PRENATAL DIAGNOSIS, VOL. **17:12:** 1107–1111 (1997)

SCREENING FOR DOWN SYNDROME USING URINE hCG FREE β-SUBUNIT IN THE SECOND TRIMESTER OF PREGNANCY

LAURENCE A. COLE[1]*, MELISSA JACOBS[1], TAICHI ISOZAKI[1], GLENN E. PALOMAKI[2], RAY O. BAHADO-SINGH[1,3] AND MAURICE J. MAHONEY[1,3]

[1]*Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, U.S.A.*
[2]*Foundation for Blood Research, Scarborough, ME, U.S.A.*
[3]*Department of Genetics, Yale University School of Medicine, New Haven, CT, U.S.A.*

Received 26 February 1997
Revised 1 May 1997
Accepted 7 May 1997

SUMMARY

Human chorionic gonadotropin (hCG) free β-subunit levels were determined in 709 control and 13 Down syndrome urine samples from the second trimester of pregnancy. Results were normalized to urine creatinine concentration and converted to multiples of the unaffected pregnancy medium (MOM). The concentration of free β-subunit in Down syndrome cases was 3·9 MOM. Seven of 13 Down syndrome pregnancies (54 per cent) had free β-subunit levels at or above the 95th centile of unaffected pregnancies. Urine free β-subunit may potentially be useful as a screening test for Down syndrome. © 1997 by John Wiley & Sons, Ltd.

Prenat. Diagn. 17: 1107–1111, 1997
No. of Figures: 1. No. of Tables: 0. No. of References: 19.

KEY WORDS: Down syndrome; free β-subunit; human chorionic gonadotropin; screening

INTRODUCTION

hCG is a glycoprotein hormone composed of two dissimilar subunits, α- and β-, linked non-covalently. In addition to hCG dimer, free α- and β-subunits can be detected in serum, and the same plus β-core fragment, a degraded β-subunit, in urine samples.

A triple test, comprising hCG, α-fetoprotein (AFP), and unconjugated oestriol, is currently used to assess risk for Down syndrome in second-trimester maternal serum. In 1990, serum free β-subunit tests were proposed as an alternative to the triple test (Macri *et al.*, 1990). Since then, serum free β-subunit–AFP combinations have been introduced, and are used as alternative tests to the triple test for screening for Down syndrome (Spencer *et al.*, 1993). Recently, Spencer *et al.* (1996) measured free β-subunit levels in second-trimester pregnancy urine samples. They found a 2·6-fold elevation of free β-subunit levels in Down syndrome cases, with a detection rate of 58 per cent at a 5 per cent false-positive rate. Urine free β-subunit was seen as a possible improvement over serum assays.

Recently, we published a prospective study examining β-core fragment levels in 722 patients attending a single centre for amniocentesis (Isozaki *et al.*, 1997). In the present study, free β-subunit measurements were analysed in those same samples.

MATERIALS AND METHODS

Urine samples were collected from women with singleton pregnancies at 12–24 weeks of gestation,

*Correspondence to: Laurence A. Cole, PhD, Associate Professor, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, U.S.A. E-mail: laurence.cole@yale.edu

CCC 0197–3851/97/121107–05 $17.50

coming from amniocentesis at the Maternal–Fetal Medicine Unit at Yale-New Haven Hospital. Between August 1995 and May 1996, urine samples were volunteered (whilst waiting or during preparation for amniocentesis). Oral consent was sought, using a protocol approved by the Yale University Human Investigation Committee. Urine samples were collected from 709 women with a normal karyotype and 13 with Down syndrome. Of 722 patients, 71 per cent came for amniocentesis because of advanced maternal age (over 35 years old); 23 per cent because of a positive triple screen test; 1·1 per cent because of identified fetal abnormalities; and 3·7 per cent because of a previous aneuploid pregnancy or other reason. The 722 patients included 7 from 11–12 weeks, 9 from 12–13 weeks, 4 from 13–14 weeks, 6 from 14–15 weeks, 277 from 15–16 weeks, 210 from 16–17 weeks, 105 from 17–18 weeks, 50 from 18–19 weeks, 26 from 19–20 weeks, 15 from 20–21 weeks, 8 from 21–22 weeks, 2 from 22–23 weeks, and 3 from 23–24 weeks of gestation (determined by ultrasound) (Isozaki *et al.*, 1997).

Urine samples were refrigerated immediately after collection. Twice each week, urine samples were carried to the laboratory for assay. Results were entered into a Microsoft Excel 7 computer spreadsheet. Gestational age, determined by ultrasound, was obtained from the Maternal–Fetal Medicine Unit computer. Approximately 2 weeks after urine collection, the karyotype was obtained from the records at the Prenatal Diagnosis Service in the Department of Genetics at Yale University. Maternal age and karyotype were also entered into the computer spreadsheet.

Free β-subunit levels were measured by immunoenzymometric assay with antibody FBT11 as described previously (Cole *et al.* 1993; Kardana and Cole, 1994; Cole *et al.*, 1994). This is a two-step sandwich assay. Briefly, microtitre plates were coated with monoclonal antibody FBT11 (a gift from Drs Bidart and Bellet, Istitut Gustave Roussy, Cedex, France). Urine samples were added and free β-subunit was extracted. Plates were washed and peroxidase-labelled purified hCGβ antisera, antisera BP052 (Bios Pacific, Emmeryville, CA, U.S.A.) were added to quantitate bound free β-subunit. After a further wash, substrate was added and peroxidase enzyme activity was measured spectrometrically in a microtitre plate reader. Urine samples were tested undiluted, or diluted 1:10 or 1:100 in serum albumin-containing buffer. The FBT11 assay was standardized with free β-subunit batch CR129β (a gift from Dr Birken, Columbia University, New York, NY, U.S.A.), calibrated according to mass by amino acid analysis. The FBT11 assay detects nicked and non-nicked free β-subunit molecules equally, with less than 0·1 per cent cross-reactivity with hCG, free α-subunit or β-core fragment (Cole *et al.*, 1993; Kardana and Cole, 1994).

Urine samples were collected at random times with no uniformity in the time of voiding. Free β-subunit levels were normalized to adjust for variations in the urine concentration by dividing by the creatinine concentration. The creatinine concentration was determined using the Sigma Chemical Co. spectrometric creatinine kit (St Louis, MO, U.S.A.) and the dictated procedures. Immunoassay results (ng/ml) were normalized to the urine creatinine concentration (ng/mg creatinine).

Results were analysed at the Foundation for Blood Research in Maine using standard statistical modelling methods (Royston and Thompson, 1992). Free β-subunit levels increase with gestational age in a log-linear manner. The observed medians are reported along with the standard deviation, estimated by the 10th–90th centile difference divided by 2·56.

RESULTS

Figure 1 shows the relationship between urine free β-subunit concentration and gestational age in 709 control and 13 Down syndrome pregnancies. The log median for unaffected samples was 0·000 and the log standard deviation was 0·39. Eleven of 13 Down syndrome cases had levels exceeding 1·0 MOM, with a median value of 3·9 MOM. As shown in Fig. 1, seven of 13 Down syndrome cases had levels exceeding the 95th centile of unaffected pregnancies (54 per cent observed detection at a 5 per cent false–positive rate).

Twenty-three per cent of the control urine samples and 1 of the 13 Down syndrome collections were from women who underwent amniocentesis subsequent to a positive triple test. We reanalysed the data excluding these samples. No change in the screening performance was observed, 54 per cent detection at a 5 per cent false–positive rate (data not shown).

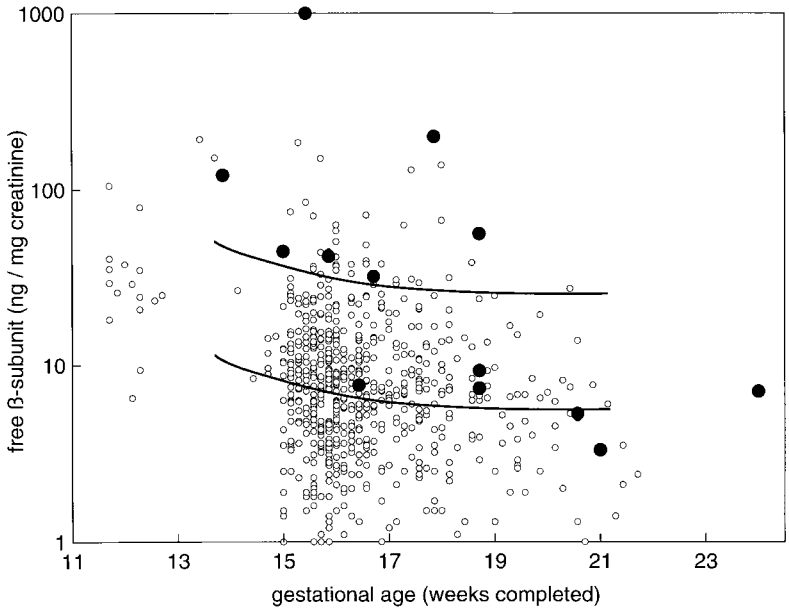

Fig. 1—Levels of free β-subunit in urine samples from 722 pregnancies, 13 affected with Down syndrome (●) and 709 controls (○). The lower line is the 50th centile for unaffected pregnancies; the upper line is the 95th centile

DISCUSSION

Urine free β-subunit was investigated as a potential marker of Down syndrome pregnancies. Fifty-four per cent detection was observed at a 5 per cent false–positive rate. This is similar to the 58 per cent detection rate reported by Spencer *et al.* (1996). We employed the FBT11 free β-subunit immunometric assay. This assay detected both nicked and non-nicked free β-subunit equally, with virtually no cross-reactivity (<0·1 per cent) with hCG or β-core fragment. Spencer *et al.* (1996) also claimed recognition of both forms of free β-subunit by their immunoassay, and similar specificity. We infer that our assays are similar and yield comparable results and detection rates.

Twenty-three per cent of the samples were from patients who underwent amniocentesis because of a positive triple test. This group could be potentially biasing the control group data, since the likelihood of elevated urine free β-subunit levels may be greater in those having higher serum hCG values (triple tests) (Kardana and Cole, 1997; Cole, 1997). When the data were reanalysed excluding these samples, no difference was observed in the screening performance.

The sample set includes 13 Down syndrome cases. Two of these cases are at the extremes of gestational age, with very few controls of corresponding gestational age. Elevated free β-subunit levels (>95th centile) are detected in one of these two cases, so that exclusion of these cases does not significantly change the suggested 54 per cent detection rate. We considered removing these samples from the study and restricting the gestational age range of control samples. This, however, reduced the number of Down syndrome cases to only 11 and limited comparison with β-core fragment levels, determined previously on all 722 samples (Isozaki *et al.*, 1997).

A detection rate of 54–58 per cent is good for a single marker. It is close to that of the triple test and may be better than any single serum marker (Canick, 1990; Spencer *et al.*, 1992, 1996). A urine test has several advantages and may be preferable to a serum assay. Urine is easier to collect than serum. It does not require venipuncture or separation, as does a blood sample. It may also be more acceptable to patients. Free β-subunit and other urine tests should be considered as alternatives to blood-based tests for Down syndrome screening. Urine tests, however, may not be able to replace serum screening methods entirely. Serum AFP

determination may still be needed for detecting neural tube defects. It is possible that AFP-related molecules may be measurable in urine samples, in which case, a multi-marker urine test may be indicated. Ultrasound might be used instead of serum AFP as a complementary test to the urine analytes for detecting neural tube defects and aneuploid pregnancies.

In the last 3 years, an alternative urine test, β-core fragment, has been evaluated as a marker of Down syndrome. Five of six studies specifically measuring this marker reported higher detection rates (80–88 per cent) than the 54 per cent reported here (Canick *et al.*, 1995, 1996; Cole *et al.*, 1996; Cuckle *et al.*, 1994, 1995). The sixth study reported a markedly lower sensitivity (Spencer *et al.*, 1996). Urine β-core fragment may be a better urine-based test for Down syndrome screening.

β-Core fragment levels were measured in the same 722 urine samples tested for free β-subunit. Sixty-two per cent detection of Down syndrome cases was indicated for β-core fragment at a 5 per cent false–positive rate (Isozaki *et al.*, 1997). While this was greater than the 54 per cent detection rate reported here for free β-subunit, it was only marginally better (8 of 13, compared with 7 of 13 true–positive Down syndrome cases).

Recently, we investigated the stability of urine analytes (Cole, 1997). Six sterile samples of urine were incubated at ambient temperature. While no change was detected in β-core fragment levels, a rise of $59 \pm 6{\cdot}5$ per cent each week was detected in free β-subunit values over the 4-week incubation period. Stability could be an issue if urine free β-subunit were to be considered for routine use as a screening test for Down syndrome pregnancies. Samples might need to be stored and shipped refrigerated, or precipitated or blotted and shipped in a solid phase. We again infer that β-core fragment may be the preferable urine screening test.

REFERENCES

Canick, J.A. (1990). Screening for Down syndrome using maternal serum alpha-fetoprotein, unconjugated estriol and hCG, *J. Clin. Immunoassay*, **13,** 30–33.

Canick, J.A., Kellner, L.H., Saller, D.N., Jr, Palomaki, G.E., Walker, R.P., Osathanondh, R. (1995). Second trimester levels of maternal urinary gonadotropin peptide in Down syndrome pregnancy, *Prenat. Diagn.*, **15,** 752–759.

Canick, J.A., Kellner, L.H., Saller, D.N., Jr, Palomaki, G.E., Tumber, M.B., Messerlian, G.L., Walker, R.P., Osathanondh, R. (1996). A second level evaluation of maternal urinary gonadotropin peptide as a marker for second trimester Down syndrome screening, *Early Hum. Dev.*, in press.

Cole, L.A. (1995). Down's syndrome screening using urine β-core fragment test; choice of immunoassay, *Prenat. Diagn.*, **15,** 679–682.

Cole, L.A. (1997). Stability of hCG free β-subunit and β-core fragment in urine, *Prenat. Diagn.*, **17,** 185–189.

Cole, L.A., Kardana, A., Andrade-Gordon, P., Gawinowicz, M.A., Morris, J.C., Bergert, E.R., O'Connor, J., Birken, S. (1991). The heterogeneity of hCG: III. The occurrence, biological and immunological activities of nicked hCG, *Endocrinology*, **129,** 1559–1567.

Cole, L.A., Kardana, A., Park, S.-Y., Braunstein, G.D. (1993). The deactivation of hCG by nicking and dissociation, *J. Clin. Endocrinol. Metab.*, **76,** 704–710.

Cole, L.A., Kardana, A., Seifer, D., Bohler, H. (1994). Urine hCG β-subunit core fragment, a sensitive test for ectopic pregnancy, *J. Clin. Endocrinol. Metab.*, **78,** 497–499.

Cole, L.A., Isozaki, T., Palomaki, G., Canick, J., Iles, R., Kellner, L., Saller, Cuckle, H. (1996). Detection of β-core fragment in second trimester Down's syndrome pregnancies, *Early Hum. Dev.*, **47,** S47–S48.

Cuckle, H.S., Iles, R., Chard, T. (1994). Urinary β-core human chorionic gonadotropin: a new approach to Down's syndrome screening, *Prenat. Diagn.*, **14,** 953–958.

Cuckle, H.S., Iles, R., Sehmi, I., Chard, T., Oakey, R., Davies, S., Ind, T. (1995). Urinary multiple marker screening for Down's syndrome, *Prenat. Diagn.*, **15,** 745–751.

Isozaki, T., Palomaki, G.E., Bahado-Singh, R.O., Cole, L.A. (1997). Screening for Down syndrome pregnancy using β-core fragment: prospective study, *Prenat. Diagn.*, **17,** 407–413.

Kardana, A., Cole, L.A. (1994). Human chorionic gonadotropin β-subunit nicking enzymes in pregnancy and cancer patient serum, *J. Clin. Endocrinol. Metab.*, **79,** 761–767.

Kardana, A., Cole, L.A. (1997). The stability of hCG and free β-subunit in serum samples, *Prenat. Diagn.*, **17,** 141–147.

Macri, J.N., Kasturi, R.V., Krantz, D.A., Cook, E.J., Moore, N.D., Young, J.A., Romero, K., Larsen, J.W., Jr. (1990). Maternal serum Down syndrome screening: free beta-protein is a more effective marker than human chorionic gonadotropin, *Am. J. Obstet. Gynecol.*, **163,** 1248–1253.

Royston, P., Thompson, S.G. (1992). Model based screening by risk with application to Down's syndrome screening, *Stats. Med.*, **11,** 257–268.

Spencer, K., Coombes, E.J., Mallard, A.S., Milford-Ward, A. (1992). Free beta human choriogonadotropin in Down's syndrome screening: a multicenter study of its role compared with other biochemical markers, *Ann. Clin. Biochem.*, **29,** 506–518.

Spencer, K., Macri, J.N., Anderson, R.W., Aitken, D.A., Berry, E., Crossley, J.A., Wood, P.J., Coombes, E.J., Stroud, M., Worthington, D.J. (1993). Dual analyte immunoassay in neural tube defect and Down's syndrome screening: results of a multicentre clinical trial, *Ann. Clin. Biochem.*, **30,** 394–401.

Spencer, K., Aitken, D.A., Macri, J.N., Buchanan, P.D. (1996). Urine free beta hCG and beta core in pregnancies affected by Down's syndrome, *Prenat. Diagn.*, **16,** 605–613.