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Maternal Urine Hyperglycosylated hCG in Pregnancies with Down Syndrome

H. S. Cuckle[1]*, S. Shahabi[2], I. K. Sehmi[1], R. Jones[3] and L. A. Cole[2]

[1]*Centre for Reproduction, Growth and Development, University of Leeds, U.K.*
[2]*Department of Obstetrics and Gynecology, Yale University, New Haven, CT, U.S.A.*
[3]*Institute of Pathology, Leeds Teaching Hospitals NHS Trust, U.K.*

Stored maternal urine samples were used to determine the distribution of hyperglycosylated human chorionic gonadotrophin (H-hCG) levels in pregnancies with Down syndrome. A total of 349 samples from singleton pregnancies, including 45 with Down syndrome, were tested at 10–19 weeks' gestation. Urinary concentration was allowed for by expressing H-hCG in ng per mmol creatinine. The median level in Down syndrome was 3.63 multiples of the gestation-specific median in unaffected pregnancies ($p<0.0001$, Wilcoxon rank–sum test, two-tail). However, creatinine levels were relatively low in cases and creatinine did not fully correct for concentration. When this bias was allowed for, the median level was 3.34 multiples of the normal median (MoM). The H-hCG elevation in affected pregnancies was more marked at 14 weeks' gestation or later: a median of 4.64 MoM and allowing for creatinine bias 4.46 MoM.

KEY WORDS: maternal urine; hCG; hyperglycosylated; Down syndrome; screening

INTRODUCTION

Several studies have shown that in Down syndrome pregnancies the maternal urine level of specific subspecies of human chorionic gonadotrophin (hCG) is raised, on average. 15 studies have reported on β-core hCG levels (Cuckle *et al.*, 1994, 1995, 1996, 1999; Canick *et al.*, 1995; Spencer *et al.*, 1996, 1997; Kellner *et al.*, 1997; Isozaki *et al.*, 1997; Lam *et al.*, 1997; Macintosh *et al.*, 1997; Korman *et al.*, 1997; Hallahan *et al.*, 1998; Hsu *et al.*, 1999; Cole *et al.*, 1999a) seven on free-β or intact hCG (Spencer *et al.*, 1996, 1997; Cole *et al.*, 1997, 1998, 1999b; Hallahan *et al.*, 1998; Hsu *et al.*, 1999) and one used an assay that measured both (Hayashi and Kozu, 1995). More recently, in a preliminary study, Cole *et al.* (1998) reported raised maternal urinary hyperglycosylated (H-)hCG levels in 10 pregnancies with Down syndrome. The assay was subsequently improved and raised levels were found in 23 affected pregnancies, including 8 from the first series (Cole *et al.*, 1999b). This prompted us to carry out a case–control study of H-hCG using a large bank of maternal urine samples.

MATERIALS AND METHODS

Maternal urine samples had been routinely collected from women having routine maternal serum screening for Down syndrome. The samples were aliquoted and stored in a bank at -40°C. In addition, samples were collected and stored after Down syndrome had been diagnosed following amniocentesis or chorionic villus sampling, or in some cases immediately before the procedure.

We retrieved from storage 349 samples from singleton pregnancies—45 cases with Down syndrome and 304 unaffected controls—with a gestational range of 10–19 completed weeks. Gestation was based on ultrasound in all but 5 cases and 19 controls; the median duration of sample storage was 3.0 years for cases (range 1.1–5.4) and 3.2 years for controls (1.2–4.0). The urine samples from cases had been taken as part of routine serum screening in 11, after prenatal diagnosis in 28 and prior to the procedure in 6. The indications for prenatal diagnosis were: positive serum screening tests (17 cases); abnormal ultrasound findings (12); advanced maternal age (3); and two with a previous chromosomal abnormality.

Samples were measured for H-hCG by an immunoassay as previously described (Cole *et al.*, 1998, 1999b) and for creatinine by the Jaffe method. H-hCG results were expressed as ng/mmol creatinine, and to allow for changes in level with gestation as multiples of the normal gestation-specific median (MoM). The normal medians were obtained for each day of gestation by regression among the controls of the observed median level on median days at each completed week. The regression was weighted for the number at controls: 9 at 10–12 weeks combined, and 26, 20, 55, 70, 68, 46, 10, respectively, for each single week from 13 to 19.

RESULTS

The best fitting regression line in the controls was log–cubic:

$$\log_{10}(h) = 38.8247 - 0.965993 \times d + 0.00817047 \times d^2 - 0.00002330 \times d^3$$

*Correspondence to: H. S. Cuckle, Reproductive Epidemiology, Centre for Reproduction, Growth and Development, School of Medicine, University of Leeds, 26 Clarendon Road, Leeds, LS2 9NZ, U.K. E-mail: h.s.cuckle@leeds.ac.uk

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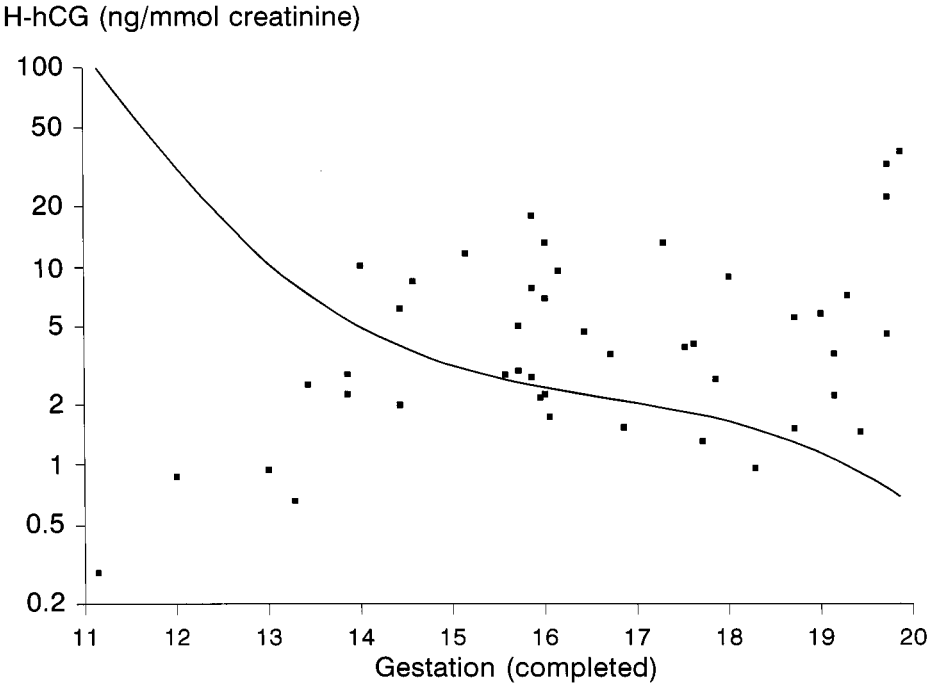

Fig. 1—Individual maternal urine H-hCG levels according to gestation for 45 cases (■) and the regressed normal median values

where *h* is H-hCG in ng/mmol creatinine and *d* is the gestation in days. The observed (and regressed) values were: 15 (16) ng/mmol for 10–12 weeks, and 8.6 (7.3), 2.8 (3.7), 2.6 (2.9), 2.8 (2.3), 1.9 (1.9), 1.2 (1.5), 1.1 (0.8) ng/mmol thereafter.

The median H-hCG level in unaffected pregnancies was 0.93 MoM with a 10th–90th centile range of 0.28–3.98 MoM; the 95th centile was 5.62 MoM. There was a strong negative association between creatinine and H-hCG in MoMs. The median H-hCG level for controls with creatinine under 4.0 mmol/ml was 1.54 MoM (59 samples), 4.0–5.9 mmol 1.22 MoM (48), 6.0–7.9 mmol 0.81 MoM (58), 8.0–9.9 mmol 0.72 MoM (38), 10.0–11.9 mmol 0.88 MoM (42), and 12 or more mmol 0.68 MoM (59).

Fig. 1 shows the individual H-hCG levels in the 45 cases together with the regressed normal median values. The overall median level was 3.63 MoM, a statistically significant elevation ($p<0.0001$, Wilcoxon rank–sum test, one-tail). The 10th–90th centile range was 0.96–13.2 MoM; 21 (47 per cent) exceeded the normal 90th centile and 16 (36 per cent) exceeded the normal 95th centile.

There appeared to be a strong association with gestation. Excluding the 7 cases under 14 weeks' gestation the median level was 4.64 MoM with 20 (53 per cent) above the normal 90th centile (4.15 MoM after excluding 35 controls under 14 weeks) and 17 (45 per cent) above the 95th centile (5.52 MoM).

DISCUSSION

We have confirmed that maternal urine H-hCG levels are increased on average in pregnancies with Down syndrome. The median level in our study of 45 cases was 3.63 MoM with 95 per cent confidence interval (CI) 2.5–5.3 MoM, consistent with the median of 5.7 MoM (CI 3.3–9.8 MoM) found in the previous study of 10 cases (Cole *et al.*, 1998) and 7.8 MoM (CI 4.4–13.7 MoM) in the extended series of 23 cases (Cole *et al.*, 1999b).

The results from the current series may have been biased due to cases having lower creatinine levels than controls. The median level in the cases was 6.3 mmol/ml compared with 7.3 mmol/ml in the controls. We attempted to allow for this by dividing the H-hCG level by the median MoM for the creatinine using the six groups above. The median adjusted values in cases and controls were 3.34 and 1.00 MoM, respectively. Also, as a result of adjustment there was an 8 per cent reduction in the normal standard deviation, in logs, so that 20 (44 per cent) cases exceeded the normal 90th centile and 15 (33 per cent) the 95th centile. Cole *et al.* (1999b) also found a negative association between H-hCG level in MoMs and creatinine. When the results were adjusted as we have done, the median level in the 23 cases of Down syndrome was reduced from 7.8 MoM to 7.3 MoM and the normal standard deviation was reduced by 6 per cent.

As with other types of urinary hCG the H-hCG elevation in affected pregnancies is more marked in the second trimester of pregnancy. After 14 completed weeks the median was 4.64 MoM and when creatinine bias was allowed for this became 4.46 MoM. Also, as found with other urinary hCG species, there was a correlation with maternal serum free-β hCG. Results were available in 30 cases and 295 controls; the correlation coefficient between log serum and urine MoM

levels after creatinine correction was 0.44 and 0.53, respectively.

Only one of the groups investigating hCG subspecies in maternal urine from Down syndrome have prospectively tested fresh samples (Isozaki *et al.*, 1997; Cole *et al.*, 1999a). One of us (L.C.) has performed laboratory experiments showing that sample storage conditions can affect immunoreactivity for some subspecies. Therefore, although H-hCG appears to be reasonably stable after storage (Cole *et al.*, 1999b) prospective studies will be needed, both to surmount the problems with creatinine correction and to confidently assess the potential of H-hCG in Down syndrome screening.

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