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Maternal serum placental protein 13 at 11–13 weeks of gestation in preeclampsia

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Objective To examine the potential value of maternal serum concentration of placental protein 13 (PP13) at 11–13 weeks' gestation in screening for preeclampsia (PE).

Methods Serum PP13, PAPP-A and uterine artery pulsatility index (PI) were determined in a case–control study of 208 cases that developed PE including 48 that required delivery before 34 weeks (early-PE) and 416 unaffected controls.

Results Serum PP13 levels, expressed as multiples of the median (MoM) in the unaffected group, were significantly reduced in early-PE (0.83 MoM) but not in late-PE (0.96 MoM). In both early- and late-PE serum PAPP-A (0.55 and 0.84 MoM) was reduced and uterine artery PI (1.61 and 1.25 MoM) was increased. In PE pregnancies there was a significant association between serum PP13 and both uterine artery PI and serum PAPP-A ($p < 0.0001$ for both). Logistic regression analysis demonstrated that serum PP13 did not improve significantly the prediction of early-PE provided by a combination of maternal factors, uterine artery PI and PAPP-A.

Conclusion PP13 is implicated in the pathogenesis of impaired placentation and subsequent development of early-PE but measurement of this placental product is unlikely to be useful in screening for the disease at 11–13 weeks. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: preeclampsia; placental protein 13; first trimester; uterine artery Doppler; screening; foetal and placental pathology

INTRODUCTION

Placental protein 13 (PP13) is a protein dimer produced by the trophoblast thought to be involved in normal placentation (Than *et al*., 1999, 2004; Burger *et al*., 2004). There is evidence from studies in the first-trimester of pregnancy that the maternal serum concentration of PP13 is reduced in women who subsequently develop preeclampsia (PE) (Table 1). However, the number of PE cases in these studies varied from 4 to 88, and the reported levels of PP13 in PE cases ranged from 0.07 MoM (multiples of the median) to 0.69 MoM. In all the studies PP13 analysis was done by solid-phase sandwich enzyme linked immunosorbent immunoassay (ELISA).

The PP13 assay has now been automated using the DELFIA (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) technique. The aim of this study was to analyse the maternal serum concentration of PP13 at 11–13 weeks of gestation in a large group of pregnancies that subsequently developed PE using the automated PP13 immunoassay, and to examine whether any possible differences in levels from normal controls are related to uterine artery pulsatility index (PI) and maternal serum pregnancy associated plasma protein-A (PAPP-A) which are markers of impaired placentation.

METHODS

Study population

This was a case–control study drawn from a large prospective study for hypertensive complications of pregnancy in women attending for their routine first hospital visit in pregnancy at King's College Hospital, London, UK. In this visit, which is held at 11 to 13 weeks and 6 days of gestation, all women have an ultrasound scan to firstly, confirm gestational age from the measurement of the foetal crown-rump length (CRL), secondly, diagnose any major foetal abnormalities and thirdly, measure foetal nuchal translucency 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 the foetal NT (nuchal translucency) to calculate the patient-specific risk for trisomy 21 (Snijders *et al*., 1998; Kagan *et al*., 2008a). We recorded maternal characteristics and medical history, measured the PI by transabdominal colour Doppler from the left and right uterine artery and recorded the lowest value (L-PI) (Poon *et al*., 2009a,b), and stored serum at $-80\,^{\circ}C$ for subsequent biochemical analysis. Written informed consent was

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Table 1—Studies reporting on the association between maternal serum placental protein 13 (PP13) concentration and preeclampsia

		Preeclampsia		Control		
Author	Gestation (weeks)	*n*	PP13 MoM	*n*	PP13 MoM	*p* value
Nicolaides *et al*., 2006	11–14	10	0.07	423	1.00	<0.001
Chafetz *et al*., 2007	9–12	47	0.20	290	1.00	<0.01
Spencer *et al*., 2007	11–14	88	0.69	446	1.00	<0.001
Romero *et al*., 2008	8–13	50	0.59	250	1.00	<0.001
Huppertz *et al*., 2008	5–10	4	0.12	41	1.00	<0.005
Gonen *et al*., 2008	6–10	20	0.30	1178	1.01	<0.001
Khalil *et al*., 2009	11–14	42	0.40	210	1.00	<0.001

MoM, Multiples of the median.

obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

The base cohort study population, wherein the present case–control study was nested, was examined between March 2006 and October 2008, and constituted 15 759 singleton pregnancies. In 298 (1.9%) cases there was subsequent development of PE, 231 (1.5%) cases developed gestational hypertension (GH) and 15 152 cases were unaffected by PE or GH. We also excluded 78 (0.5%) pregnancies, in which there was at least one episode of hypertension but on the basis of the available data it was not possible to determine if the diagnosis was PE. Stored blood was available in 208 of the 298 cases that developed PE, and maternal serum PP13 was measured in all these 208 cases, which included 48 cases that required delivery before 34 weeks (early-PE) and 160 cases of late-PE. Each case of PE was matched with two controls, who had blood collected on the same day and delivered a phenotypically normal neonate appropriate for gestational age at term and did not develop any hypertensive disorder of pregnancy. None of the samples were previously thawed and refrozen. This study is part of a research programme on the early prediction of pregnancy complications and some of the data from these patients on serum PAPP-A and uterine artery L-PI were included in previous publications (Poon *et al*., 2009b,c).

Maternal history

Patients were asked to complete a questionnaire on maternal age, racial origin, cigarette smoking during pregnancy, method of conception, medical history, medication, parity, obstetric history and family history of PE in the mother. The questionnaire was then reviewed by a doctor together with the patient. The maternal weight and height were measured and the body mass index (BMI) was calculated in kg/m^2.

Outcome measures

The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy (Brown *et al*., 2001). The diastolic blood pressure should be 90 mmHg or more on at least two occasions 4 h apart developing after 20 weeks of gestation together with significant proteinuria in previously normotensive women. Significant proteinuria is defined by 300 mg or more in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection is available. In PE superimposed on chronic hypertension significant proteinuria (as defined above) should develop after 20 weeks of gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks of gestation in the absence of trophoblastic disease).

Sample analysis

DELFIA research reagents (Perkin Elmer Life and Analytical Sciences, Turku, Finland) were used to measure PP13 in maternal serum samples (25 μL/well in duplicate). The concentration of PP13 measured was directly proportional to the fluorescence measured on time-resolved fluorometer at 615 nm. The coefficient of variation (CV) was 4.1% at a PP13 concentration of 16.6 pg/mL, 2.0% at 60.4 pg/mL and 2.7% at 136.2 pg/mL. Samples with duplicate CVs greater than 10% were reanalysed.

Maternal serum PAPP-A was measured using the DELFIA XPRESS analyser (PerkinElmer Life and Analytical Sciences, Waltham, USA). The variation of the DELFIA XPRESS PAPP-A assay was determined in 20 runs with two replicates using this system. The calibration curve of the first run was used as a reference curve during the 14-day period. The intra- and inter-assay variations were 1.2 and 2.1%, respectively, at a PAPP-A concentration of 462 mU/L, 1.4 and 2.3% at 2124 mU/L, and 1.3 and 2.5% at 5543 mU/L.

Statistical analysis

The following steps were taken. First, the distributions of PP-13, uterine artery L-PI and PAPP-A were made Gaussian after logarithmic transformation. Distributions were confirmed to be Gaussian using Kolmogorov–Smirnov test. Second, multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of log PP13 in the unaffected group. Then the

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distribution of log PP13, expressed as (MoM) of the unaffected group, was determined in the PE group. The measured uterine artery L-PI was converted into MoM after adjustment for gestation, maternal age, BMI and racial origin, as previously described (Poon *et al*., 2009b). Similarly, the measured PAPP-A was converted into MoM after adjustment of gestation, maternal age, racial origin, weight, parity, cigarette smoking status and method of conception as previously described (Kagan *et al*., 2008b). Third, Kruskall–Wallis test with *post hoc* Dunn's procedure was used to compare median MoM of PP13, uterine artery L-PI and PAPP-A between the outcome groups. Fourth, regression analysis was used to determine the significance of association among log PP13 MoM, log uterine artery L-PI MoM and log PAPP-A MoM in the outcome groups. Fifth, the maternal factor-derived *a priori* risks for PE were determined as previously decribed and were then logarithmically transformed (Poon *et al*., 2009a). Logistic regression analysis was used to determine if the log transformed maternal factor-derived *a priori* risks, log PP13 MoM, log PAPP-A MoM and log uterine artery L-PI MoM had a significant contribution in predicting PE. The detection and false-positive rates were calculated as the respective proportions of PE (detection rate) and unaffected pregnancies (false-positive rate) with MoM values were above given cut-offs. The performance of screening was determined by receiver-operating characteristic (ROC) curves analysis.

The statistical software package SPSS 16.0 (SPSS Inc.,Chicago, IL, USA), MedCalc for windows, version 9.6.2.0 (MedCalc Software, Mariakerke, Belgium) and XLSTAT-Pro 2008 (Addinsoft, USA) were used for data analyses.

RESULTS

The maternal characteristics of each of the outcome groups are compared in Table 2. In the groups that developed early- and late-PE compared to the unaffected group, women had a significantly higher BMI, there were more Black women, more women had PE in their previous pregnancy, were chronic hypertensives on antihypertensive medication and their mother had PE.

Unaffected group

Multiple regression analysis in the unaffected group demonstrated that for log PP13 significant independent contributions were provided by maternal weight and smoking but not by racial origin ($p = 0.594$), parity ($p = 0.870$) or foetal CRL ($p = 0.707$):

log expected PP13 $= 2.089 - 0.004\times$ maternal weight in kilograms $+$ (-0.214 if smoker, 0 if non-smoker); $R^2 = 0.154$, $p < 0.0001$.

In each patient we used this formula to derive the expected log PP13 and then expressed the observed value as an MoM of the expected (Table 3). Similarly, we used previously derived formulae for uterine artery L-PI and PAPP-A to calculate respective MoM values (Poon *et al*., 2009b,c).

Preeclampsia group

In those who subsequently developed early-PE, compared to controls, serum PP13 and PAPP-A were significantly decreased and uterine L-PI was increased (Table 3). In those who subsequently developed late-PE, compared to controls, PAPP-A was decreased and uterine L-PI was increased but serum PP13 was not significantly different.

In the PE group there was a significant association between serum PP13 and both uterine artery L-PI ($r = -0.342$, $p < 0.0001$) and serum PAPP-A ($r = 0.328$, $p < 0.0001$). In the unaffected group there was a significant association between serum PP13 and serum PAPP-A ($r = 0.269$, $p < 0.0001$) but not uterine artery L-PI ($r = -0.043$, $p = 0.376$).

Screening for preeclampsia

Logistic regression analysis demonstrated that in the prediction of early-PE there were significant contributions from log maternal factor-derived *a priori* risk [odds ratio (OR)15.9, 95% confidence interval (CI) 6.1–41.5], log uterine artery L-PI MoM (OR $5.1E^4$, 95% CI $1243.4–2.1E^6$; $p < 0.0001$) and log MoM PAPP-A (OR 0.07, 95% CI 0.02–0.27; $p < 0.0001$). Although log PP13 MoM did provide significant contribution to prediction of early-PE, it did not improve significantly the performance of screening for early-PE provided by the combination of the maternal factor-derived *a priori* risk, uterine artery L-PI and serum PAPP-A.

The patient-specific risk for early-PE is calculated from the formula: odds/(1+odds), where odds $= e^Y$ and Y is derived from multivariate logistic regression analysis of the disease-specific maternal factor-derived *a priori* risk, uterine artery L-PI MoM and PAPP-A MoM.

The estimated detection rates of early-PE at fixed false-positive rates of 5 and 10%, and their respective areas under the receiver-operating characteristic curves in screening by maternal factor-derived *a priori* risk, PP13, PAPP-A, uterine artery L-PI and by their combinations are shown in Table 4. The estimated detection rate of screening for early-PE by PP13 independently was 20.8 and 37.5% at respective false-positive rates of 5 and 10%. The addition of PP13 did not improve the detection rate of early-PE that was achieved by a combination of maternal factor-derived *a priori* risk, uterine artery PI and serum PAPP-A.

The maternal serum PP13 in late-PE was not significantly different from controls and therefore did not add value in screening for late-PE.

DISCUSSION

The findings of this study demonstrate that at 11–13 weeks of gestation, women who subsequently

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Table 2—Maternal characteristics in the three outcome groups

Maternal characteristics	Control ($n = 416$)	Early preeclampsia ($n = 48$)	Late preeclampsia ($n = 160$)
Maternal age in years, median (IQR)	32.5 (28.4–36.3)	30.0 (23.7–34.4)	31.7 (27.7–36.7)
Body mass index in kg/m^2, median (IQR)	24.6 (22.2–27.8)	27.1 (23.2–31.8)*	27.3 (23.8–31.7)*
Crown–rump length in mm, median (IQR)	64.3 (60.2–70.1)	67.2 (58.2–72.7)	62.0 (56.9–69.0)*
Racial origin			
White, *n* (%)	279 (67.1)	19 (39.6)	75 (46.9)
Black, *n* (%)	94 (22.6)	24 (50.0)*	64 (40.0)*
Indian or Pakistani, *n* (%)	28 (6.7)	2 (4.2)	10 (6.3)
Chinese or Japanese, *n* (%)	6 (1.4)	0	3 (1.9)
Mixed, *n* (%)	9 (2.2)	3 (6.3)	8 (5.0)
Parity			
Nulliparous, *n* (%)	188 (45.2)	24 (50.0)	90 (56.3)
Parous—no previous PE, *n* (%)	222 (53.4)	13 (27.1)*	47 (29.4)*
Parous—previous PE, *n* (%)	6 (1.4)	11 (22.9)*	23 (14.4)*
Family history of PE—Mother (*n*, %)	17 (4.1)	7 (14.6)*	17 (10.6)*
Cigarette smoker, *n* (%)	22 (5.3)	1 (2.1)	12 (7.5)
Conception			
Spontaneous, *n* (%)	401 (96.4)	43 (89.6)	150 (93.8)
Ovulation drugs, *n* (%)	6 (1.4)	4 (8.3)	6 (3.7)
In vitro fertilization, *n* (%)	9 (2.2)	1 (2.1)	4 (2.5)
Medical history			
None, *n* (%)	409 (98.3)	40 (83.3)	150 (93.8)
Chronic hypertension, *n* (%)	1 (0.2)	6 (12.5)*	7 (4.4)*
Diabetes mellitus, *n* (%)	2 (0.5)	1 (2.1)	2 (1.2)
Thrombophilia, *n* (%)	3 (0.7)	1 (2.1)	1 (0.6)
Others, *n* (%)	1 (0.2)	0	0
Medication during pregnancy			
None, *n* (%)	390 (93.8)	39 (81.3)	142 (88.8)
Anti-hypertensives, *n* (%)	0	4 (8.3)*	5 (3.1)*
Insulin, *n* (%)	2 (0.5)	1 (2.1)	2 (1.3)
Aspirin, *n* (%)	4 (0.9)	1 (2.1)	0
Others, *n* (%)	20 (4.7)	3 (6.3)	11 (6.9)

Comparisons between each outcome group with controls (Chi-square test and Fisher exact test for categorical variables and Kruskall–Wallis test with *post hoc* Dunn's procedure for continuous variables).
BMI, body mass index.
* $p < 0.05$.

Table 3—Median placental protein 13 (PP13), uterine artery L-PI (lowest pulsatility index) and pregnancy associated plasma protein-A (PAPP-A) in the outcome groups

	Unaffected ($n = 416$)	Early preeclampsia ($n = 48$)	Late preeclampsia ($n = 160$)
Serum PP13 (median, IQR)			
MoM	1.02 (0.78–1.30)	0.83 (0.55–1.15)*	0.96 (0.75–1.25)
pg/mL	66.6 (49.3–86.5)	52.4 (36.3–70.5)	58.3 (44.1–81.3)
Uterine artery L-PI (median, IQR)			
MoM	0.97 (0.77–1.22)	1.61 (1.31–1.73)*	1.25 (0.88–1.52)*
Unit	1.32 (1.04–1.66)	2.26 (1.80–2.44)	1.75 (1.22–2.16)
Serum PAPP-A (median, IQR)			
MoM	1.08 (0.75–1.48)	0.55 (0.37–0.94)*	0.84 (0.55–1.18)*
mU/L	3.26 (1.93–5.15)	2.16 (1.04–3.47)	2.42 (1.33–4.01)

Comparisons between outcome groups by Kruskall–Wallis test with *post hoc* Dunn's procedure.
IQR, interquartile range; MoM, multiples of the median.
* $p < 0.0167$.

develop PE have reduced maternal serum concentration of PP13 and PAPP-A and increased uterine artery PI. These findings are compatible with previous studies which reported reduced levels of maternal serum PP13 and PAPP-A, and increased uterine artery PI in the first-trimester of pregnancy in women destined to develop

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Table 4—Detection rates of early preeclampsia at fixed false-positive rates (FPR) of 5 and 10%, and comparison of screening performance by receiver-operating characteristic curve analysis in screening by maternal risk factor, placental protein 13 (PP13), pregnancy associated plasma protein-A (PAPP-A), uterine artery L-PI (lowest pulsatility index) and by their combinations

Screening test	FPR 5%	FPR 10%	AUROC (95% CI)
Maternal risk factor	39.0 (25.4–54.3)	49.0 (34.5–63.5)	0.785 (0.745–0.822)
PP13	20.8 (10.5–35.0)	37.5 (24.0–52.6)	0.652 (0.606–0.695)
PAPP-A	27.1 (15.3–41.8)	37.5 (24.0–52.6)	0.744 (0.701–0.783)
Uterine artery L-PI	45.8 (31.4–60.8)	68.7 (53.7–81.3)	0.863 (0.829–0.893)
Maternal risk factor plus			
PP13	37.5 (24.0–52.6)	52.1 (37.2–66.7)	0.818 (0.779–0.852)
PAPP-A	47.9 (33.3–62.8)	54.2 (39.2–68.6)	0.872 (0.838–0.901)
PP13 and PAPP-A	52.1 (37.2–66.7)	60.4 (45.3–74.2)	0.878 (0.845–0.906)
Uterine artery L-PI	58.3 (43.2–72.4)	75.0 (60.4–46.3)	0.920 (0.891–0.943)
Uterine artery L-PI and PP13	66.7 (51.6–79.6)	77.1 (62.7–88.0)	0.924 (0.896–0.946)
Uterine artery L-PI and PAPP-A	64.6 (49.5–77.8)	81.2 (67.4–91.0)	0.936 (0.910–0.957)

AUROC, area under receiver-operating characteristic curve; CI, confidence interval.

PE (Table 1) (Poon *et al*., 2009b,c). The PP13 levels are significantly lower in early-PE but not in late-PE compared to controls, whereas serum PAPP-A is reduced and uterine artery PI is increased in both early-PE and late-PE.

In the unaffected controls, the measured concentration of maternal serum PP13 decreased with maternal weight and was lower in smokers compared to non-smokers. Consequently, as in the case of serum PAPP-A and uterine artery L-PI the measured concentration of PP13 must be adjusted for these variables before comparing with pathological pregnancies (Poon *et al*., 2009b,c). A longitudinal study of 41 normotensive pregnancies at 5–40 weeks of gestation reported that the maternal serum concentration of PP13 increases with gestational age and then declines to undetectable levels after 8–12 weeks of delivery (Huppertz *et al*., 2008). In our study there was no change with foetal CRL within the narrow gestational range of 11–13 weeks.

PP13 is a protein dimer produced by the trophoblast. There is evidence from *in vitro* studies that PP13 binds to extracellular matrix proteins between the placenta and the endometrium and is therefore thought to be involved in normal placentation (Than *et al*., 1999, 2004; Burger *et al*., 2004). The scientific basis for the measurement of serum PP13 in the prediction of PE was the finding that expression of PP13 m-RNA in placentae from patients with established PE is reduced (Sammar *et al*., 2005). Studies in the first-trimester of pregnancy using solid-phase sandwich ELISA reported reduced maternal serum concentration of PP13 in women destined to develop PE, but the studies varied widely with regard to the number of PE cases and levels of PP13 in the affected group (Table 1). We examined a large number of affected pregnancies and were therefore able to differentiate between early- and late-PE demonstrating reduced levels of PP13 only in early-PE. This finding is consistent with the results of *in vitro* studies, which reported that placental PP13 m-RNA levels were reduced in patients with preterm-PE but not in those with term-PE (Than *et al*., 2008). In our study, we used the automated DELFIA technique to measure PP13 because in a pilot study we found that the reproducibility of the ELISA method was poor. This problem with the ELISA was highlighted in a previous study which reported that the inter-assay CV was 19.5% (Romero *et al*., 2008).

In pregnancies destined to develop PE there is evidence of impaired placentation manifested in increased uterine artery PI and reduced serum PAPP-A at 11–13 weeks. The finding that in the PE group there was a significant association between maternal serum PP13 and both serum PAPP-A and uterine artery PI supports the postulated role of PP13 in placentation (Than *et al*., 1999, 2004; Burger *et al*., 2004).

Effective first-trimester screening for early-PE is provided by a combination of maternal factors, uterine artery L-PI and serum PAPP-A with a detection rate of about 80% at a false-positive rate of 10%. In the combined data of three previous studies on a total of 30 cases of early-PE first-trimester screening by serum PP13 would identify about 70% of cases at a false-positive rate of 10% (Nicolaides *et al*., 2006; Khalil *et al*., 2009; Romero *et al*., 2008;). However, in our study, maternal serum PP13 alone would detect only 38% of pregnancies destined to develop early-PE, and logistic regression analysis demonstrated that serum PP13 did not improve the detection achieved by the combination of maternal factors, uterine artery L-PI and serum PAPP-A. Similarly, Spencer *et al*(2007) reported that serum PP13 at 11–13 weeks detected 36% of cases of early-PE for a false-positive rate of 10%, and PP13 did not improve the 70% detection rate achieved by uterine artery Doppler at 22–24 weeks. One area requiring further investigation is the possibility of measuring PP13 both in the first and in the second trimester. One study examining 20 women who subsequently developed PE reported that serum PP13 at 6–10 weeks was reduced, but at 16–20 weeks was increased (Gonen *et al*., 2008).

In summary, the maternal serum concentration of PP13 at 11–13 weeks is significantly lower in pregnancies destined to develop early-PE, and there is a significant association of this protein with established markers of impaired placentation, such as uterine artery PI and serum PAPP-A. However, measurement of serum PP13 at 11–13 weeks does not improve the performance

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of screening for early-PE achieved by a combination of maternal factors, uterine artery PI and serum PAPP-A.

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