

Maternal serum human placental growth hormone at 11 to 13 weeks in trisomy 21 and trisomy 18 pregnancies

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Objective To investigate the maternal serum concentration of human placental growth hormone (hPGH) in trisomy 21 and trisomy 18 pregnancies at 11 to 13 weeks of gestation and to examine the possible association between fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotrophin (β -hCG) and pregnancy-associated plasma protein-A (PAPP-A).

Methods The maternal serum concentration of hPGH at 11 to 13 weeks was measured in a case–control study from 28 pregnancies with fetal trisomy 21, 28 with trisomy 18 and 112 pregnancies with euploid fetuses. The median hPGH multiple of the median (MoM) in trisomy 21 and trisomy 18 pregnancies were compared with euploid pregnancies.

Results Serum hPGH was significantly lower in trisomy 21 (0.93 MoM) and trisomy 18 (0.62 MoM) compared to euploid pregnancies (1.02 MoM). There was a significant association between serum hPGH and PAPP-A in both the euploid ($r = 0.258$, $p = 0.006$) and trisomy 21 pregnancies ($r = 0.410$, $p = 0.030$) but not in trisomy 18 pregnancies ($p = 0.445$).

Conclusion In the first trimester, serum hPGH in trisomy 21 and trisomy 18 pregnancies is reduced. This is the opposite of findings in previous studies reporting that in the second trimester, trisomy 21 and 18 pregnancies have increased hPGH. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: human placental growth hormone; first trimester screening; trisomy 21; trisomy 18; nuchal translucency; pregnancy-associated plasma protein-A

INTRODUCTION

The human placenta synthesizes human placental growth hormone (hPGH), which differs from the pituitary growth hormone by only 13 amino acids (Igout *et al.*, 1988; Scippo *et al.*, 1993; Lacroix *et al.*, 2005). The maternal serum concentration of hPGH increases with gestation and this placental product gradually replaces the secretion of the pituitary growth hormone and takes over its somatotrophic, lactogenic and lipolytic effects (Frankenne *et al.*, 1988; Alsat *et al.*, 1998; Chellakooty *et al.*, 2004; Fuglsang and Ovesen, 2006).

In pregnancies with fetal trisomy 21, the concentration of hPGH during the second trimester in both maternal serum and amniotic fluid is increased by 1.6 to 2.4-fold (Moghadam *et al.*, 1998; Baviera *et al.*, 2004; Papadopoulou *et al.*, 2008; Sifakis *et al.*, 2009). Similarly, in second trimester trisomy 18 pregnancies, the maternal serum hPGH is increased by 1.7-fold (Moghadam *et al.*, 1998).

The aim of this study was to investigate the maternal serum concentration of hPGH in trisomy 21 and trisomy 18 pregnancies at 11 to 13 weeks of gestation and to examine the possible association with fetal

nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotrophin (β -hCG) and pregnancy-associated plasma protein-A (PAPP-A).

METHODS

Study population

This was a case–control study drawn from a large prospective study to identify potential biomarkers of pregnancy complications in women attending for their routine first hospital visit in pregnancy at King's College Hospital, London, UK. In this visit, which was held at 11⁺⁰ to 13⁺⁶ weeks of gestation, all women had an ultrasound scan to: firstly, confirm gestational age from the measurement of the fetal crown-rump length (CRL); secondly, diagnose any major fetal abnormalities; and thirdly, measure fetal NT thickness as part of screening for chromosomal abnormalities. In addition, the maternal serum PAPP-A and free β -hCG are determined and the results are combined with maternal age and fetal NT to calculate the patient-specific risk for trisomy 21 (Snijders *et al.*, 1998; Kagan *et al.*, 2008a).

Maternal demographic characteristics, ultrasonographic measurements and biochemical results were recorded in a computer database. Karyotype results and details on pregnancy outcomes were added into the database as soon as they became available. Written

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informed consent was obtained from the women agreeing to participate in the study, which was approved by the King's College Hospital Ethics Committee. They agreed for aliquots of their serum used for the measurement of free β -hCG and PAPP-A to be stored at -80°C for future studies.

The case-control study population comprised of 28 cases with fetal trisomy 21, 28 cases with trisomy 18 and 112 euploid controls. Each case was matched with two controls who had blood collected on the same day, had a normal karyotype and delivered a phenotypically normal neonate. None of the samples in the case-control study were previously thawed and refrozen.

Sample analysis

Duplicate serum samples were used to measure hPGH concentration by a quantitative enzyme-linked immunoassay (ELISA) technique using DSL-10-19 200 hPGH assay (Diagnostic systems laboratories, Inc. Webster, Texas, USA). The lower limit of detection of the assay was 8 pg/mL and the average coefficient of variation in all the batches was less than 0.08%.

Statistical analysis

The distribution of maternal serum hPGH was made Gaussian using the square root transformation $Y = \sqrt{\text{hPGH}}$, and the distribution was confirmed to be Gaussian using the Kolmogorov-Smirnov test. Multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of $\sqrt{\text{hPGH}}$ in the control group. In each case and control, the measured maternal serum free β -hCG and PAPP-A were converted into MoMs after adjustment for gestation, maternal age, ethnicity, weight, parity and method of conception as previously described (Kagan *et al.*, 2008b). In each case and control, the measured NT was expressed

as a difference from the expected normal mean for CRL (delta value) (Wright *et al.*, 2008). Non-parametric analysis (Mann-Whitney U-test) was used to compare median values of hPGH, free β -hCG, PAPP-A and delta NT in trisomy 21 and trisomy 18 pregnancies with euploid controls. Regression analysis was used to determine the significance of association between hPGH MoM with free β -hCG MoM, PAPP-A MoM and delta NT in the outcome groups.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL) was used for all data analyses.

RESULTS

The maternal characteristics of the trisomy 21, trisomy 18 and euploid pregnancies are compared in Table 1.

Multiple regression analysis in the euploid pregnancies demonstrated that for $\sqrt{\text{hPGH}}$ significant independent contributions were provided by fetal CRL but not by maternal age ($p = 0.631$), racial origin ($p = 0.358$), maternal weight ($p = 0.596$), smoking ($p = 0.810$) or method of conception ($p = 0.337$):

$$\text{Expected } \sqrt{\text{hPGH}} = 24.227 + 0.287$$

$$\times \text{fetal CRL in mm; } R^2 = 0.051, p = 0.010.$$

In each patient we used this formula to derive the expected $\sqrt{\text{hPGH}}$ and then expressed the observed value as a MoM of the expected.

In trisomy 21 and trisomy 18, compared to the euploid controls, maternal serum hPGH and PAPP-A were significantly decreased and serum free β -hCG and delta NT were increased (Table 2). The median serum hPGH MoM did not significantly change with fetal CRL in trisomy 21 ($p = 0.466$) and trisomy 18 ($p = 0.686$) pregnancies (Figure 1).

In the euploid pregnancies there was a significant association between maternal serum hPGH and PAPP-A ($r = 0.258$, $p = 0.006$) but not with serum free

Table 1—Maternal characteristics in the trisomy 21, trisomy 18 and euploid pregnancies

Maternal characteristics	Euploid ($n = 112$)	Trisomy 21 ($n = 28$)	Trisomy 18 ($n = 28$)
Maternal age in years, median (range)	35.9 (32.5–40.3)	37.3 (34.4–39.6)	39.0 (36.0–40.6)
Maternal weight in kg, median (range)	63.0 (57.4–73.6)	67.5 (60.0–74.3)	73.3 (65.3–80.8)*
Crown-rump length in mm, median (range)	69.1 (63.5–76.1)	62.7 (59.3–70.5)*	58.9 (55.2–65.0)*
Racial origin			
White, n (%)	95 (84.8)	25 (89.3)	24 (85.7)
Black, n (%)	7 (6.3)	2 (7.1)	0
Indian or Pakistani, n (%)	5 (4.5)	1 (3.6)	3 (10.7)
Chinese or Japanese, n (%)	4 (3.6)	0	0
Mixed, n (%)	1 (0.9)	0	1 (3.6)
Cigarette smoker, n (%)	11 (9.8)	3 (10.7)	2 (7.1)
Conception			
Spontaneous, n (%)	88 (78.6)	21 (75.0)	20 (71.4)
Ovulation drugs, n (%)	21 (18.8)	6 (21.4)	8 (28.6)
In vitro fertilization, n (%)	3 (2.7)	1 (3.6)	0

Comparisons with controls (Chi-square test and Fisher exact test for categorical variables and Mann-Whitney U-test for continuous variables): * $p < 0.05$.

Table 2—Median (interquartile range) of maternal serum hPGH MoM, free β -hCG MoM, PAPP-A MoM and delta NT thickness in trisomy 21, trisomy 18 and euploid pregnancies

	Euploid ($n = 112$)	Trisomy 21 ($n = 28$)	Trisomy 18 ($n = 28$)
Serum hPGH (median, IQR)			
MoM	1.02 (0.75–1.35)	0.93 (0.54–1.19)*	0.62 (0.48–1.02)*
pg/mL	1951.4 (1455.4–2714.5)	1667.5 (989.5–2123.0)	1052.2 (800.7–1700.4)
Serum free β -hCG (median, IQR)			
MoM	1.07 (0.74–1.65)	2.64 (1.78–3.69) [†]	0.20 (0.14–0.27) [†]
Unit	40.3 (27.5–53.4)	103.8 (56.1–146.4)	7.10 (5.10–11.5)
Serum PAPP-A (median, IQR)			
MoM	1.00 (0.49–1.56)	0.43 (0.28–0.65) [†]	0.20 (0.15–0.25) [†]
mU/L	3.33 (1.58–5.02)	1.09 (0.65–1.95)	0.36 (0.28–0.52)
Delta NT (median, IQR)			
Delta value	0.4 (–0.04 to 1.0)	2.2 (1.0–4.3) [†]	4.4 (0.7–6.8) [†]
Observed value	2.1 (1.7–2.8)	3.8 (2.8–6.0)	6.1 (2.4–8.2)

Comparisons with controls: Mann–Whitney U-test: * $p < 0.05$, [†] $p < 0.0001$.

hPGH, human placental growth hormone; MoM, multiple of the median; β -hCG, beta-human chorionic gonadotrophin; PAPP-A, pregnancy associated plasma protein-A; NT, nuchal translucency.

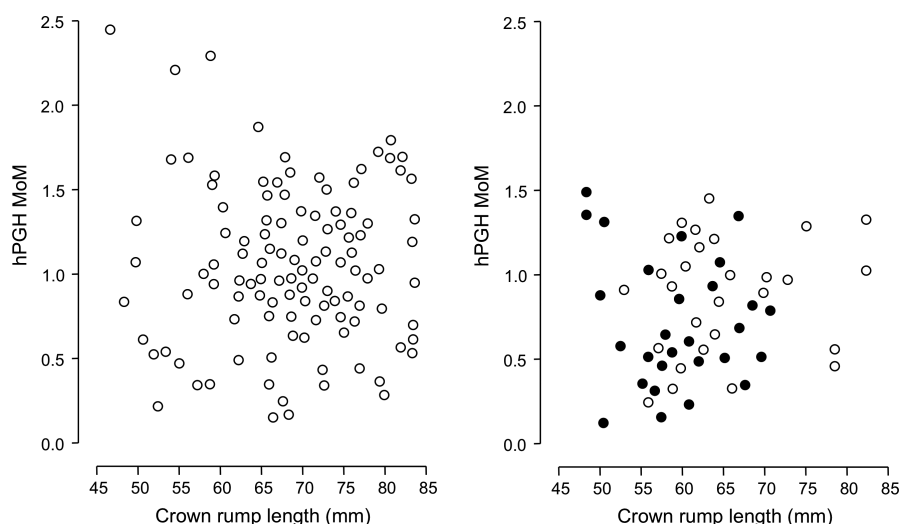


Figure 1—Relationship of human placental growth hormone (hPGH) multiple of the median (MoM) with fetal crown-rump length (CRL) in euploid pregnancies (left) and aneuploid pregnancies (right). The open circles on the right represent the results of trisomy 21 and the closed circles represent the results of trisomy 18

β -hCG ($p = 0.160$) and delta NT ($p = 0.340$). Similarly, in the trisomy 21 pregnancies there was a significant association between maternal serum hPGH and PAPP-A ($r = 0.410$, $p = 0.030$) but not with serum free β -hCG ($p = 0.754$) and delta NT ($p = 0.705$). In trisomy 18 pregnancies, there was no significant association between serum hPGH and PAPP-A ($p = 0.445$), free β -hCG ($p = 0.095$) or delta NT ($p = 0.227$).

DISCUSSION

The findings of this study demonstrate that in trisomy 21 and trisomy 18 pregnancies the maternal serum concentration of hPGH at 11 to 13 weeks of gestation is reduced. This is in contrast to the findings from second-trimester studies which reported increased

levels of hPGH in trisomy 21 and trisomy 18. (Moghadam *et al.*, 1998; Baviera *et al.*, 2004; Papadopoulou *et al.*, 2008; Sifakis *et al.*, 2009). In trisomy 21 pregnancies, the difference between affected and euploid pregnancies is small and there is a significant association between hPGH and PAPP-A. It is, therefore, unlikely that inclusion of this placental product in the existing first trimester combined screening by maternal age, fetal NT and serum free β -hCG and PAPP-A would improve the 85% to 90% detection at the false-positive rate of less than 5% (Kagan *et al.*, 2008a).

The lower maternal serum levels of hPGH in trisomy 21 pregnancy is compatible with the findings of Frenzo *et al.* (2000), who demonstrated that placental production and secretion of hPGH in affected pregnancies is dramatically decreased. There is no obvious explanation for the results of previous studies that in trisomy 21 pregnancies during the second trimester, the

concentration of hPGH in both maternal serum and amniotic fluid is increased (Moghadam *et al.*, 1998; Baviera *et al.*, 2004; Papadopoulou *et al.*, 2008; Sifakis *et al.*, 2009). The serum concentration of several placental products, including PAPP-A, hCG and ADAM 12, in trisomy 21 relative to euploid pregnancies changes between the first and second trimester (Christiansen *et al.*, 2004; Donalson *et al.*, 2008; Spencer *et al.*, 2008). It is possible that this change is the consequence of altered placental production with gestation or post-transcriptional modification of the placental products. Frendo *et al.* (2000) highlighted this paradox in the case of hCG of reduced placental production (Brizot *et al.*, 1995) and increased maternal serum levels, and suggested that the latter may be a consequence of post-transcriptional switch from glycosylated to a hyperglycosylated form which has a different half-life. The same may also be true for hPGH which has also been reported to exist in glycosylated and non-glycosylated isoforms (Fuglsang and Ovesen, 2006).

The maternal serum hPGH levels in trisomy 21 and trisomy 18 pregnancies did not change significantly with fetal CRL within the narrow gestational window of 11 to 13 weeks. Nevertheless, since the levels are lower at this gestation and higher in later pregnancy, it is possible that like PAPP-A and ADAM-12, the maternal serum concentration of hPGH at 8 to 10 weeks may be substantially lower than euploid pregnancies. In such case, measurement of serum hPGH at 8 to 10 weeks could potentially be useful in improving screening for these chromosomal abnormalities. The extent to which this will prove to be the case remains to be determined.

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