-hCG at this time.

After the initial observation by Cole *et al.* (1997) of abnormal oligosaccharides on hCG from pregnancies affected by Down syndrome, there have been a handful of studies investigating the potential use of HhCG as a screening tool. These studies, predominantly in urine in the second trimester, are summarized in Table 5. The studies of Cole and colleagues consistently show high median MoMs with approaching 80% or more of cases greater than the 95th centile. The study by Cuckle *et al.* (1999b) showed a much lower median MoM with only 38% above the 95th centile. When examined in the first trimester, Weinans *et al.* (2000) have shown a lower median MoM than in the second trimester and Cuckle *et al.* (1999b) in their study also showed lower MoMs in cases prior to 14 weeks. In serum the two studies thus far in the second trimester seem to show closer agreement with median MoMs of 2.2 and 3.9 but with greater than 60% of cases above the 95th centile.

Table 2—Median marker MoMs in the study populations and the significance of the difference

Marker	Down syndrome	Unaffected	Probability
HhCG	1.97	1.00	<0.001
Free β-hCG	2.09	1.00	<0.001
ThCG	1.34	1.00	<0.001
PAPP-A	0.47	1.00	<0.001
NT	2.45	1.00	<0.001

hCG; Human chorionic gonadotrophin; HhCG, hyperglycosylated hCG; ThCG, total hCG; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

The present data in the first trimester shows a significant elevation of HhCG with 44% above the 95th centile, being superior to both ThCG and free β-hCG. The observation of an age-dependent increase in HhCG MoM is consistent with the findings of Weinans *et al.* (2000) and Cuckle *et al.* (1999b) for urine and is similar to the temporal changes reported for ThCG (Spencer *et al.*, 2000a) and for free β-hCG (Spencer *et al.*, 1999).

In combination with other first trimester markers of

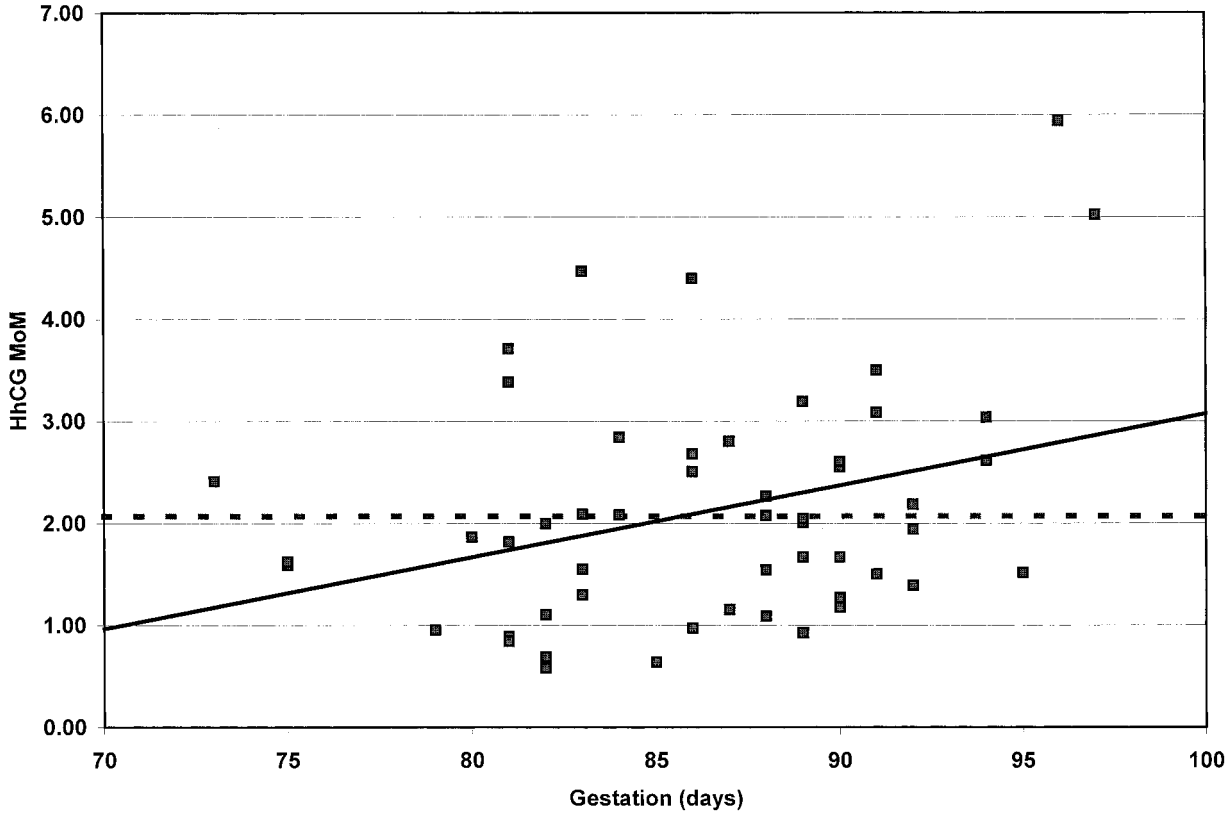

Figure 3—Hyperglycosylated human chorionic gonadotrophin (HhCG) in 54 cases of Down syndrome in the first trimester. The dotted line is the 95th centile in unaffected pregnancies. The solid line is the regression line for HhCG against gestation

aneuploidy, namely free β-hCG, PAPP-A and fetal NT, although HhCG performed as well as free β-hCG, in combination with other markers, the significant correlation with the other biochemical markers reduces its effectiveness. Replacing free β-hCG with HhCG would lead to a 5% reduction in detection rate at a 5% false-positive rate.

Little is known about the long-term stability of HhCG. One possible criticism of the present study is that cases and controls had different average storage times, with the cases having been stored for a longer period. Although cases and controls were stored in aliquots and had not previously been thawed since collection, it is possible that this difference in long-term storage may have influenced the present results.

In conclusion, whilst maternal serum HhCG is elevated in the first trimester, levels do not approach the supra elevated levels reported by Cole and colleagues

Table 3—Statistical parameters for the HhCG population

	Down syndrome cases	Controls
Mean $\log_{10}$ HhCG MoM	0.264	0.004
SD $\log_{10}$ HhCG MoM	0.2326	0.264
5th–95th centile MoM	0.82–4.43	0.43–2.07
10th–90th centile MoM	0.90–3.50	0.53–1.76
r v Free β-hCG	0.626	0.583
r v ThCG	0.905	0.712
r v PAPP-A	0.536	0.061
r v NT	0.148	0.182

hCG; Human chorionic gonadotrophin; HhCG, hyperglycosylated hCG; ThCG, total hCG; MoM, multiples of the median; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A; r v, correlation coefficient; SD, standard deviation.

Table 4—Detection rates at a fixed 5% false-positive rate for various marker combinations in conjunction with maternal age in the first trimester

Markers	Detection rate (%)
ThCG	37.0
HhCG	48.6
ThCG and PAPP-A	49.0
Free β-hCG	49.5
PAPP-A	50.8
HhCG and PAPP-A	61.2
Free β-hCG and PAPP-A	69.5
NT	73.5
NT and ThCG	75.5
NT, ThCG and PAPP-A	78.7
NT and HhCG	79.9
NT and free β-hCG	80.1
NT and PAPP-A	81.2
NT, HhCG and PAPP-A	83.0
NT, free β-hCG and PAPP-A	88.9

hCG; Human chorionic gonadotrophin; HhCG, hyperglycosylated hCG; ThCG, total hCG; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

Table 5—Studies of HhCG in screening for Down syndrome

Study	Gestation (weeks)	Cases/controls	Median MoM	Cases > 95th centile (%)	Fluid	Assay
Cole *et al*. (1998)	11–21	10/142	5.7	90	Urine	Immunoassay
Cole *et al*. (1999a)	11–22	23/1134	7.8	78	Urine	Immunoassay
Shahabi *et al*. (1999)	15–22	10/66	3.9	60	Serum	Immunoassay
Cuckle *et al*. (1999b)	10–19	45/304	3.63	36	Urine	Immunoassay
	>14		4.64			
Cole *et al*. (1999)	14–22	21/1059[a]	8.44	81	Urine	Immunoassay
		18/389	9.94	82		
		39/1448	9.50	80		
Weinans *et al*. (2000)	10–12	8/55	3.6	63	Urine	Immunoassay
Abushoufa *et al*. (2000)	16–18	39/105	2.2	74	Serum	Lectin immunoassay

[a]Repeat of samples from study by Cole *et al*. (1999a).

for HhCG in urine during the second trimester. It remains to be seen if the early promise of significant discrimination can be maintained in studies from other centres or whether like with urine beta core (Cuckle *et al*., 1999a; Hsu *et al*., 1999) assay difficulties, sample instability and renal concentrating issues lead to another cul-de-sac in our search for improved screening markers.

REFERENCES

Abushoufa RA, Talbot JA, Brownbill K, Rafferty B, Kane JW, Robertson WR. 2000. The development of a sialic acid specific lectin-immunoassay for the measurement of human chorionic gonadotrophin glycoforms in serum and its application in normal and Down's syndrome pregnancies. *Clin Endocrinol* **52**: 499–508.

Birken S, Krichevsky A, O'Connor J, *et al*. 1999. Development and characterization of antibodies to a nicked and hyperglycosylated form of hCG from a choriocarcinoma patient: generation of antibodies that differentiate between pregnancy hCG and choriocarcinoma hCG. *Endocr J* **10**: 137–144.

Cole LA. 1997. Immunoassay of hCG, its free subunits and metabolites. *Clin Chem* **43**: 2233–224.

Cole LA, Cermik D, Bahado Singh RO. 1997. Oligosaccharide variants of hCG-related molecules: potential screening markers for Down syndrome. *Prenat Diagn* **17**: 1188–1190.

Cole LA, Omrani A, Cermik D, Bahado-Singh RO, Mahoney MJ. 1998. Hyperglycosylated hCG, a potential alternative to hCG in Down syndrome screening. *Prenat Diagn* **18**: 926–933.

Cole LA, Shahabi S, Oz UA, *et al*. 1999a. Urinary screening tests for fetal Down syndrome: II. Hyperglycosylated hCG. *Prenat Diagn* **19**: 351–359.

Cole LA, Shahabi S, Oz UA, Bahdo-Singh R, Mahoney MJ. 1999b. Hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen) immunoassay: a new basis for gestational Down syndrome screening. *Clin Chem* **45**: 2109–2119.

Crossley JA, Aitken DA, Berry E, Connor JM. 1994. Impact of a regional screening programme using maternal serum AFP and hCG on the birth incidence of Down's syndrome in the west of Scotland. *J Med Screen* **1**: 180–183.

Cuckle HS, Iles RK, Chard T. 1994. Urinary β-core human chorionic gonadotrophin: a new approach to Down's syndrome screening. *Prenat Diagn* **14**: 953–958.

Cuckle HS, Iles RK, Sehmi IH, *et al*. 1995. Urinary multiple marker screening for Down's syndrome. *Prenat Diagn* **15**: 745–751.

Cuckle HS, Canick JA, Kellner LH. 1999a. Collaborative study of maternal urine β-core human chorionic gonadotrophin screening for Down syndrome. *Prenat Diagn* **19**: 911–917.

Cuckle HS, Shahabi S, Sehmi IK, Jones R, Cole LA. 1999b. Maternal urine hyperglycosylated hCG in pregnancies with Down syndrome. *Prenat Diagn* **19**: 918–920.

Elliott M, Kardana A, Lustbader JW, Cole LA. 1997. Carbohydrate and peptide structure of the α- and β-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. *Endocrine* **7**: 15–32.

Hsu JJ, Spencer K, Aitken DA, *et al*. 1999. Urinary free beta hCG, beta core fragment and total oestriol as markers of Down syndrome in the second trimester of pregnancy. *Prenat Diagn* **19**: 148–158.

Macri JN, Spencer K. 1996. Towards the optimal protocol for Down's syndrome screening. *Am J Obstet Gynecol* **174**: 1668–1669.

Neveux LM, Palomaki GE, Larrivee DA, Knight GJ, Haddow JE. 1996. Refinements in managing maternal weight adjustment for interpreting prenatal screening results. *Prenat Diagn* **16**: 1115–1119.

Nicolaides KH, Snijders RJM, Cuckle HS. 1998. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* **18**: 519–521.

Office for National Statistics. 1997–1999. 1997, 1998, 1999 Birth Statistics Series FM1, number 26, 27, 28. London: Her Majesty's Stationery Office (HMSO).

Ong CYT, Liao AW, Spencer K, Munim S, Nicolaides KH. 2000. First trimester maternal serum free β-human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *Br J Obstet Gynaecol* **107**: 1265–1270.

Royston P, Thompson SG. 1992. Model based screening by risk with application to Down's syndrome screening. *Stat Med* **111**: 257–268.

Shahabi S, Oz UA, Bahado-Singh RO, *et al*. 1999. Serum hyperglycosylated hCG: a potential screening test for fetal Down syndrome. *Prenat Diagn* **19**: 488–489.

Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. 1998. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. *Lancet* **352**: 343–346.

Snijders RJ, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. 1999. Maternal age- and gestation-specific risk for trisomy 21. *Ultrasound Obstet Gynecol* **13**: 167–170.

Spencer K. 1999. Second trimester prenatal screening for Down's syndrome using alpha-fetoprotein and free beta hCG: a seven year review. *Br J Obstet Gynaecol* **106**: 1287–1293.

Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. 1999. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* **13**: 231–237.

Spencer K, Berry E, Crossley JA, Aitken DA, Nicolaides KH. 2000a. Is maternal serum total hCG a marker of trisomy 21 in the first trimester of pregnancy. *Prenat Diagn* **20**: 311–317.

Spencer K, Spencer CE, Power M, Moakes A, Nicolaides KH. 2000b. One stop clinic for assessment of risk for fetal anomalies: a report of the first year of prospective screening for chromosomal anomalies in the first trimester. *Br J Obstet Gynaecol* **107**: 1271–1275.

Wald NJ, Kennard A, Hackshaw A, McGuire A. 1997. Antenatal screening for Down's syndrome. *J Med Screen* **4**: 181–246.
Weinans MJN, Butler SA, Mantingh A, Cole LA. 2000. Urinary hyperglycosylated hCG in first trimester screening for chromosomal abnormalities. *Prenat Diagn* **20**: 976–978.