PRENATAL DIAGNOSIS
Prenat Diagn 2000; **20**: 390–394.

Maternal serum free β-hCG and PAPP-A in fetal sex chromosome defects in the first trimester

Kevin Spencer[1]*, Natasha Tul[2] and Kypros H. Nicolaides[2]

[1]*Endocrine Unit, Clinical Biochemistry Department, Harold Wood Hospital, Gubbins Lane, Romford, Essex RM3 0BE, UK*
[2]*Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, London SE5 8RX, UK*

We have studied maternal serum free β-hCG and PAPP-A, and fetal nuchal translucency (NT) in a series of 46 cases of fetal Turner's syndrome, 13 cases of other sex chromosomal anomalies and compared these with 947 control pregnancies in the first trimester. In cases of Turner's syndrome (45,X) the median fetal NT was significantly higher than in controls (4.76 MoM), the median PAPP-A was significantly lower (0.49 MoM), whilst the free β-hCG was not significantly different (1.11 MoM). For NT, 93% (43/46) of cases were equal to or greater than the 95th centile of controls, for PAPP-A 35% (16/46) of cases were less than or equal to the 5th centile of controls and for free β-hCG 15% (7/46) of cases were equal to or greater than the 95th centile of controls. For other sex chromosomal anomalies (47XXX, XXY, XYY) the median NT was increased (2.07 MoM) whilst PAPP-A was not significantly decreased (0.88 MoM) and free β-hCG was not significantly different (1.07 MoM) from controls. Using a previously derived multivariate risk algorithm for trisomy 21, incorporating NT, PAPP-A, free β-hCG and maternal age, 96% of the Turner's cases and 62% of the other sex chromosomal anomalies would have been identified. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: biochemical screening; Turner's syndrome; nuchal translucency

INTRODUCTION

First trimester screening for trisomy 21 by a combination of maternal serum free β-hCG and pregnancy associated plasma protein A (PAPP-A) and fetal nuchal translucency thickness (NT) can identify about 90% of affected pregnancies for a 5% false positive rate (Spencer *et al.*, 1999). Similarly, by combining biochemical and NT measurements it is possible to identify about 90% of trisomy 18 pregnancies for a 1% false positive rate (Tul *et al.*, 1999). The use of rapid analytical techniques for the measurement of biochemical markers has allowed the introduction of a one stop clinic for assessment of risk for fetal abnormalities (OSCAR) in which during a 1 h visit the patient undergoes pre-test counselling, biochemical and ultrasound assessment and combined risk estimation, prior to receiving post-test counselling (Spencer, 1999; Spencer *et al.*, 1999).

Fetal sex chromosome abnormalities (47,XXX; 47,XXY; 47,XYY; 45,X) are found in 2–3 per 1000 pregnancies at 10–14 weeks of gestation (Snijders *et al.*, 1995) and are the most common chromosomal abnormalities in live births . In Turner syndrome (45,X) presenting with fetal hydrops, second trimester biochemical screening has shown elevations in maternal serum levels of intact hCG (Saller *et al.*, 1992; Wenstrom *et al.*, 1994, 1996), free β-hCG (Laundon *et al.*, 1996), inhibin A (Lambert-Messerlian *et al.*, 1998) and progesterone (Lambert-Messerlian *et al.*, 1999), but it is uncertain if these changes are a consequence of the fetal hydrops rather than Turner syndrome as such (Laundon *et al.*, 1996). As far as the other sex chromosome abnormalities there is no published data but in our series of 10 cases of Kleinfelter syndrome (45,XXY), maternal serum free β-hCG was marginally increased (median MoM, 1.44; Spencer, 1999—unpublished data).

As prenatal screening for chromosomal abnormalities is clearly moving into the first trimester with a combination of fetal NT and maternal serum biochemistry, we have undertaken a study of the biochemical markers in sex chromosome disorders in order that these can be evaluated alongside the previous observation of elevated levels of NT at 10–14 weeks (Sebire *et al.*, 1998).

MATERIALS AND METHODS

The study population was derived from two groups of women. The first comprised women with singleton pregnancies who were referred to the Harris Birthright Research Centre for Fetal Medicine for fetal karyotyping, because screening by a combination of maternal age and fetal NT at 10–14 weeks in their hospital identified these patients as being at high risk for a chromosomal abnormality (Snijders *et al.*, 1998). The second group comprised self-referred women for assessment of risk by a combination of maternal age and fetal NT. Blood samples were collected from women at the time of ultrasound assessment and the serum was aliquoted and stored at −20°C prior to blinded retrospective analysis. Gestational age was determined by measurement of fetal crown–rump length (CRL). Pregnancy outcome was ascertained in all women.

Serum samples from 59 pregnancies affected by sex

*Correspondence to: K. Spencer, Endocrine Unit, Clinical Biochemistry Department, Harold Wood Hospital, Gubbins Lane, Romford, Essex RM3 0BE, UK. E-mail: Kevin_Spencer@Compuserve.com

Received: 12 October 1999
Revised: 3 February 2000
Accepted: 8 February 2000

Table 1—Median (range) for maternal age, gestational age, fetal crown–rump length, maternal weight and sample storage time in the various study groups

	Controls	45,X	47,XXX, 47,XXY, 47,XYY
Number of cases	947	46	13
Maternal age (years)	36 (15–47)	31 (16–42)	32 (26–43)
Gestational age (days)	86 (76–95)	86 (74–96)	87 (75–93)
Crown–rump length (mm)	60 (38–85)	56 (40–78)	61 (47–72)
Maternal weight (kg)	67 (38–112)	60 (41–86)	62 (50–86)
Sample storage time (days)	546 (102–1811)	768 (53–1382)	1025 (25–1380)

chromosome disorders were available for biochemical analysis. These comprised 46 cases of 45,X, nine cases of 47,XXY, two cases of 47,XYY and two cases of 47,XXX. A series of 946 samples from pregnancies resulting in the birth of an unaffected baby, which were used in a previous study (Spencer *et al*., 1999), formed the basis of the control population.

Maternal serum free β-hCG and PAPP-A were measured on the Kryptor analyser—a rapid random access immunoassay analyser using time-resolved amplified cryptate emission (TRACE) technology—and the CIS automated immunofluorescent assays (CIS UK Ltd, High Wycombe, Bucks, UK). The performance of these assays has been described before (Spencer *et al*., 1999).

Statistical analysis

All analyte measurements were converted to MoMs using the derived medians from unaffected pregnancies at the same gestation (in days) obtained from our previous study (Spencer *et al*., 1999). Correction of each MoM for maternal weight was performed using the reciprocal-linear regression weight correction procedure of Neveux *et al*. (1996). For NT the measured values were converted to MoMs using the median relationship with CRL described by Nicolaides *et al*. (1998). Statistical analysis of data was carried out using Excel and Analyse-It, a statistical software add in for Excel (Smart Software, Leeds, UK).

RESULTS

The maternal age, gestational age, fetal crown–rump length, maternal weight and sample storage time in the various study groups are shown in Table 1 and the the statistical parameters for the various marker distributions in affected pregnancies compared with those for controls established in our previous study (Spencer *et al*., 1999) are shown in Table 2.

In the 45,X pregnancies, the median fetal NT (4.76 MoM) was significantly higher than that in the control group ($p<0.0001$, Mann Whitney U test) and the NT MoM was equal to or greater than the 95th centile of the controls in 43 (93%) of 46 cases (Figure 1). The median PAPP-A (0.49 MoM) was significantly lower than that in the control group ($p<0.001$, Mann Whitney U test) and the PAPP-A MoM was equal to or less than the 5th centile of the controls in 16 (35%) of the cases (Figure 2). The median free β-hCG (1.11 MoM) was not significantly different from that of the control group ($p=0.932$, Mann Whitney U test) and the free β-hCG MoM was equal to or greater than the 95th centile of the controls in only seven (15%) of the cases (Figure 3).

In the group with 47,XXX, 47,XXY or 47,XYY, the median NT (2.07 MoM) was significantly increased ($p<0.05$, Mann Whitney U test) and the NT MoM was equal to or greater than the 95th centile in six (46%) of 13 cases (Figure 4). The median PAPP-A (0.88 MoM) was not significantly lower than that in the control group ($p=0.539$, Mann Whitney U test) and the PAPP-A MoM was equal to or less than the 5th centile in only one (8%) case. The median free β-hCG (1.07 MoM) was not significantly different from that of the control group ($p=0.610$, Mann Whitney U test) and the free β-hCG MoM was equal to or greater than the 95th centile of the controls in only 1 (8%) case.

Using a previously derived multivariate risk algorithm for trisomy 21, incorporating NT, PAPP-A, free β-hCG and maternal age (Spencer *et al*., 1999) and the *apriori* trisomy 21 maternal age risks of Snijders *et al*. (1995), 44 (96%) of the 46 cases of 45,X and eight (62%) of the 13 cases with the other sex chromosomal anomalies would have been identified using a risk cut-off of 1 in 300. If NT alone had been used the detection rate would have been 96% (44 of 46) for 45,X and 77% (10 of 13) for the other sex chromosomal anomalies.

On the basis of the maternal age distribution of the study population at 12 weeks of gestation the expected number of cases with 45,X was 59 (Snijders *et al*., 1995). The policy of screening for trisomy 21 by

Table 2—Statistical parameters for the various marker distributions in the two affected groups and the control distributions from a previous study (Spencer *et al*., 1999)

	Free β-hCG	PAPP-A	NT
Log_{10} mean controls	0.004	−0.004	0.000
Log_{10} SD controls	0.2558	0.2431	0.120
Log_{10} mean 45,X	0.0623	−0.317	0.640
Log_{10} SD 45,X	0.2965	0.2634	0.2024
Log_{10} mean others	0.006	−0.064	0.261
Log_{10} SD others	0.2879	0.3226	0.2561
Median 45,X	1.111	0.490	4.756
Median others	1.073	0.877	2.067

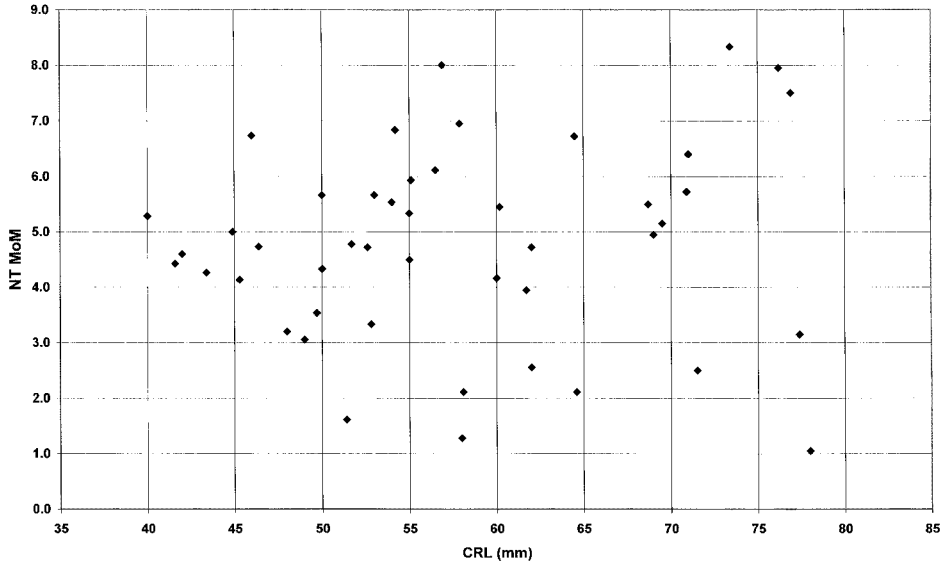

Figure 1—Nuchal translucency in 46 cases of Turner's syndrome in the first trimester

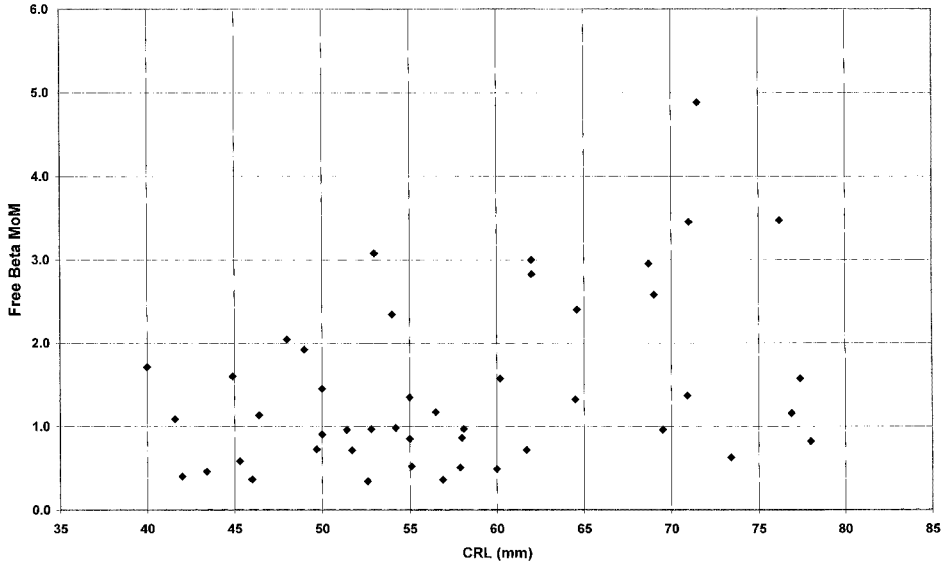

Figure 2—Maternal serum free β-hCG in 46 cases of Turner's syndrome in the first trimester

maternal age, fetal NT and maternal serum biochemistry, using a risk cut-off of 1 in 300 would detect 96% (44 of 46) of the known cases of 45,X, but on the assumption that at 12 weeks there were another 13 cases which were not identified, the actual population detection rate would be 75% (44 of 59).

DISCUSSION

The findings of this study confirm our previous observation that 45,X is associated with increased fetal NT at 10–14 weeks of gestation (Sebire *et al.*, 1998). Furthermore, the data indicate that in 45,X pregnancies maternal serum PAPP-A is significantly reduced. In contrast to a previous study of eight affected pregnancies, where maternal serum free β-hCG was increased (Spencer *et al.*, 1997), in this larger study free β-hCG was not significantly different from normal. In pregnancies with other sex chromosomal defects maternal serum PAPP-A and free β-hCG were not significantly different from normal.

An OSCAR screening programme at 10–14 weeks of gestation can identify about 90% of trisomy 21 pregnancies for a false positive rate of 5% (Spencer *et al.*, 1999). Our findings suggest that this programme can also detect more than 90% of fetuses with Turner syndrome and the majority of those with other sex chromosomal abnormalities. However, as discussed by others (Sebire *et al.*, 1998), the likely impact on live birth prevalence and the probability that NT (and biochemical screening) will identify the majority of 45,X affected fetuses at 10–14 weeks, but only about 20% of those destined to be live born, will require consideration, along with other issues, when counselling women (Christian *et al.*, 2000).

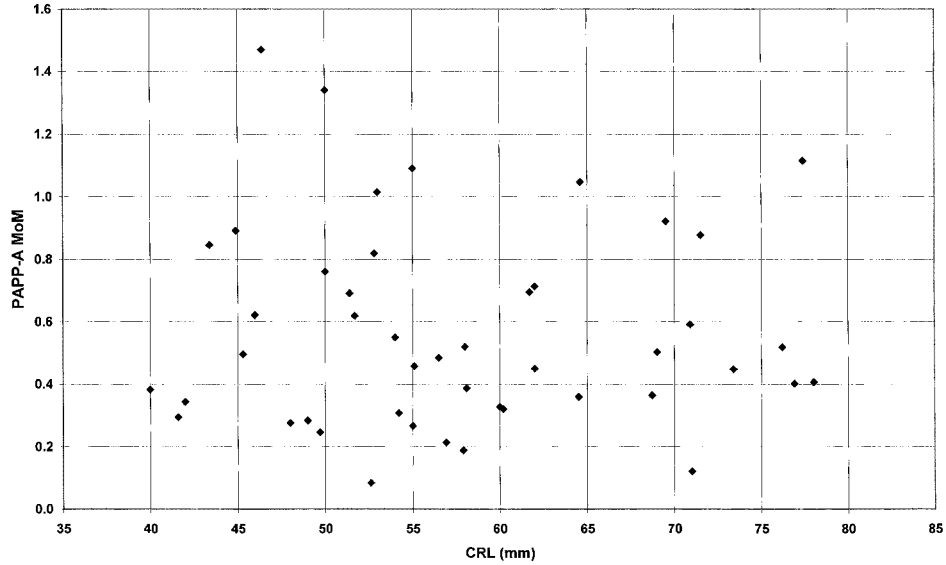

Figure 3—Maternal serum PAPP-A in 46 cases of Turner's syndrome in the first trimester

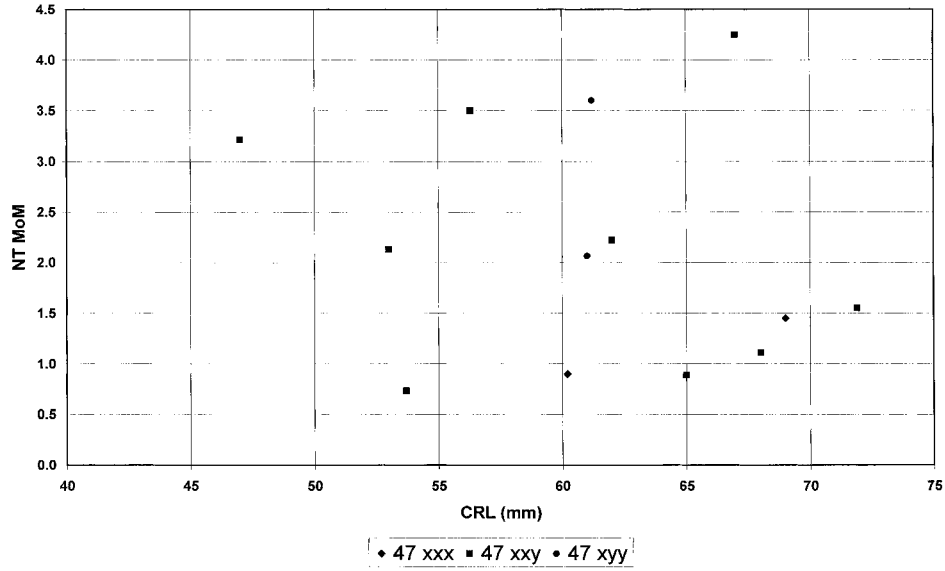

Figure 4—Nuchal translucency in cases of 47,XXX (◆), 47,XXY (■) and 47,XYY (●) in the first trimester

ACKNOWLEDGEMENTS

The work at the Harris Birthright Research Centre for Fetal Medicine is supported by a grant from the Fetal Medicine Foundation (Charity no. 1037116). We thank CIS for the provision of the Kryptor and the reagents for this study.

REFERENCES

Christian SM, Koehn D, Pillay R, MacDougall A, Wilson RD. 2000. Parental decisions following prenatal diagnosis of sex chromosome aneuploidy: a trend over time. *Prenat Diagn* **20**: 37–40.

Lambert-Messerlian GM, Saller DN, Tumber MB, French CA, Peterson CJ, Canick JA. 1998. Second-trimester maternal serum inhibin A levels in fetal trisomy 18 and Turner syndrome with and without hydrops. *Prenat Diagn* **18**: 1061–1067.

Lambert-Messerlian GM, Saller DN, Tumber MB, French CA, Peterson CJ, Canick JA. 1999. Second-trimester maternal serum progesterone levels in Turner syndrome with and without hydrops and in trisomy 18. *Prenat Diagn* **19**: 476–479.

Laundon CH, Spencer K, Macri JN, Anderson RW, Buchanan PD. 1996. Free beta-hCG screening of hydropic and non-hydropic Turner syndrome pregnancies. *Prenat Diagn* **16**: 853–856.

Neveux LM, Palomaki GE, Larivee 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.

Saller DJ, Canick JA, Schwartz S, Blitzer MG. 1992. Multiple-marker screening in pregnancies with hydropic and nonhydropic Turner syndrome. *Am J Obstet Gynecol* **167**: 1021–1024.

Sebire NJ, Snijders RJM, Brown R, Southall T, Nicolaides KH. 1998. Detection of sex chromosome abnormalities by nuchal translucency screening at 10–14 weeks. *Prenat Diagn* **18**: 581–584.

Snijders RJM, Sebire NJ, Nicolaides KH. 1995. Maternal age and gestational age specific risk for chromosomal defects. *Fetal Diagn Ther* **10**: 356–367.

Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH, for the Fetal Medicine Foundation First-trimester Screening Group. 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* **351**: 343–346.

Spencer K. 1999. One-stop clinic for assessment of risk for fetal abnormalities. *Downs Screening News* **6**: 10.

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.

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

Tul N, Spencer K, Noble P, Chan C, Nicolaides KH. 1999. Screening for trisomy 18 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* **19**: 1035–1042.

Wenstrom KD, Williamson RA, Grant S. 1994. Detection of fetal Turner syndrome with multiple marker screening. *Am J Obstet Gynecol* **170**: 570–573.

Wenstrom KD, Boots LR, Cosper PC. 1996. Multiple marker screening test: identification of fetal cystic hygroma, hydrops and sex chromosome aneuploidy. *J Matern Fetal Med* **5**: 31–35.