PRENATAL DIAGNOSIS
Prenat Diagn 2001; **21**: 718–722.
DOI: 10.1002/pd.148

First trimester maternal serum placenta growth factor (PIGF) concentrations in pregnancies with fetal trisomy 21 or trisomy 18

Kevin Spencer[1]*, Adolfo W. Liao[2], Charas Y. T. Ong[2], Lut Geerts[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, Denmark Hill, London SE5 8RX, UK*

Placenta growth factor (PIGF), an angiogenic factor belonging to the vascular endothelial growth factor family, pregnancy-associated plasma protein A (PAPP-A) and free β-human chorionic gonadotrophin (β-hCG) were measured in maternal serum from 45 pregnancies with trisomy 21, 45 with trisomy 18 and 493 normal controls at 10–13 completed weeks of gestation. In the normal pregnancies maternal serum PIGF levels increased exponentially with gestation. The median multiple of the median (MoM) PIGF concentration in the trisomy 21 group (1.26 MoM) was significantly higher ($p<0.0001$) than in the control group (1.00 MoM). In the trisomy 18 group the median PIGF was lower (0.889 MoM) but this did not quite reach significance ($p=0.064$). The corresponding median MoM values for PAPP-A were 1.00 MoM for the controls, 0.49 MoM for trisomy 21 and 0.16 MoM for trisomy 18. The median MoM values for free β-hCG were 1.00 MoM for the controls, 2.05 MoM for trisomy 21 and 0.38 MoM for trisomy 18. In the control group there was a small but significant correlation of PIGF with free β-hCG ($r=+0.1024$) and PAPP-A ($r=+0.2288$). In the trisomy 18 group there was a significant association between PIGF and free β-hCG ($r=+0.2629$) but not with PAPP-A ($r=+0.0038$). In the trisomy 21 group there was a small but significant association with PAPP-A ($r=+0.1028$) but not with free β-hCG ($r=+0.0339$). The separation of affected and unaffected pregnancies in maternal serum PIGF is small, and therefore it is unlikely that measurement of PIGF would improve screening for these abnormalities provided by the combination of fetal nuchal translucency and maternal serum PAPP-A and free β-hCG. Copyright © 2001 John Wiley & Sons, Ltd.

KEY WORDS: prenatal screening; Down syndrome; free β-hCG; PAPP-A; placental growth factors

INTRODUCTION

Angiogenesis and vascular transformation are important processes in the normal development of the placenta. Placenta growth factor (PIGF), a homodimeric glycoprotein (45–50 kDa) belonging to the vascular endothelial growth factor (VEGF) family, is a potent angiogenic factor (Ziche *et al*., 1997) and is expressed by cytotrophoblasts and syncytiotrophoblasts (Shore *et al*., 1997). In addition to its role as a paracrine angiogenic factor within the placenta, the presence of PIGF receptors on trophoblasts suggest that PIGF may also have an autocrine function in regulating trophoblast function (Khaliq *et al*., 1996). Maternal serum concentrations of placental growth factors are altered in pregnancies complicated by fetal growth restriction (Miell *et al*., 1997). Trisomy 18 is invariably associated with fetal growth restriction that is evident from the first trimester of pregnancy (Sherrod *et al*., 1997). In contrast, trisomy 21 is associated with normal fetal growth. In these chromosomal defects the maternal serum concentration of free β-hCG, both in the second and in the first trimester, is altered with values being on average twice and one-third of the normal value in trisomy 21 and trisomy 18, respectively (Spencer *et al*., 1992a,b, 1993, 1999a; Tul *et al*., 1999). Pregnancy-associated plasma protein A (PAPP-A) in maternal serum however is reduced in both trisomy 21 and trisomy 18 (Spencer *et al*., 1999a; Tul *et al*., 1999) in the first trimester. In the second trimester levels continue to fall in cases of trisomy 18 (Spencer *et al*., 1999b) whilst in trisomy 21 levels reach normal (Spencer *et al*., 1994).

PAPP-A and free β-hCG are produced by placental trophoblasts and trophoblast function may at least in part be controlled by PIGF (Khaliq *et al*., 1996). The aim of the present study was to examine maternal serum levels of PIGF in trisomy 21 and trisomy 18 pregnancies and examine the possible interrelation to free β-hCG and PAPP-A.

PATIENTS AND METHODS

At the Harris Birthright Research Centre for Fetal Medicine, London and the antenatal clinic at Harold Wood Hospital, Essex maternal serum samples from pregnancies in the first trimester (10–13 completed weeks of gestation) have been collected as part of screening for trisomy 21 and other chromosomal anomalies by a combination of fetal nuchal translucency and maternal serum free β-hCG and PAPP-A (Spencer *et al*., 1999a, 2000). All serum was aliquoted and stored at -20°C within 30 min of sample collection. From the stored database of normal pregnancies and those affected by trisomy 21 or

*Correspondence to: K. Spencer, Endocrine Unit, Clinical Biochemistry Department, Harold Wood Hospital, Gubbins Lane, Romford, Essex, RM3 0BE, UK. E-mail: KevinSpencer1@cs.com

Received: 12 January 2001
Revised: 3 April 2001
Accepted: 10 April 2001

trisomy 18, 45 cases of trisomy 21 and 45 cases of trisomy 18 were selected along with 493 control unaffected pregnancies. Data summarising the clinical details of these pregnancies were extracted from the fetal database. These cases and controls have been part of previous studies (Spencer *et al.*, 2001a,b and Spencer *et al.*, in press).

Maternal serum PIGF was measured in all samples in duplicate using a commercial quantitative enzyme immunoassay technique (R & D Systems Europe Ltd, Abingdon, UK) with cases and controls blinded to the analyst. The inter-assay precision was 7% and intra-assay precision was 6% at 80 pg/ml. At 200 pg/ml the inter-assay and intra-assay precision was 4% and at 600 pg/ml was 3%. Maternal serum PAPP-A and free β-hCG results were available from previous studies (Spencer *et al.*, 2001a and Spencer *et al.*, in press) when they were measured using the Kryptor system as previously described (Spencer *et al.*, 1999a).

Statistical analysis

Median PIGF concentrations were established across the gestational window of 10–13 completed weeks using gestational age determined by crown–rump length. All PIGF results were converted to MoMs from the specific gestational median regression formula. Statistical analysis was carried out using Analyse-It (Smart Software, Leeds, UK), a statistical software add-in for Microsoft Excel 7. The Kolmogorov–Smirnov test was used to confirm a Gaussian distribution of raw or log transformed data. The significance of marker levels in the trisomy 21 and trisomy 18 group was analysed using *t*-tests of unequal variance on Gaussian-confirmed marker distributions.

Free β-hCG and PAPP-A MoMs for cases and controls were available from previous studies (Spencer *et al.*, 2001a and Spencer *et al.*, in press).

RESULTS

Table 1 outlines the characteristics of the trisomy 21, trisomy 18 and the control group. Maternal serum PIGF levels in unaffected pregnancies increased exponentially with increasing gestation [median PIGF = 2.9257 * exp(gestation (decimal weeks) * 0.2269), $r = 0.39$, $p < 0.0001$] as shown in Figure 1. The $\log_{10}$ MoMs fitted a Gaussian distribution in the trisomy 21, trisomy 18 and the control group with a probability better than 0.01 using the Kolmogorov–Smirnov test or the Anderson–Darling test. The median MoM PIGF in the trisomy 21 group (1.257 MoM) was significantly higher ($p < 0.0001$) than in the control group (0.999 MoM). In the trisomy 18 group the median PIGF was lower (0.889 MoM) but this did not quite reach significance ($p = 0.0642$). The $\log_{10}$ mean in controls was 0.002 compared with 0.100 in the trisomy 21 group and -0.038 in the trisomy 18 group and the $\log_{10}$ SDs were 0.1855, 0.1746 and 0.1310, respectively. PIGF was above the 95th centile of normal in only 3/45 cases (7%) of trisomy 21 and below the 5th centile of normal in 1/45 cases (2%) of trisomy 18. Figures 2 and 3 show the distribution of PIGF in cases of trisomy 21 and trisomy 18 with gestational age. The median MoM values for free β-hCG were 1.00 MoM for the controls, 2.05 MoM for trisomy 21 and 0.38 MoM for trisomy 18. The corresponding median MoM values for PAPP-A were 1.00 MoM for the controls, 0.49 MoM for trisomy 21 and 0.16 MoM for trisomy 18. In the control group there was a small but significant correlation of PIGF with free β-hCG ($r = +0.1024$) and PAPP-A ($r = +0.2288$). In the trisomy 18 group there was a significant association between PIGF and free β-hCG ($r = +0.2629$) but not with PAPP-A ($r = +0.0038$). In the trisomy 21 group there was a small but significant association with PAPP-A ($r = +0.1028$) but not with free β-hCG ($r = +0.0339$).

DISCUSSION

The present study demonstrated that at 10–14 weeks, maternal serum PIGF increases with gestation and that in trisomy 21 pregnancies levels are increased, whereas in trisomy 18 levels are reduced. However, the separation of affected and unaffected pregnancies in maternal serum PIGF is small and therefore it is unlikely that measurement of PIGF would improve screening for these abnormalities provided by the combination of fetal nuchal translucency and maternal serum PAPP-A and free β-hCG (Spencer *et al.*, 1999a).

In the development of the placental vasculature, PIGF, VEGF and intraplacental oxygenation and their relative balance seem to have an important role. Ahmed *et al.* (2000) have shown that in placentae from pregnancies in which the fetus has growth restriction, the levels of expression of PIGF and VEGF are

Table 1—Characteristics of the control, trisomy 21 and trisomy 18 study populations

	Controls ($n = 493$)	Trisomy 21 ($n = 45$)	Trisomy 18 ($n = 45$)
Maternal age (years)	28.1 (SD 5.2)	36.62 (SD 5.28)	35.00 (SD 5.65)
Maternal weight (kg)	68.0 (SD 13.6)	67.5 (SD 10.6)	61.8 (SD 8.5)
Caucasian (%)	430 (87.2%)	41 (91%)	38 (84.4%)
Cigarette smokers (%)	78 (15.8%)	5 (11.1%)	8 (17.8%)
Primigravidae (%)	240 (48.7%)	13 (28.9%)	13 (28.9%)
Gestational age (weeks)	12 (SD 0.8)	12 (SD 0.6)	12 (SD 0.9)

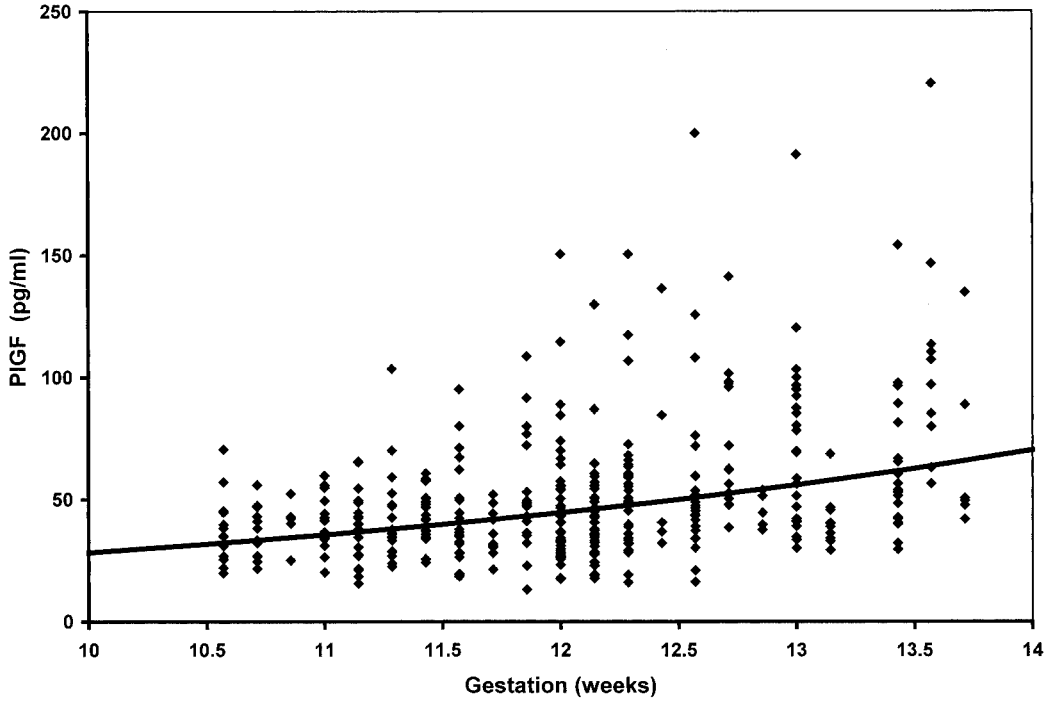

Figure 1—Distribution of PIGF in unaffected cases at 10–13 completed weeks of gestation. The solid line represents the median from the regression equation

disturbed. PIGF becomes more dominant, causing increased trophoblast proliferation and inhibiting endothelial cell growth. In pre-eclampsia, placental ischaemia is thought to be of great importance in the pathophysiology of the disease. Reduced levels of PIGF and VEGF have been reported in pregnancies with pre-eclampsia (Shore *et al.*, 1997; Reuvekamp *et al.*, 1999). It is suggested that these reduced levels might in part explain the shallow placentation described in pre-eclampsia.

In chromosomally normal fetuses with growth restriction there is Doppler evidence of impaired placental perfusion with increased impedance to flow in both the uterine and umbilical arteries. In contrast, in trisomy 18 fetuses with growth restriction placental perfusion is normal, suggesting that in this

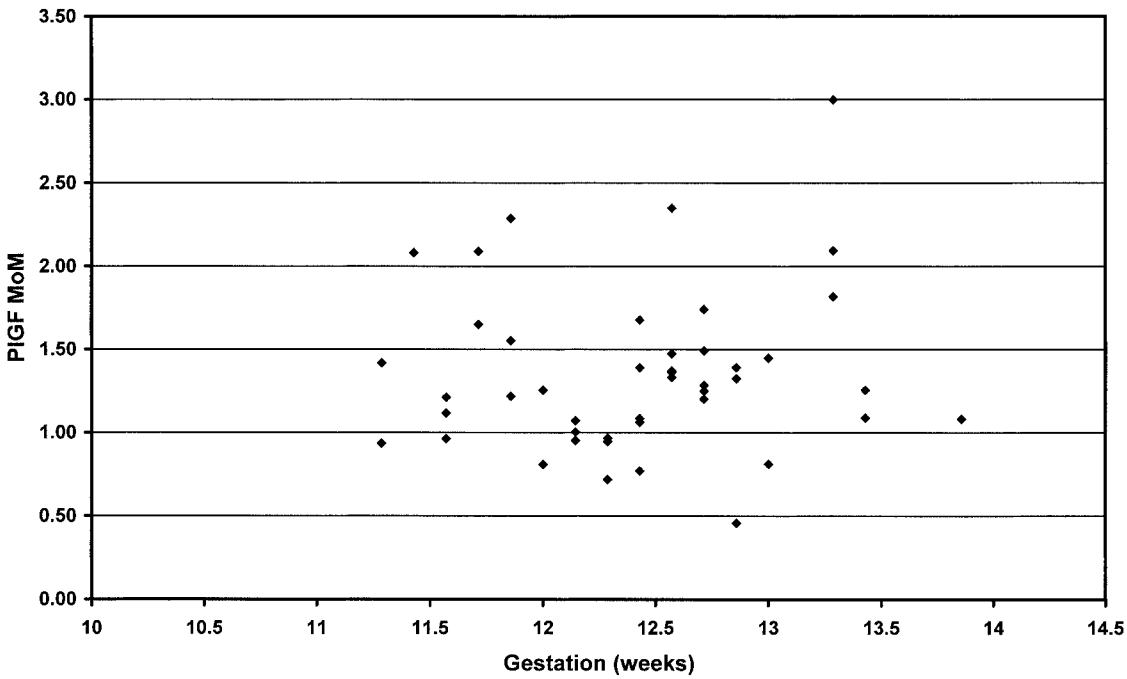

Figure 2—Distribution of PIGF MoM in 45 cases of trisomy 21 at 10–13 completed weeks of gestation

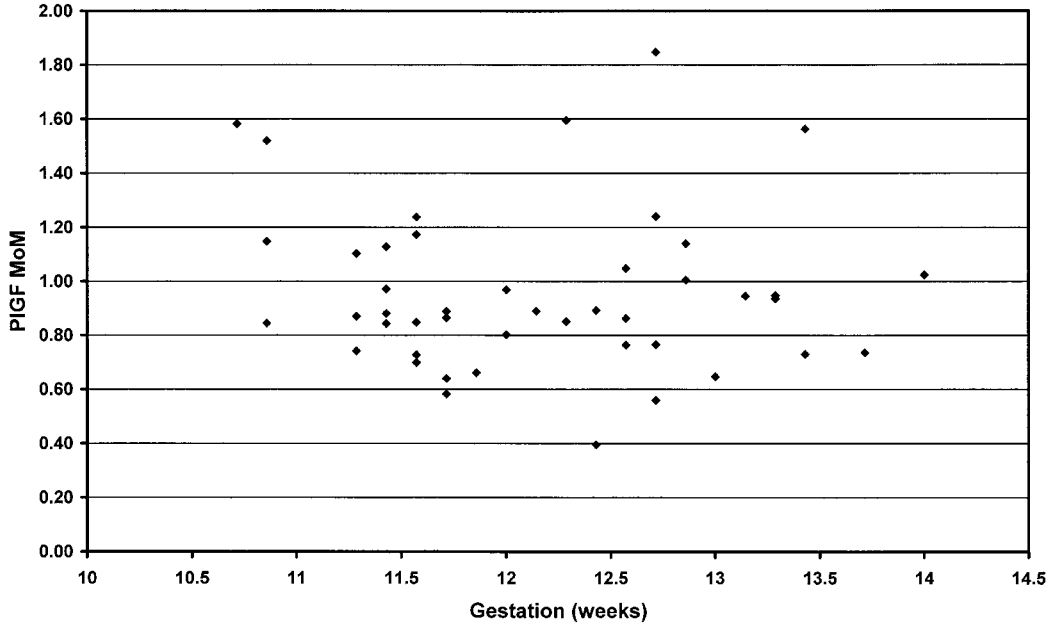

Figure 3—Distribution of PIGF MoM in 45 cases of trisomy 18 at 10–13 completed weeks of gestation

abnormality the origin of impaired growth is fetal rather than placental (Snijders *et al.*, 1993). Nevertheless it is also possible that there may be a certain degree of placental dysfunction, as reflected in the reduced levels of maternal serum PIGF. If, however, the maternal serum concentration of a placental angiogenic growth factor was a marker of placental function and fetal growth, then the increased PIGF observed in trisomy 21 pregnancies would imply that these fetuses would be macrosomic, which is not the case. A more likely explanation for the observed pattern of deviation from normal in maternal serum concentrations of PIGF in trisomies 18 and 21 is the interrelation in the control of production of PIGF, the effect this has on trophoblast function and production of PAPP-A and hCG, reflected in the significant positive associations between maternal serum levels of these substances. However, this does not explain the finding in normal pregnancy where at 10–14 weeks there is a gestation-related increase in PIGF concentration and decrease in free β-hCG.

ACKNOWLEDGEMENT

This study was supported by a grant from the Fetal Medicine Foundation (Charity No. 1037116).

REFERENCES

Ahmed A, Dunk C, Ahmad S, Khaliq A. 2000. Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF) and soluble Flt-1 by oxygen–a review. *Placenta* **21** (Suppl. A): S16–S24.

Khaliq A, Li XF, Shams M, *et al.* 1996. Localisation of placenta growth factor (PIGF) in human term placenta. *Growth Factors* **13**: 243–250.

Miell JP, Langford KS, Jones J, *et al.* 1997. The maternal IGF and IGF-binding protein response to trisomic pregnancy during the first trimester: a possible diagnostic tool for trisomy 18 pregnancies. *J Clin Endocrinol Metab* **82**: 287–292.

Reuvekamp A, Velsing-Aarts FV, Poulina IEJ, Capello JJ, Duits AJ. 1999. Selective deficit of angiogenic growth factors characterises pregnancies complicated by pre-eclampsia. *Br J Obstet Gynaecol* **106**: 1019–1022.

Sherrod C, Sebire NJ, Soares W, Snijders RJ, Nicolaides KH. 1997. Prenatal diagnosis of trisomy 18 at the 10–14-week ultrasound scan. *Ultrasound Obstet Gynecol* **10**: 387–390.

Shore VH, Wang TH, Wang CL, Torry RJ, Caudle MR, Torry DS. 1997. Vascular endothelial growth factor, placenta growth factor and their receptors in isolated human trophoblasts. *Placenta* **18**: 657–665.

Snijders RJM, Sherrod C, Gosden CM, Nicolaides KH. 1993. Severe fetal growth retardation: associated malformations and chromosomal abnormalities. *Am J Obstet Gynecol* **168**: 547–555.

Spencer K, Coombes EJ, Mallard AS, Ward AM. 1992a. Free beta human choriogonadotropin in Down's syndrome screening: a multicentre study of its role compared with other biochemical markers. *Ann Clin Biochem* **29**: 506–518.

Spencer K, Macri JN, Aitken DA, Connor JM. 1992b. Free β-hCG as first trimester marker for fetal trisomy. *Lancet* **339**: 1480.

Spencer K, Mallard AS, Coombes EJ, Macri JN. 1993. Prenatal screening for trisomy 18 with free β-hCG as a marker. *Br Med J* **307**: 1455–1458.

Spencer K, Aitken DA, Crossley JA, *et al.* 1994. First trimester biochemical screening for trisomy 21: the role of free beta hCG, alpha fetoprotein and pregnancy associated plasma protein A. *Ann Clin Biochem* **31**: 447–454.

Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. 1999a. 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 Gynaecol* **13**: 231–237.

Spencer K, Crossley JA, Green K, Worthington DJ, Brownbill K, Aitken DA. 1999b. Second trimester levels of pregnancy associated plasma protein A in cases of trisomy 18. *Prenat Diagn* **19**: 1127–1134.

Spencer K, Spencer CE, Power M, Moakes A, Nicolaides KH. 2000. 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.

Spencer K, Liao AW, Skentou H, Ong CYT, Nicolaides KH. 2001a. Maternal serum levels of total activin-A in first trimester trisomy 21 pregnancies. *Prenat Diagn* **21**: 270–273.

Spencer K, Liao AW, Ong CYT, Geerts L, Nicolaides KH. 2001b.

Maternal serum levels of dimeric inhibin A in pregnancies affected by trisomy 21 in the first trimester. *Prenat Diagn* **21**: 441–444.
Spencer K, Liao AW, Ong CYT, Flack NJ, Nicolaides KH. Maternal serum activin A and inhibin A in trisomy 18 pregnancies at 10–14 weeks. *Prenat Diagn* **21** (in press).
Tul N, Spencer K, Noble P, Chan C, Nicolaides KH. 1999. Screening for trisomy 18 by fetal nuchal translucency and maternal serum free β-hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* **19**: 1035–1042.
Ziche M, Maglione R, Ribatti D, *et al*. 1997. Placenta growth factor-1 is chemotactic, mitogenic and angiogenic. *Lab Invest* **76**: 517–531.