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First-trimester maternal placental protein 13 levels in pregnancies resulting in adverse outcomes

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Background In a previous study, reduced levels of maternal serum placental protein 13 (PP13) in the first trimester have been correlated with adverse pregnancy outcomes. The objective of this study was to compare first-trimester PP13 levels in control pregnancies with pregnancies resulting in one or more of the following adverse outcomes: intrauterine growth restriction (IUGR), small and very small (3rd, 5th, 10th centile) for gestational age (SGA), low (<1.5 and <2.5 kg) birth weight (LBW), macrosomia (the >90th centile), large birth weight (>4.5 kg), preterm (35–36 weeks) and very early (<34 weeks) delivery (PTD), and intrauterine fetal demise (IUFD).

Methods Maternal serum samples from 1940, 11 to 14 weeks singleton pregnancies, were assayed for PP13 and the concentrations were corrected for gestational age, maternal weight, smoking status, and ethnic origin. A comparison of the levels of PP13 in 364 controls and 1576 adverse outcome categories was made. PP13 levels were expressed in terms of both concentration and multiple of medians (MoMs).

Results Comparison of PP13 MoMs from SGA, PTD, and low birth weight samples with control pregnancy samples showed no statistically significant difference. In macrosomic pregnancies (>90th centile), levels of PP13 were significantly higher than controls ($p = 0.0263$) although the number of cases in this study was small.

Conclusion Decreased levels of PP13 were not significantly correlated with the studied adverse pregnancy outcomes of IUGR, PTD low birth weight, and IUFD. Further studies are required to evaluate if measurement of PP13 has any value in the early assessment of pregnancies. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: intrauterine growth restriction; preterm delivery; fetal death; low birth weight; macrosomia

INTRODUCTION

Adverse pregnancy outcomes caused by abnormal placental development include intrauterine growth restriction (IUGR), small for gestational age (SGA), low birth weight (LBW), and preterm delivery (PTD). These are not only the key causes of perinatal and maternal morbidity and mortality, but, when related to placental insufficiency during pregnancy, they may also be linked to childhood and adult disease later on in life, including noninsulin dependent diabetes mellitus and hypertension (Barker *et al*., 1993; Newnham, 1998; Rich-Edwards *et al*., 1999).

Accurate early pregnancy markers for placental dysfunction that predict impending adverse outcomes would allow efficient clinical management and surveillance of women at risk, thereby reducing the gravity of these conditions. Potential early pregnancy markers under investigation include Doppler ultrasound of the uterine artery in the first trimester (Dugoff *et al*., 2005; Spencer *et al*., 2005), decreased first-trimester pregnancy associated plasma protein - A (PAPP-A) (Dugoff *et al*., 2004; Spencer *et al*., 2005; Cowans and Spencer, 2007), and a disintegrin and metaloprotease (ADAM12s) (Cowans and Spencer, 2007).

Placental protein 13 (PP13) is a 32-kDa homodimeric placental protein believed to be involved in placental implantation and maternal artery remodeling (Burger *et al*., 2004). Median first-trimester maternal serum PP13 levels have been shown to be reduced in pregnancies resulting in preeclampsia, in particular, early preeclampsia (Burger *et al*., 2004; Nicolaides *et al*., 2006) although the size of this decrease varied with different studies (Chafetz *et al*., 2007; Spencer *et al*., 2007). The effect on IUGR was less significant, especially for IUGR cases not associated with preeclampsia (Chafetz *et al*., 2007). A recent study has shown that first-trimester maternal serum PP13 have markedly reduced MoMs of 0.2, 0.6, and 0.6 in preeclampsia, IUGR, and PTD, respectively, compared with a control MoM of 1.0 (Chafetz *et al*., 2007).

This nested case-control study performed here aims to compare first-trimester maternal serum PP13 in control pregnancies with pregnancies that went on to develop one or more of the following adverse outcomes: SGA (<3rd, <5th, and <10th centiles), macrosomia (>90th centile), LBW (<1.5 kg and <2.5 kg), large birth weight (>4.5 kg), PTD (24–34 and 35–36 weeks), and also intrauterine fetal demise (IUFD).

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MATERIALS AND METHODS

Maternal serum samples were collected at a routine first-trimester prenatal screening clinic at Harold Wood Hospital, Essex, as part of a one stop clinic for assessment of risk (OSCAR) for fetal anomalies. Ethics approval and patient consent were obtained for research to be carried out on the remaining excess serum that was frozen at −20 °C for further analysis.

Demographic, biochemical, and ultrasound data for a range of patients with a singleton pregnancy, screened between 11 and 14 weeks of gestation were extracted from the fetal database (ViewPoint, Weßling, Germany). Pregnancy outcome data were obtained from the cytogenetics laboratories, national chromosomal anomaly register, the patient themselves, their general practitioners, or the maternity unit. Data were also obtained from the hospital midwifery and patient administration databases and matched to the prenatal screening records by a locally derived record linkage software.

The sample group was created by searching the database for first-trimester singleton pregnancies screened between September 1999 and August 2003 in which one or more of the following pregnancy complications occurred: IUFD at or after 24 weeks, PTD (subclassified as between 24 and 34 weeks and between 35 and 36 weeks), very low birth weight (<1.5 kg), low birth weight (<2.5 kg), large birth weight (>4.5 kg), birth weight below the 3rd centile, birth weight below the 5th centile, birth weight below the 10th centile, and birth weight above the 90th centile for gestational age (Yudkin *et al*., 1987). There was insufficient data in all pregnancies to produce individual customized growth measures (De Jong *et al*., 2000). One control in which the pregnancy resulted in the delivery of a live karyotypical normal baby, unaffected by hypertensive disorders or any of the conditions under investigation, was selected for every four test cases, matched for length of sample storage and gestational age with the cases. The same sample group was used in a previous study (Cowans and Spencer, 2007).

The study population comprised a total of 1940 cases. Of these, 1576 (81.2%) fell into one or more of the adverse outcome categories and the remaining 364 (18.8%) acted as controls. Classification by specific pregnancy complication resulted in some pregnancies being classified in more than one group, thus, for example, pregnancies with a birth weight of 1.2 kg would be classified in both the <1.5 kg and <2.5 kg groups and those with a birth centile of 2% would appear in the <3rd, <5th, and <10th centile groups.

Serum PP13 levels were determined using a solid phase sandwich enzyme linked immunosorbent assay (ELISA) kit (Diagnostic Technologies, Haifa, Israel) described previously (Burger *et al*., 2004). Briefly, standards, controls, and samples were loaded in duplicate onto microtitre plates coated with monoclonal antibody (Mab) 27-2-3 and incubated overnight. After washing three times with PBS-Tween, biotin-conjugated MAb 215-28-3 was added for 1 h 30 min. After a further washing step, horse radish peroxidase (HRP) was added and incubated for 40 min. Following a final washing step, the wells were developed with tetramethyl-benzidine (TMB) for up to 25 min, then stopped with hydrochloric acid. The optical density was measured at 450 nm (with a background reading at 620 nm), and translated to a PP13 concentration using a calibration curve derived from recombinant PP13. Results with coefficients of variance (CV) above 10% were repeated; following this, results with CV above 15% were rejected. All samples were analyzed blind to the researcher.

Raw PP13 concentrations were converted to multiples of the median (MoM) by dividing each individual result by the expected median marker in a control pregnancy at that gestational day, calculated using the control group medians from this study population. To account for hemodilution, the control group was clustered into 10 kg bands by maternal weight, and the median log PP13 MoM for each cluster was plotted against the median weight for each cluster to derive an equation to determine the weight corrected MoM, as outlined previously (Spencer *et al*., 2003).

While the distribution of PP13 values is not Gaussian either as raw values or after taking MoM and adjustment to various confounders, PP13 MoM values were $\log_{10}$ transformed and tested for a Gaussian distribution using the Kolmogorov–Smirnov goodness of fit test. The analysis showed a good fit to a Gaussian distribution [Shapro–Wilk coefficient 0.9974 ($p < 0.0001$) in the controls]. PP13 was compared in each test group against the control group. Following this, the effect of ethnic origin and smoking status were examined, and the data was reanalyzed following correction for these factors where necessary.

Parametric two-tailed Student's *t*-tests were used to compare PP13 MoM $\log_{10}$ transformed means. Where this was not appropriate, the Mann–Whitney test was used to compare PP13 MoM medians. Chi-squared tests were used to explore differences in proportions of ethnic origin and smoking status in test groups compared to the controls. All statistical analyses were performed using Microsoft Excel, Analyse-It (Analyse-It, Leeds, UK) and SPSS (SPSS Inc, Chicago, Illinois).

RESULTS

Demographics

Tables 1 and 2 show the demographic data for the control and each adverse outcome group along with the *p* statistic for Mann–Whitney or Chi-squared tests. Not all demographic data were available: maternal age was documented in all records, parity in 42.2% of the records, BMI in 38.7%, and smoking status in 98.4% of the records.

A significantly lower maternal BMI was found in SGA pregnancies and a significant increase was found in macrosomic pregnancies. Smoking prevalence was significantly higher in IUFD, LBW (<2.5 kg), and SGA.

When the distribution of ethnic origins in the control group and in each disorder group were examined there

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Table 1—Demographics; Maternal age, parity, BMI and smoking status distributions, with p statistic from Mann–Whitney test for each group of adverse outcomes, compared to the control pregnancy group

	n	Maternal age	Parity	BMI	% Smoke
Control	364	28.75	0.00	23.80	15.93
IUFD	56	29.63	0.00	24.20	30.36*
24–34 weeks	297	29.95**	0.00	25.35*	22.22
35–36 weeks	485	29.61	1.00	24.20	18.14
<1.5 kg	114	30.31*	0.00	26.40	22.81
<2.5 kg	724	29.28	0.00	23.70	25.28**
>4.5 kg	5	32.04*	0.50	28.40	0.00
<3rd	284	28.93	0.00	22.80*	29.23***
<5th	488	28.51	0.00	22.90*	27.46**
<10th	860	28.61	0.00	23.00*	25.58**
>90th	123	30.03***	1.00	25.90**	17.07

Asterisks denote statistical significance $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

was a statistically significant difference in ethnic group distributions when compared to the control group with the disorders IUFD, 24 to 34 week PTD, LBW, <10th centile SGA, and in macrosomic pregnancies.

Gestational age, maternal weight, and other marker corrections

As shown in Figure 1, from 11 to 14 weeks, there was a very small correlation of 0.23 ($p = 0.31$) with a small increase in control group maternal serum PP13 with gestational age, which loosely fitted the linear equation. The linear equation found best to fit this had a r^2 value of 0.035. However, MoM values were calculated using the expected PP13 concentrations at each gestational age derived from the following formula.

$$\text{MoM} = \text{PP13 concentration (pg/mL)}/$$
$$[1.8689 \times \text{gestational age (days)} + 28.481]$$

Log PP13 MoMs were found to be correlated to maternal weight (correlations of -0.14 ($p < 0.01$), as shown in Figure 2, and the log-linear equation was used to correct for this:

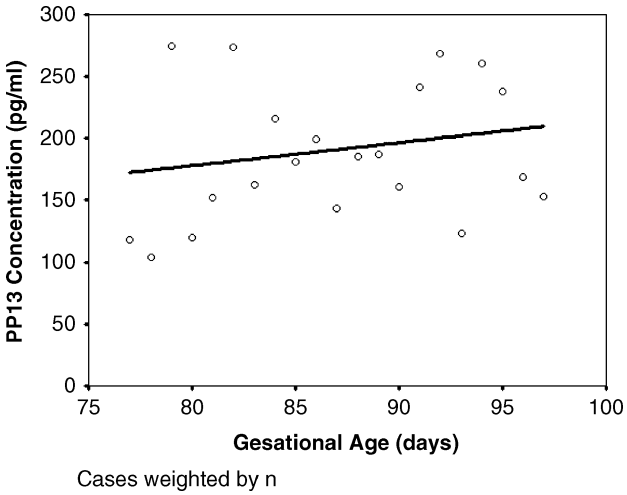

Figure 1—Variation of PP13 through 11to 14 weeks gestational age. Median PP13 concentration per gestational day, weighted by number of cases (shown). Linear regression ($r^2 = 0.035$). MoM = PP13 concentration/[1.8689 × gestational age (days) + 28.481]

$$\text{Corrected MoM} = \text{MoM}/10\ ^{\wedge}$$
$$\{0.2531 - [0.004 \times \text{weight (kg)}]\}$$

The control population was studied to see if smoking and ethnicity had an effect on PP13 levels. Nonsmokers had a median weight corrected PP13 MoM of 1.04, which decreased to 0.87 in smokers (mean log MoM 0.001 vs -0.090, $p = 0.0750$). Table 3 shows the effect of ethnicity on PP13 marker levels. The variation with ethnicity was not statistically significant, although some of the groups had small numbers and the change in Afro-Caribbean PP13 levels may become significant in a larger study group.

Adverse outcomes

Table 4 shows the median corrected MoM and p statistic from the Kologmogorov–Smirnov test, to determine

Table 2—Demographics; Percentage distributions of ethnic origins with p statistic from Chi-squared test for each group of adverse outcomes, compared to the control pregnancy group

	n	Caucasian	Afro-Caribbean	Asian	Other	Unknown	P
Control	364	72.80	4.40	17.86	4.95	0.00	
IUFD	56	67.86	10.71	8.93	12.50	0.00	**0.0126**
24–34 weeks	297	72.39	11.45	13.47	2.69	0.00	**0.0020**
35–36 weeks	485	74.64	6.39	14.02	4.95	0.00	0.3149
<1.5 kg	114	63.16	14.91	17.54	4.39	0.00	**0.0017**
<2.5 kg	724	66.02	8.70	20.44	4.70	0.14	**0.0340**
>4.5 kg	5	100.00	0.00	0.00	0.00	0.00	0.6023
<3rd	284	66.90	8.45	20.07	4.58	0.00	0.1386
<5th	488	66.80	7.58	21.31	4.30	0.00	0.1153
<10th	860	63.72	8.02	23.26	4.88	0.12	**0.0095**
>90th	123	79.67	8.94	6.50	4.88	0.00	**0.0077**

Bold denotes statistical significance.

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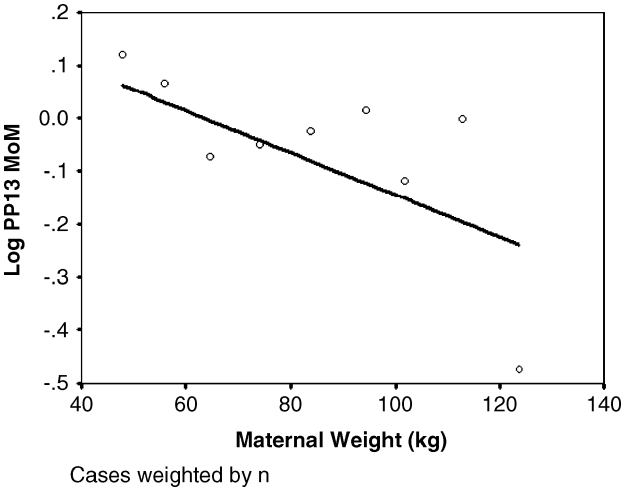

Figure 2—Variation of log PP13 MoM with maternal weight. Median log PP13 MoM per 10 kg band, weighted by number of cases (shown). Linear regression ($r^2 = 0.413$). Corrected MoM = MoM/{10 ^ [−0.004 × weight (kg) + 0.2531]}

Table 3—The effect of ethnicity on PP13 marker levels

Ethnicity	Median weight corrected MoM	Mean log MoM	*P*
All	1.00	−0.014	
Caucasian	1.00	−0.013	0.9763
Asian	1.08	−0.024	0.8392
Afro-Caribbean	0.58	−0.133	0.1988
Other	1.41	0.114	0.1412

parametric or nonparametric nature of each distribution, and of either the *t*-test or Mann–Whitney test for each group versus the control's log PP13 MoM. Since PP13 concentration was shown to be affected by smoking status and possibly ethnic origin, the data are shown after correction for this, gestational age, and maternal weight.

First-trimester median PP13 MoM maternal serum levels in pregnancies that resulted in birth weights in the lowest 3rd, 5th and 10th centiles for their gestational age were all slightly lower than the controls, although the difference was not significant. Low birth weight pregnancies also had slightly lower first-trimester maternal median PP13 MoM, although again, not significantly. When we reexamined the data by comparing those with SGA delivered at term and those delivered preterm (prior to 37 weeks) versus controls, again there was no significant difference in PP13 MoM. Pregnancies that resulted in macrosomic newborns (>90th centile) had a significantly larger median PP13 MoM, both before and after the demographic corrections (Table 4). There was no difference in PP13 MoMs for pregnancies that resulted in PTD or IUFD when compared with controls.

DISCUSSION

The demographic data relating to the current data set have been previously discussed (Cowans and Spencer,

Table 4—Median PP13 MoM and mean log PP13 MoM values in each group of adverse outcomes (after correction for gestational age, maternal weight, smoking status, and ethnicity), with the *p* statistic from either Student's *t*-test or Mann–Whitney test, compared with the control pregnancy group. Bold denotes statistical significance

	All	Control	IUFD	24–34 weeks	35–36 weeks	<1.5 kg	<2.5 kg	>4.5 kg	<3rd	<5th	<10th	>90th
Median PP13 MoM	1.096	1.083	1.053	1.141	1.111	0.956	1.057	0.914	0.978	1.058	1.051	1.288
Mean log PP13 MoM	0.021	0.005	0.096	0.050	0.034	−0.007	0.022	−0.103	−0.009	0.002	0.003	0.068
SD log PP13 MoM	0.378	0.355	0.306	0.375	0.341	0.340	0.371	0.252	0.390	0.428	0.406	0.354
Kolmogorov–Smirnov (*p*)		0.1104	0.0682	0.0216	>0.15	0.0912	0.0126	>0.15	0.0302	<0.01	<0.01	<0.01
t-test/Mann–Whitney (*p*)		n/a	0.1461	0.1187	0.2422	0.7449	0.6004	0.3942	0.6044	0.9213	0.9306	**0.0263**

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2007). Briefly, PTD and IUGR pregnancies were found to be associated with higher maternal age, as previously described (Newburn-Cook and Onyskiw, 2005). Macrosomic pregnancies were linked to increased maternal BMI, and SGA pregnancies with decreased maternal BMI, as previously found (Wolfe *et al*., 1991). The incidence of smoking was significantly higher in IUFD and IUGR pregnancies, as expected (Bernstein *et al*., 2005; Vielwerth *et al*., 2007).

Median maternal serum PP13 in the control group increased slightly during 11 to 14 weeks of gestation. Previous reports have also shown a small but significant increase in PP13 levels in control pregnancies during this gestational window (Nicolaides *et al*., 2006; Spencer *et al*., 2007). Although only a loosely fitting linear regression was found, since PP13 has been shown to increase throughout the whole of pregnancy (Burger *et al*., 2004), it was decided to correct for gestational age in this study. We also corrected PP13 for smoking status and ethnic origin, although the latter showed no statistically significant effect.

First-trimester IUGR and low birth weight median PP13 MoMs appeared slightly lower than the controls. However, there were no statistically significant differences between PP13 concentrations in IUGR, low birth weight, nor PTD pregnancies. This conflicts with the findings of Chafetz *et al*. (2007), who reported median MoMs of 0.6 for both IUGR ($n = 42$, <5th centile) and PTD ($n = 46$) ($p < 0.01$ compared with controls). Comparing raw data, we found no significant variation in PP13 levels in IUGR ($n = 488$, <5th centile) nor PTD ($n = 297$, 24–34 weeks, $n = 485$, 35–36 weeks; Table 4), whereas Chafetz *et al*. found PP13 in the first trimester to be 132.5 pg/mL in control pregnancies but 86.6 pg/mL in IUGR and 84.9 pg/mL in PTD.

Interestingly, macrosomic pregnancies (>90th centile) had significantly higher levels of PP13 in the first trimester. Macrosomia increases the risk of birth trauma, therefore, an early marker is also of potential use. If PP13 is involved in implantation and maternal artery remodeling, it is possible that when levels are higher than normal this leads to mechanisms that result in macrosomia.

Our data showing increased PP13 in macrosomic pregnancies support its proposed role in fetal growth and development. However, we found the decrease in PP13 levels in IUGR pregnancies to be statistically insignificant, in disagreement with the findings of Chafetz *et al*., (2007). Therefore, we believe that further studies are required to determine the true worth of PP13 as a marker for IUGR and PTD.

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