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Maternal serum placental growth factor at 11 + 0 to 13 + 6 weeks of gestation in the prediction of pre-eclampsia

R. AKOLEKAR, E. ZARAGOZA, L. C. Y. POON, S. PEPES and K. H. NICOLAIDES

Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK

KEYWORDS: first-trimester screening; placental growth factor; pre-eclampsia; uterine artery Doppler

ABSTRACT

Objective *To investigate the potential value of maternal serum placental growth factor (PlGF) in first-trimester screening for pre-eclampsia (PE).*

Methods *The concentration of PlGF at 11 + 0 to 13 + 6 weeks' gestation was measured in samples from 127 pregnancies that developed PE, including 29 that required delivery before 34 weeks (early PE) and 98 with late PE, 88 cases of gestational hypertension (GH) and 609 normal controls. The distributions of PlGF multiples of the median (MoM) in the control and hypertensive groups were compared. Logistic regression analysis was used to determine the factors with a significant contribution for predicting PE.*

Results *In the control group significant independent contributions for log PlGF were provided by fetal crown–rump length, maternal weight, cigarette smoking and racial origin, and after correction for these variables the median MoM PlGF was 0.991. In the early-PE and late-PE groups PlGF (0.611 MoM and 0.822 MoM, respectively;* $P < 0.0001$*) and pregnancy-associated plasma protein-A (PAPP-A) (0.535 MoM;* $P < 0.0001$ *and 0.929 MoM;* $P = 0.015$*, respectively) were reduced but in GH (PlGF: 0.966 MoM; PAPP-A: 0.895 MoM) there were no significant differences from controls. Significant contributions for the prediction of PE were provided by maternal characteristics and obstetric history, serum PlGF and uterine artery pulsatility index (PI) and with combined screening the detection rates for early PE and late PE were 90% and 49%, respectively, for a false-positive rate of 10%.*

Conclusion *Effective screening for PE can be provided by a combination of maternal characteristics and obstetric history, uterine artery PI and maternal serum PlGF at 11 + 0 to 13 + 6 weeks' gestation.*

INTRODUCTION

Pre-eclampsia (PE), which affects about 2% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality[1–3]. The condition is associated with reduced production of the pro-angiogenic protein placental growth factor (PlGF), and several studies have reported that during the clinical phase of PE the maternal serum PlGF concentration is reduced[4–11]. These reduced levels of serum PlGF precede the clinical onset of the disease and are evident from both the second and first trimesters of pregnancy[12–19].

The underlying mechanism for PE is thought to be impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries, documented by the findings of both histological studies and Doppler ultrasound studies of the uterine arteries[20–23]. In addition, the maternal serum concentration of pregnancy-associated plasma protein-A (PAPP-A), which is thought to be involved in placental growth and development, is reduced at 11 + 0 to 13 + 6 weeks' gestation in pregnancies resulting in PE[24–26]. The likelihood of developing PE can be predicted by a combination of factors in the maternal history, including black racial origin, high body mass index (BMI) and prior or family history of PE, the measurement of uterine artery pulsatility index (PI) and the maternal serum level of PAPP-A at 11 + 0 to 13 + 6 weeks[23,26].

Previous studies have demonstrated that prediction of PE can be provided by uterine artery Doppler in the second trimester of pregnancy[27], and this can be improved

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK (e-mail: fmf@fetalmedicine.com)

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by combining the Doppler findings with maternal serum concentration of PlGF[28] and the anti-angiogenic protein soluble fms-like tyrosine kinase 1 (sFlt-1)[29]. Although in pregnancies developing PE reduced levels of PlGF are evident from the first trimester, significant increases in levels of sFlt-1 become apparent only about 5 weeks before the onset of PE[30].

The aim of this study was to investigate further the levels of maternal serum PlGF in the first trimester of pregnancy in cases that subsequently developed PE, to examine the relation of these levels to uterine artery PI and maternal serum PAPP-A levels and to estimate the potential performance of screening for PE by a combination of maternal factors, uterine artery PI and maternal serum PAPP-A and PlGF.

METHODS

Study population

This was a case-control study. Screening for adverse pregnancy outcomes was performed in women attending for routine assessment of risk for chromosomal abnormalities by measurement of fetal nuchal translucency thickness and maternal serum PAPP-A and free β-human chorionic gonadotropin (β-hCG) at 11 + 0 to 13 + 6 weeks of gestation[31,32]. Gestational age was determined from the known date of the last menstrual period and confirmed by the sonographic measurement of the fetal crown–rump length (CRL). We recorded maternal characteristics and medical history, measured the uterine artery PI by transabdominal color Doppler[23] and stored serum at −80°C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

In the screening study the prevalence of PE was 1.8%. The case-control study population comprised 127 pregnancies that subsequently developed PE, including 29 that required delivery before 34 weeks (early PE) and 98 with late PE, 88 with gestational hypertension (GH), 296 cases that delivered small-for-gestational-age (SGA) neonates, 57 cases with spontaneous preterm delivery before 34 weeks and 41 cases of trisomy 21. Each case was matched with one control case that had blood collected and stored on the same day that did not develop any pregnancy complications and resulted in the live birth of phenotypically normal neonates. The results of the subanalysis of the hypertensive complications of pregnancy are presented in this paper.

Maternal history

Patients were asked to complete a questionnaire on maternal age, racial origin (white, black, Indian or Pakistani, Chinese or Japanese and mixed), cigarette smoking during pregnancy (yes or no), method of conception (spontaneous, use of ovulation drugs and *in-vitro* fertilization), medical history (including chronic hypertension, diabetes mellitus, anti-phospholipid syndrome, thrombophilia, human immunodeficiency virus infection and sickle cell disease), medication (including antihypertensive, antidepressant, antiepileptic, anti-inflammatory, aspirin, β-mimetic, insulin, steroids, thyroxin), parity (parous or nulliparous if no delivery beyond 23 weeks), obstetric history (including previous pregnancy with PE) and family history of PE (mother). The maternal weight and height were measured and the BMI was calculated in kg/m^2.

Outcome measures

The definitions of PE and GH were those of the International Society for the Study of Hypertension in Pregnancy[33]. In GH the diastolic blood pressure should be 90 mmHg or more on at least two occasions 4 h apart developing after 20 weeks of gestation in previously normotensive women in the absence of significant proteinuria and in PE, there should be GH with proteinuria of 300 mg or more in 24 h or two readings of at least ++protein 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

Duplicate serum samples of 100 μL were used to measure PlGF concentrations by a quantitative enzyme linked immunoassay (ELISA) technique using Quantikine® human PlGF immunoassay (R&D systems Europe Ltd, Abingdon, UK). The assays were performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Absorbance readings were taken on a VICTOR3^{TM} plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland) and PlGF concentrations were determined using MultiCalc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The lower limit of detection of the assay was 7 pg/mL and the between-batch imprecision was 8.3% at a PlGF concentration of 48 pg/mL, 5.6% at 342 pg/mL and 5.1% at 722 pg/mL. Samples whose coefficient of variation of the duplicates exceeded 15% were reanalyzed.

Statistical analysis

The measured concentration of PlGF was log transformed to make the distribution Gaussian. Multiple regression analysis was then used to determine which of the factors among the maternal characteristics and fetal CRL were significant predictors of log PlGF in the control group and from the regression model the value in each case

and control was expressed as a multiple of the expected median in the control group (MoM). Box-and-whisker plots of PlGF MoM of each outcome group were created. The Mann–Whitney test was used to determine the significance of differences in the median MoM in each outcome group from that in the controls.

In each case and control the measured PAPP-A and uterine artery PI were converted into MoMs after adjustment for gestation, maternal age, racial origin, BMI or weight, parity, previous history of PE and method of conception as previously described[26,34]. Regression analysis was then used to determine the significance of association between log PlGF MoM and log PAPP-A MoM, log uterine artery PI MoM, birth weight percentile and gestation at delivery in each outcome group.

The patient-specific risks for PE (%) were calculated from the formula: Risk = odds/(1 + odds), where odds = e^Y. Y was derived from logistic regression analysis.

Logistic regression analysis was used to determine which of the factors among the maternal characteristics, log PlGF MoM, log PAPP-A MoM and log uterine artery PI MoM had a significant contribution to predicting PE. The performance of screening was determined by receiver–operating characteristics (ROC) curves.

The statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL, USA) was used for all data analyses.

RESULTS

The maternal characteristics of each of the outcome groups are summarized in Table 1.

Control group

Multiple regression analysis in the control group demonstrated that for log PlGF significant independent

Table 1 Maternal characteristics in the four outcome groups

Maternal characteristic	*Control* (n = *609*)	*Early pre-eclampsia* (n = *29*)	*Late pre-eclampsia* (n = *98*)	*Gestational hypertension* (n = *88*)
Maternal age (years, median (range))	32.7 (16–45)	32.7 (17–49)	31.5 (18–44)	33.3 (18–46)
Weight (kg, median (range))	65.0 (42–143)	72.0 (54–105)*	69.5 (44–140)†	71.0 (50–147)‡
Crown–rump length (mm, median (range))	64.0 (45–84)	67.0 (52–84)	62.3 (46–84)*	62.5 (47–83)
Racial origin (*n* (%))				
White	443 (72.7)	11 (37.9)†	41 (41.8)‡	67 (76.1)
Black	97 (15.9)	14 (48.3)‡	41 (41.8)‡	16 (18.2)
Indian or Pakistani	34 (5.6)	2 (6.9)	7 (7.1)	—*
Chinese or Japanese	13 (2.1)	—	2 (2.0)	1 (1.1)
Mixed	22 (3.6)	2 (6.9)	7 (7.1)	4 (4.5)
Parity (*n* (%))				
Nulliparous	278 (45.6)	15 (51.7)	64 (65.3)‡	49 (55.7)
Parous–no previous pre-eclampsia	315 (51.7)	7 (24.1)*	23 (23.5)‡	29 (33.0)†
Parous–previous pre-eclampsia	16 (2.6)	7 (24.1)‡	11 (11.2)†	10 (11.4)†
Cigarette smoker	30 (4.9)	0	6 (6.1)	7 (8.0)
Family history of pre-eclampsia–mother	22 (3.6)	3 (10.3)	12 (12.2)†	9 (10.2)*
Conception (*n* (%))				
Spontaneous	594 (97.5)	25 (86.2)*	94 (95.9)	85 (96.6)
Ovulation drugs	10 (1.6)	3 (10.3)*	3 (3.1)	—
In-vitro fertilization	5 (0.8)	1 (3.4)	1 (1.0)	3 (3.4)
Medical history (*n* (%))				
None	599 (98.4)	24 (82.8)†	93 (94.9)*	85 (96.6)
Chronic hypertension	1 (0.2)	4 (13.8) ‡	4 (4.1)*	—
Diabetes mellitus	4 (0.7)	—	—	2 (2.3)
Antiphospholipid syndrome	3 (0.5)	—	1 (1.0)	1 (1.1)
Thrombophilia	—	1 (3.4)*	—	—
Sickle cell disease	1 (0.2)	—	—	—
Human immunodeficiency viral infection	1 (0.2)	—	—	—
Medication during pregnancy (*n* (%))				
None	572 (93.9)	25 (86.2)	90 (91.8)	76 (86.4)*
Antihypertensives	—	2 (6.9)*	2 (2.0)*	—
Insulin	3 (0.5)	—	—	2 (2.3)
Steroids	1 (0.2)	—	—	—
β-mimetics	11 (1.8)	—	4 (4.1)	4 (4.5)
Thyroxin	9 (1.5)	1 (3.4)	1 (1.0)	2 (2.3)
Aspirin	3 (0.5)	—	—	2 (2.3)
Antiepileptic	2 (0.3)	—	—	1 (1.1)
Antidepressant	6 (1.0)	1 (3.4)	—	1 (1.1)
Anti-inflammatory	2 (0.3)	—	1 (1.0)	—

Comparison with unaffected group (Chi-square or Fisher exact test for categorical variables and *t*-test for continuous variables): *$P < 0.05$. †$P < 0.01$. ‡$P < 0.0001$.

contributions were provided by fetal CRL, maternal weight, cigarette smoking and racial origin:

$$\begin{aligned}\log \text{ expected PlGF} = {} & 1.150 + (0.008 \times \text{CRL in mm}) \\ & - (0.002 \times \text{weight in kg}) \\ & + (0.199 \text{ if smoker, 0 if not}) \\ & + (0.177 \text{ if black,} \\ & 0.100 \text{ if Indian or Pakistani,} \\ & 0 \text{ if other racial origin);} \\ & r^2 = 0.237,\ P < 0.0001.\end{aligned}$$

In each patient we used this formula to derive the expected log PlGF and then expressed the observed value as a MoM of the expected value (Figure 1, Table 2).

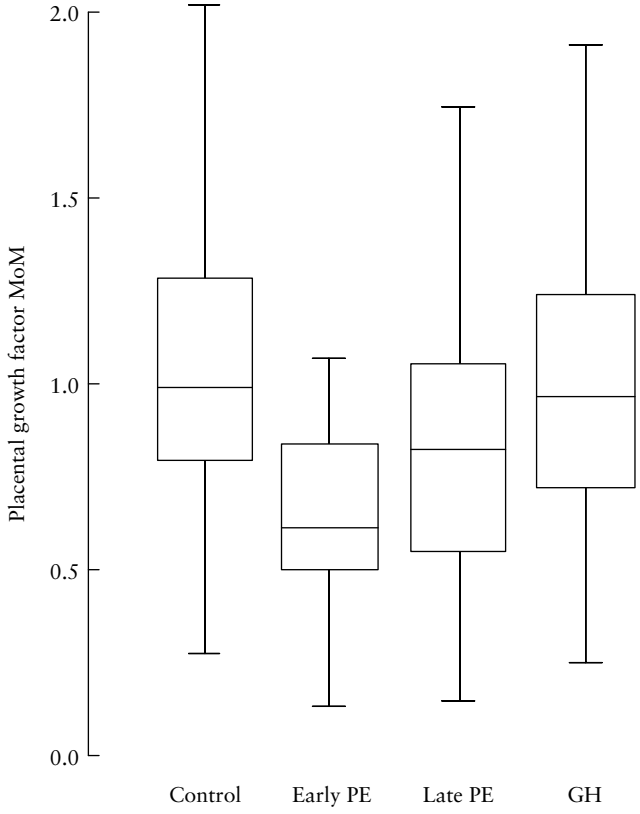

Figure 1 Box-and-whisker plot (median, interquartile range and range) of placental growth factor multiples of the median (MoM) in the pregnancy outcome groups: controls, early pre-eclampsia (PE), late PE and gestational hypertension (GH).

There was a significant association between log PlGF MoM and log PAPP-A MoM ($r = 0.264$, $P < 0.0001$; Figure 2); log uterine artery PI MoM ($r = 0.102$, $P = 0.012$; Figure 3); birth weight percentile ($r = 0.114$, $P = 0.005$); but not gestational age at delivery ($P = 0.960$).

Pre-eclampsia group

In both the early-PE and late-PE groups PlGF and PAPP-A were lower and uterine artery PI was higher than in the controls (Figure 1, Table 2). There was a significant association between log PlGF MoM and log PAPP-A MoM ($r = 0.325$, $P < 0.0001$; Figure 2); log uterine artery PI MoM ($r = 0.279$, $P = 0.001$; Figure 3); gestational age at delivery ($r = 0.256$, $P = 0.004$); and birth weight percentile ($r = 0.338$, $P < 0.0001$).

Logistic regression analysis demonstrated that significant contributions for the detection of early PE were provided from maternal factors, PlGF, PAPP-A and uterine artery PI:

$$\begin{aligned}Y = {} & -5.620 - (4.717 \times \log \text{ PlGF MoM}) \\ & - (1.865 \times \log \text{ PAPP-A MoM}) \\ & + (14.519 \times \text{uterine artery PI MoM}) \\ & + (5.471 \text{ if history of chronic hypertension}) \\ & + (1.159 \text{ if black, 0 if other racial origin}); \\ & r^2 = 0.500,\ P < 0.0001.\end{aligned}$$

Logistic regression analysis demonstrated that significant contributions for the detection of late PE were provided from maternal factors, PlGF and uterine artery PI but not PAPP-A ($P = 0.933$):

$$\begin{aligned}Y = {} & -5.136 - (2.400 \times \log \text{PlGF MoM}) \\ & + (2.641 \times \log \text{ uterine artery PI MoM}) \\ & + (0.108 \times \text{BMI in kg/m}^2) \\ & + (1.441 \text{ if patient's mother had PE}) \\ & + (1.366 \text{ if black, } 1.083 \text{ if Indian or Pakistani,} \\ & 1.549 \text{ if mixed, 0 if other racial origin}) \\ & + (-1.281 \text{ if parous and no previous PE,} \\ & 0 \text{ if parous with previous PE or nulliparous}); \\ & r^2 = 0.290,\ P < 0.0001.\end{aligned}$$

Table 2 Median (interquartile range) of maternal serum placental growth factor (PlGF) multiples of the median (MoM), pregnancy-associated plasma protein-A (PAPP-A) MoM and uterine artery pulsatility index (PI) MoM in the four outcome groups: control, early pre-eclampsia, late pre-eclampsia and gestational hypertension

Outcome group	*PlGF MoM*	*PAPP-A MoM*	*Uterine artery PI MoM*
Control	0.991 (0.799–1.286)	1.070 (0.735–1.455)	1.030 (0.839–1.242)
Early pre-eclampsia	0.611 (0.480–0.839)†	0.535 (0.391–0.961)†	1.512 (1.204–1.653)†
Late pre-eclampsia	0.822 (0.550–1.056)†	0.929 (0.574–1.310)*	1.220 (0.927–1.448)†
Gestational hypertension	0.966 (0.712–1.246)	0.895 (0.622–1.442)	1.100 (0.885–1.287)

Mann–Whitney test to compare each group with controls: *$P < 0.05$; †$P < 0.0001$.

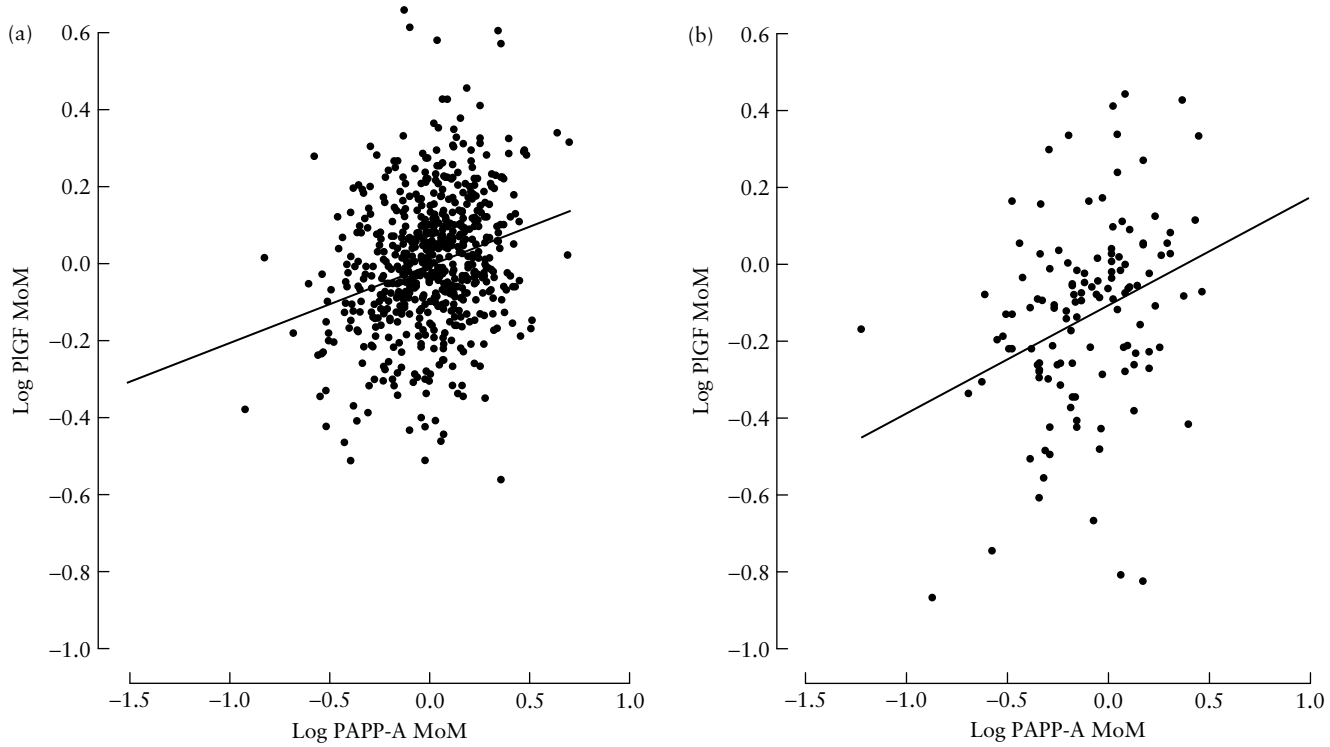

Figure 2 Relationship between log placental growth factor (PlGF) multiples of the median (MoM) and log pregnancy-associated plasma protein-A (PAPP-A) MoM in the control group (a) and the pre-eclampsia group (b).

The areas under the ROC curves and detection rates of early PE and late PE for different false-positive rates in screening by maternal factors, serum PlGF, serum PAPP-A, uterine artery PI and by their combinations are given in Tables 3 and 4.

Gestational hypertension

In the GH group, compared to the controls, there were no significant differences in PlGF, PAPP-A or uterine artery PI (Figure 1, Table 2).

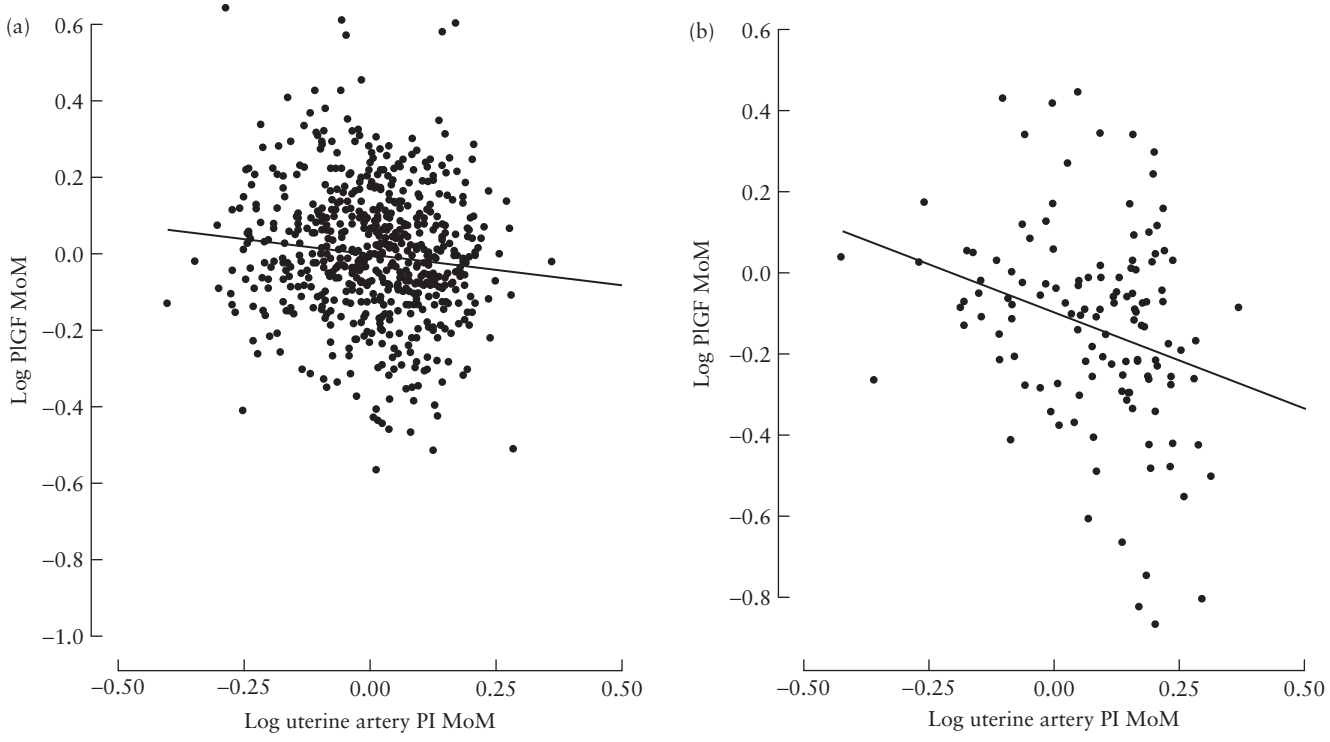

Figure 3 Relationship between log placental growth factor (PlGF) multiples of the median (MoM) and log uterine artery pulsatility index (PI) MoM in the control group (a) and the pre-eclampsia group (b).

Table 3 Comparison of the performance of screening for pre-eclampsia by maternal obstetric history and characteristics, placental growth factor (PlGF), pregnancy-associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI) and by their combinations, by receiver–operating characteristics curve analysis

	Area under receiver–operating characteristics curve (95% CI)	
Screening test	*Early pre-eclampsia*	*Late pre-eclampsia*
History/characteristics	0.762 (0.654–0.870)	0.788 (0.742–0.834)
PlGF	0.797 (0.705–0.888)	0.652 (0.589–0.714)
PAPP-A	0.742 (0.639–0.846)	0.576 (0.513–0.639)
Uterine artery PI	0.826 (0.740–0.912)	0.626 (0.560–0.692)
History/characteristics plus:		
PlGF	0.881 (0.817–0.944)	0.817 (0.775–0.859)
PAPP-A	0.842 (0.747–0.937)	0.788 (0.741–0.834)
Uterine artery PI	0.902 (0.833–0.971)	0.801 (0.753–0.849)
PAPP-A, uterine artery PI	0.907 (0.882–0.929)	—
PlGF, uterine artery PI	0.941 (0.889–0.994)	0.817 (0.773–0.861)
PlGF, PAPP-A, uterine artery PI	0.936 (0.882–0.989)	—

Table 4 Comparison of the detection rate of early and late pre-eclampsia at fixed false-positive rates (FPR) of 5% and 10% in screening by maternal characteristics and obstetric history, placental growth factor (PlGF), pregnancy-associated plasma protein-A (PAPP-A), uterine artery pulsatility index (PI) and by their combinations

	Detection rate (% (95% CI))			
	Early pre-eclampsia		*Late pre-eclampsia*	
Screening test	*FPR 5%*	*FPR 10%*	*FPR 5%*	*FPR 10%*
History/characteristics	39.0 (17.5–62.5)	49.0 (20.0–70.0)	29.6 (20.8–39.7)	43.9 (33.9–54.3)
PlGF	27.6 (12.8–47.2)	51.7 (32.5–70.5)	19.4 (12.1–29.6)	32.7 (23.5–42.9)
PAPP-A	24.1 (10.3–43.5)	41.4 (23.5–61.1)	8.2 (3.6–15.5)	18.4 (11.3–27.5)
Uterine artery PI	37.9 (20.7–57.7)	65.5 (45.7–82.0)	16.3 (9.6–25.2)	27.6 (19.0–37.5)
History/characteristics plus:				
PlGF	55.2 (35.7–73.5)	62.1 (42.3–79.3)	28.6 (19.9–38.6)	52.0 (41.7–62.2)
PAPP-A	51.7 (32.5–70.5)	69.0 (49.2–84.7)	29.6 (20.8–39.7)	46.9 (36.8–57.3)
Uterine artery PI	69.0 (49.2–84.7)	75.9 (56.5–89.7)	29.6 (20.8–39.7)	51.0 (40.7–61.3)
PAPP-A, uterine artery PI	69.0 (49.2–84.7)	72.4 (52.8–87.2)	—	—
PlGF, uterine artery PI	75.9 (56.5–89.7)	89.7 (72.6–97.7)	29.6 (20.8–39.7)	49.0 (38.7–59.3)
PlGF, PAPP-A, uterine artery PI	75.9 (56.5–89.7)	86.2 (68.3–96.0)	—	—

DISCUSSION

The findings of this study demonstrate that the maternal serum PlGF concentration at 11 + 0 to 13 + 6 weeks of gestation in normal pregnancies increased with fetal CRL and therefore gestational age, decreased with maternal weight, and was higher in black than in white women and in cigarette smokers than in non-smokers. Consequently, as in the case of PAPP-A[34], the measured concentration of PlGF must be adjusted for these variables before comparing results with pathological pregnancies. Previous studies comparing PE with controls either have made no corrections for the measured PlGF or they corrected only for gestation[4–17]. In common with PlGF, the serum concentration of PAPP-A also increases with fetal CRL, decreases with maternal BMI and is higher in black than in white women[34]. However, in cigarette smokers there is an apparent dissociation in the relationship between these two placental products with a decrease in serum PAPP-A and increase in PlGF.

In pregnancies developing PE the maternal serum PlGF concentration at 11 + 0 to 13 + 6 weeks' gestation was lower than in normotensive pregnancies. Furthermore, there was a significant association between PlGF and the severity of PE defined by both the gestation at which iatrogenic delivery was carried out and the birth-weight centile of the neonates. These results are in agreement with most previous studies, which reported reduced serum PlGF not only during the clinical phase of the disease but also during the second and first trimesters of pregnancy[4–18]. Those studies comparing values between patients who developed early PE and late PE also showed that the levels were lower in early PE[8–10,17,18].

The finding of an interrelationship between serum levels of PlGF and PAPP-A with uterine artery PI is compatible with the postulated roles of PlGF and PAPP-A in placental development and the reflection of impaired placentation in increased impedance to flow in the uterine arteries. Abnormalities in the biochemical and Doppler indices of placentation are substantially more common in women

developing early PE than late PE. This is particularly important because it is early rather than late disease that is associated with increased risk of perinatal mortality and morbidity and both short-term and long-term maternal complications[35–37].

In early screening for PE there were significant independent contributions from maternal characteristics and obstetric history, uterine artery PI, maternal serum PlGF and PAPP-A. The association between black race, obesity, family history of PE and personal history of chronic hypertension or PE with increased risk of developing PE is well documented[38]. We estimated that screening by a combination of maternal characteristics and obstetric history, uterine artery PI and maternal serum PlGF, with or without maternal serum PAPP-A, would identify about 90% and 50% of patients developing early PE and late PE, respectively, at a false-positive rate of 10%.

Identification of women at high risk for PE could potentially improve pregnancy outcomes because intensive maternal and fetal monitoring in such patients would lead to an earlier diagnosis of the clinical signs of the disease and the associated fetal growth restriction and avoid the development of serious complications through such interventions as the administration of antihypertensive medication and early delivery. The proposed combined screening test could also be used for effective identification of the high-risk group for future studies investigating the potential role of pharmacological interventions starting from the first trimester to improve placentation and reduce the prevalence of the disease.

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