PRENATAL DIAGNOSIS
Prenat Diagn 2000; **20**: 976–978.

Urinary hyperglycosylated hCG in first trimester screening for chromosomal abnormalities

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Hyperglycosylated human chorionic gonadotrophin (H-hCG), also known as Invasive Trophoblast Antigen or ITA, is a unique metabolic variant of hCG with more complex oligosaccharide side chains. Concentrations are independent of regular hCG. Urine H-hCG has recently proved to be a highly sensitive marker for Down syndrome screening in the second trimester of pregnancy. We evaluated H-hCG as a potential marker in the first trimester of pregnancy. Maternal urine samples were collected from 10^{+0} to 11^{+6} weeks of gestation prior to genetic analysis and stored in frozen form. Samples from eight cases of Down syndrome, two cases of trisomy 13, one case of trisomy 18, and 55 control pregnancies were hand-carried frozen to the USA and tested blindly. Samples were tested in a specific H-hCG immunoassay and values were normalized to creatinine concentration. Values were plotted against gestational age, and multiples of control pregnancy median (MoM) calculated. The median level of the MoMs of the eight Down syndrome cases was 3.6 MoM. Five of the eight Down syndrome cases exceeded the 90th centile of the 55 unaffected cases. The MoMs of the trisomy 13 and 18 pregnancies were 0.2, 0.2 and 0.3. All three cases were under the 10th centile of unaffected pregnancies. The results of this study indicate that H-hCG testing may be useful in screening for Down syndrome in the first trimester of pregnancy. Further studies are needed to assess the potential screening values of urine H-hCG and the combination of this test with free β-subunit, PAPP-A and other markers for Down syndrome in the first trimester of pregnancy. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome; hyperglycosylated hCG; screening; first trimester; invasive trophoblast antigen (ITA)

INTRODUCTION

Hyperglycosylated human chorionic gonadotrophin (H-hCG) (commercial test name Invasive Trophoblast Antigen or ITA) is a unique carbohydrate variant of hCG with more complex N- and O-linked oligosaccharide side chains with additional sialyl-N-acetyllactosamine antennae. It is produced by poorly differentiated or invasive trophoblast cells (Cole *et al.*, 1997, 1999a, b). H-hCG production is independent of regular hCG synthesis (Cole *et al.*, 1999a, b). Production is greatest at the beginning of pregnancy, when H-hCG accounts for up to 100% of hCG-related molecules (O'Connor *et al.*, 1998). This percentage rapidly diminishes as pregnancy progresses, being only a minor component of hCG-related molecules ($<2.9\%$) from 8 weeks of gestation until term (Cole *et al.*, 1999b). An assay has been developed that detects only H-hCG (Birken *et al.*, 1999).

Shahabi *et al.* (1999) have shown that H-hCG is detectable in the serum and urine samples of pregnant women. However, problems have been encountered with obtaining accurate measurements of H-hCG in serum samples with separator tubes and protein aggregation (Cole *et al.*, 1999b). This has, for the interim, restricted the number of studies on serum samples. Losses have also been noted in urine H-hCG concentrations after multiple freezing–thawing cycles.

Cole *et al.* (1999b) measured hCG in urine samples collected at a single center from 1448 control and 39 Down syndrome pregnancies at 14–22 weeks of gestation. The samples had been frozen and thawed once only. The median Down syndrome sample was 9.5 MoM of the control samples. The H-hCG test detected 80% of Down syndrome cases at a 5% false positive rate. Urine H-hCG measurements are very promising as a new high-sensitivity screening test for maternal Down syndrome.

Cuckle *et al.* (1999) carried out a case control study on H-hCG in Down syndrome pregnancies. However, the study was conducted with samples collected under diverse conditions with multiple freezing and thawing. Less promising detection rates were reported. A strong association was indicated with gestational age, indicating that H-hCG may not be elevated in Down syndrome cases prior to 12 weeks of gestation.

In this paper, for the first time, we investigated the value of H-hCG measurements in very early pregnancy (prior to 12 weeks' gestation), using urine samples that had been frozen and thawed once only.

So far, there have been only five studies published regarding urinary screening, principally or solely in the first trimester of pregnancy (Cuckle *et al.*, 1996; Kornman *et al.*, 1997; Macintosh *et al.*, 1997; Spencer

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Received: 28 April 2000
Revised: 31 July 2000
Accepted: 13 August 2000

et al., 1997; Hallahan *et al.*, 1998). In these studies β-core hCG, free β-hCG and/or total oestriol were used as urinary markers.

MATERIALS AND METHODS

From October 1997 to May 1999, maternal urine samples were collected consecutively prior to chorionic villous sampling (CVS) at the Antenatal Diagnosis Unit of the University Hospital Groningen, The Netherlands and stored frozen at −20°C. Almost all the CVS procedures were performed because of advanced maternal age.

Urine samples were selected from 11 women diagnosed as having autosomal aneuploidy: eight with trisomy 21, two with trisomy 13 and one with trisomy 18. For each chromosomally abnormal pregnancy, five matched controls were chosen on the basis of gestational age (within 4 days), maternal age (within 3 years), maternal weight (within 10%), duration of storage (within 3 months) and smoking history. The gestational age when the samples had been collected ranged between 10^{+0} and 11^{+6} weeks.

All the urine samples were coded and hand-carried frozen to the University of New Mexico. Samples were thawed for the first time and tested blindly for H-hCG and creatinine using previously described procedures (Cole *et al.*, 1999a, b). Values were expressed as nanogram H-hCG per milligram creatinine and were adjusted for creatinine correction error. Gestational age-specific multiples of the normal median (MoM) values were determined and centiles were calculated as described previously (Cole *et al.*, 1999b).

RESULTS

H-hCG levels were determined in urine samples from 55 unaffected pregnancies and from eight Down syndrome, two trisomy 13 and one trisomy 18 pregnancies. Results were normalized to creatinine and plotted against gestational age (Figure 1). During the 2-week interval (10^{+0} to 11^{+6} weeks of gestation) a virtually flat relationship was observed between the H-hCG results and gestational age (ga) in days, median H-hCG = $116 \times (1.0084^{ga})$, $r^2 = 0.001$.

MoM values were calculated. The log SD in the unaffected pregnancies was 0.32. The 5th, 10th, 90th and 95th centiles were 0.35, 0.40, 2.68 and 4.40 MoM, respectively. The individual values for the aneuploid pregnancies are shown in Table 1. The median MoM of the Down syndrome samples was 3.6. Five of the eight (63%) Down syndrome samples exceeded the 90th centile of the control samples, while three of the eight samples (37%) exceeded the 95th centile. The trisomy 13 and 18 cases had extremely low H-hCG levels. All three were below the 5th centile. Six of the 11 trisomies (55%) showed positive results (three

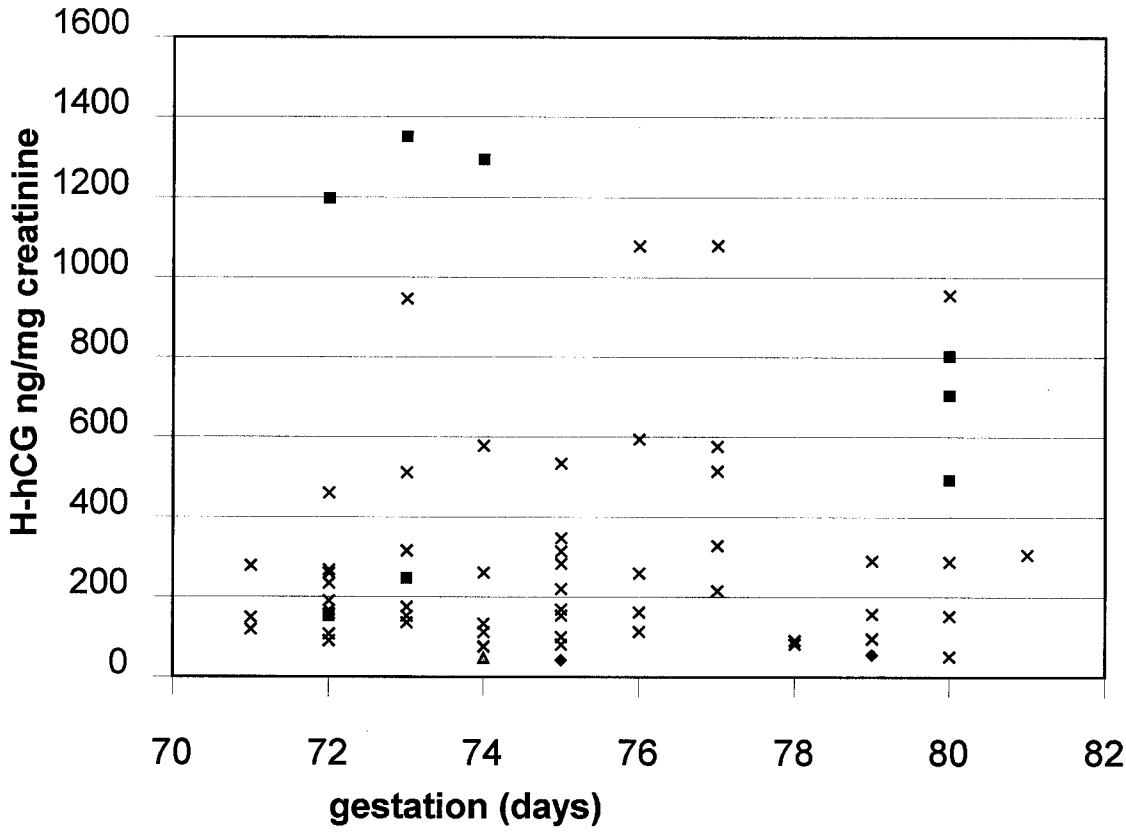

Figure 1—Maternal urine H-hCG levels versus gestational age in 55 unaffected and 11 aneuploid pregnancies. ■, Down syndrome; ◆, trisomy 13; △, trisomy 18; ×, controls

Table 1—H-hCG results in aneuploid pregnancies

Karyotype	Gestational age (days)	MoM
Trisomy 13	75	0.19 (<5th centile)
Trisomy 13	79	0.26 (<5th centile)
Trisomy 18	74	0.22 (<5th centile)
Trisomy 21	72	0.71
Trisomy 21	73	1.14
Trisomy 21	80	2.28
Trisomy 21	80	3.26 (>90th centile)
Trisomy 21	80	3.72 (>90th centile)
Trisomy 21	72	5.56 (>95th centile)
Trisomy 21	74	6.00 (>95th centile)
Trisomy 21	73	6.26 (>95th centile)

Down syndrome $>95^{th}$ centile, three other trisomies $<5^{th}$ centile).

DISCUSSION

Our results indicated that H-hCG values are raised in Down syndrome cases in the first trimester of pregnancy. This study examined 55 unaffected and eight Down syndrome pregnancies from the 10th and 11th weeks of gestation. The median H-hCG level in the Down syndrome cases was 3.6 MoM with a detection rate of 38% at a 5% false positive rate. Cole *et al.*(1999b) examined 1448 unaffected and 39 Down syndrome pregnancies from the 14th to 21st week of gestation. Samples were collected under very similar conditions. They noted a much greater elevation in Down syndrome cases (median 9.5 MoM) while the detection rate was 80% at a 5% false positive rate. We conclude that although H-hCG is a very sensitive marker for gestational Down syndrome in the second trimester of pregnancy, it is less sensitive at 10 and 11 weeks of gestation. In the absence of additional data, an intermediate sensitivity might be predicted at 12 and 13 weeks of gestation.

Multiple freezing and thawing of urine samples led to suppression of the H-hCG levels in Down syndrome pregnancies (Cole *et al.*, 1999b). The samples used in the present study and in the one by Cole *et al.*(1999b) were frozen and thawed once only. Even better results might have been obtained in both studies with fresh samples.

Cuckle *et al.* (1999), measured H-hCG in variously stored urine samples. H-hCG concentrations were less strikingly elevated in Down syndrome pregnancies in the second trimester. A strong association was indicated with gestational age, but in the first trimester samples collected before 12 weeks of pregnancy there was no elevation in the concentrations. The latter is in contrast with our results (3.6 MoM in Down syndrome pregnancies before 12 weeks of gestation). It is possible that the less striking results reported by Cuckle *et al.* (1999) in first and second trimester urine samples were caused by differences in storage and handling.

In this study, the two trisomy 13 cases and the single trisomy 18 case had H-hCG values that were below the 5th centile. In the study by Cole *et al.* (1999b), four of the six trisomy 18 cases had levels that were below the 5th centile in the second trimester. Based on these preliminary observations, H-hCG might also be useful in the first and second trimesters for detecting trisomy 13 and 18.

Finally, none of the urinary markers described in the previous first trimester studies (Cuckle *et al.*, 1996; Kornman *et al.*, 1997; Macintosh *et al.*, 1997; Spencer *et al.*, 1997; Hallahan *et al.*, 1998) performed as well as H-hCG in our study. However, this study was performed on a limited number of Down syndrome cases. Larger, prospective studies are needed to assess the value of H-hCG as a future urinary marker in Down syndrome screening in the first trimester of pregnancy. Further studies are also needed to assess the potential screening value of the combination of H-hCG and free β-subunit, PAPP-A and other markers for Down syndrome in the first trimester of pregnancy.

ACKNOWLEDGEMENTS

We wish to thank Robert de Vrij for his technical assistance. This research was supported by NIH Grant HD-35654 to LAC.

REFERENCES

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. *Endocrine* **10**: 137–144.

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

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, Bahado-Singh RO, Mahony MJ. 1999b. Hyperglycosylated human chorionic gonadotropin (Invasive Trophoblast Antigen) immunoassay: a new basis for gestational Down syndrome screening. *Clin Chem* **45**: 2109–2119.

Cuckle HS, Canick JA, Kellner LH, *et al.* 1996. Urinary β-core hCG screening in the first trimester. *Prenat Diagn* **16**: 1057–1059.

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

Hallahan TW, Kranz DA, Tului L, *et al.* 1998. Comparison of urinary free beta (hCG) and beta core (hCG) in prenatal screening for chromosomal abnormalities. *Prenat Diagn* **18**: 893–900.

Kornman LH, Morssink LP, Wortelboer MJM, *et al.* 1997. Maternal urinary β-core hCG in chromosomal abnormal pregnancies in the first trimester. *Prenat Diagn* **17**: 135–139.

Macintosh MCM, Nicolaides KH, Noble P, Chard T, Gunn L, Iles R. 1997. Urinary β-core hCG: screening for aneuploidies in early pregnancy (11–14 weeks' gestation). *Prenat Diagn* **17**: 401–405.

O'Connor JF, Ellish N, Kakuma T, Schlatterer J, Kobalebskaya G. 1998. Differential urinary gonadotropin profiles in early pregnancy loss. *Prenat Diagn* **18**: 1232–1240.

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

Spencer K, Noble P, Snijders RJM, Nicolaides KH. 1997. First trimester urine free beta hCG, beta core, and total oestriol in pregnancies affected by Down's syndrome: implications for first-trimester screening with nuchal translucency and serum free beta hCG. *Prenat Diagn* **17**: 525–538.