

Maternal endothelial function and serum concentrations of placental growth factor and soluble endoglin in women with abnormal placentation

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KEYWORDS: Doppler; FMD; PlGF; pre-eclampsia; sEng

ABSTRACT

Objectives To determine whether maternal serum concentrations of placental growth factor (PlGF) and soluble endoglin (sEng) are altered in women who subsequently develop pre-eclampsia (PE) or have small-for-gestational-age (SGA) infants, and whether these changes are associated with maternal endothelial dysfunction.

Methods Maternal serum PlGF and sEng were measured in two groups of pregnant women at 23–25 weeks' gestation: Group A (n = 40), with normal uterine artery Doppler waveforms and Group B (n = 43) with abnormal Doppler. Maternal endothelial dysfunction was assessed by flow-mediated dilatation (FMD) of the brachial artery. Comparisons between groups were performed using one-way analysis of variance.

Results In Group B, 16 women had normal outcome, 15 delivered SGA infants and 12 developed PE. Women who developed PE had lower levels of PlGF (154.8 ± 150.8 vs. 423.3 ± 230.5 pg/mL; $P < 0.001$) (data given as mean \pm SD) and higher levels of sEng (8.1 (7.0–14.1) vs. 6.5 (4.9–7.9) pg/mL; $P < 0.05$) (data given as median (interquartile range)) than Group A. Similar were the findings in women who delivered SGA infants. In women who subsequently developed PE, there was no correlation between FMD and either PlGF or sEng.

Conclusions Maternal serum concentrations of PlGF and sEng are altered in women who develop PE. However, these alterations do not correlate directly with maternal endothelial dysfunction. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Pre-eclampsia (PE) is an important cause of maternal and perinatal mortality and morbidity worldwide¹. The placenta has a central role in PE and it is conceivable that a failure of normal trophoblastic invasion and remodeling of the spiral arteries lead to a high-resistance uteroplacental circulation that can be detected non-invasively by Doppler ultrasound evaluation of the uterine arteries at 23–25 weeks' gestation^{2,3}. A similar abnormality is also associated with fetal growth restriction^{2,3}. The under-perfused placenta is then thought to release humoral, pre-eclamptic factors into the maternal circulation that cause endothelial dysfunction and end-organ ischemia leading to hypertension and proteinuria⁴.

A potentially important process in the pathogenesis of PE is an imbalance between placenta-derived proangiogenic and antiangiogenic proteins. The proangiogenic proteins vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are involved in the regulation of placental vascular development and maternal endothelial function during pregnancy⁵. Each exerts a potentially favorable effect on the endothelium including promoting the release of nitric oxide (NO) by binding to cell surface receptor fms-like tyrosine kinase-1 (Flt-1). The receptor also exists in a secreted circulating form lacking a cytoplasmic domain (sFlt-1)^{6,7}. Circulating sFlt-1 is a potent antagonist of PlGF and VEGF because it binds to these proangiogenic proteins, thereby preventing their interaction with their receptors⁸. Higher than normal sFlt-1 levels and lower than normal circulating PlGF levels have been demonstrated both in the clinical phase of PE and approximately 5 weeks before its clinical diagnosis^{5,9}.

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Accepted: 7 March 2008

Another novel antiangiogenic protein, soluble endoglin (sEng), has also been implicated in the pathogenesis of PE¹⁰. Soluble Eng is a cell-surface co-receptor for transforming growth factor (TGF)- β 1 and TGF- β 3 isoforms, and is highly expressed in endothelial cells and syncytiotrophoblasts^{11–13}. It is elevated in women with established PE and prior to the development of the condition, and increases with the severity of the disease^{10,14}. Experimental *in-vitro* studies have suggested that both of these antiangiogenic factors are involved directly in the maternal endothelial dysfunction that characterizes PE¹⁰.

Systemic endothelial function can be assessed non-invasively and reliably using flow-mediated dilatation (FMD) of the brachial artery^{15,16}. FMD depends on the endothelial release of NO, because it can be partially blocked by NG-monomethyl-L-arginine, a specific inhibitor of NO synthesis¹⁷. Furthermore, FMD of the brachial artery reflects systematic endothelial function¹⁸ and is decreased in subjects with endothelial dysfunction¹⁵. Using this technique, we have shown that maternal endothelial dysfunction precedes the development of PE by about 10 weeks¹⁹.

The aims of this study were firstly, to assess whether alterations in the levels of PlGF and sEng occur in advance of the clinical onset of PE or delivery of small-for-gestational-age (SGA) infants in women at high risk for these complications, and secondly – and more importantly – to determine if such alterations are associated with maternal endothelial dysfunction.

METHODS

Study participants

This was a retrospective study including healthy women with singleton pregnancies at 23–25 weeks' gestation, recruited consecutively from the ultrasound department of King's College Hospital, where color Doppler examination of the uterine arteries is routinely performed. All women had been included in a previous study assessing the levels of sFlt-1 and VEGF²⁰. Two groups of women were examined: Group A included 40 women with normal uterine artery Doppler waveforms and normally grown fetuses and Group B included 43 with abnormal uterine artery Doppler waveforms (presence of early diastolic notch bilaterally) and normally grown fetuses.

Maternal age, ethnic group, smoking status, parity, body mass index and blood pressure were recorded. Blood pressure was measured in the right arm with the subject seated using an ambulatory blood pressure monitor (SpaceLabs Medical 90 207, WA, USA). Three measurements were taken and averaged. The study was approved by the institutional review board and all women gave written informed consent.

Measurements of PlGF, sEng and FMD of the brachial artery

All the women had a venous blood sample taken and serum was separated by centrifugation, frozen and stored

at -70°C . Maternal serum PlGF and sEng were measured using a commercial enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, USA) by personnel who were unaware of the outcome of the pregnancy. All the assays were conducted in duplicate and the mean value of the duplicate samples was reported. Minimal detectable levels in the assays for PlGF and sEng were 7 pg/mL for both, and the intra- and inter-assay coefficients of variation at the concentrations measured were below 10%. All the women had measurements of sFlt-1 performed, as previously described²⁰, and these values were used in order to calculate the sFlt-1 : PlGF and (sFlt-1 + sEng) : PlGF ratios, as a measure of the balance between antiangiogenic and proangiogenic proteins.

In a subgroup of women (37 from Group A and 36 from Group B) maternal endothelial function was assessed using FMD of the brachial artery, an established method of assessment of endothelial function *in vivo*, as previously described¹⁹.

Definition of clinical outcome

Information on pregnancy outcome, including gestation at delivery, birth weight and sex was obtained from examination of individual patient hospital records. The diagnosis of PE was made according to the criteria of the International Society for the Study of Hypertension in Pregnancy. Under this classification, PE is defined as diastolic blood pressure of at least 110 mmHg on one occasion or diastolic blood pressure of at least 90 mmHg on two consecutive occasions more than 4 h apart, in combination with proteinuria (≥ 300 mg total protein in a 24-h urine collection or, if this is not available, $\geq 2+$ proteinuria by dipstick test on two consecutive occasions at least 4 h apart) developing after 20 weeks of gestation in previously normotensive women²¹. The diagnosis of SGA was based on the delivery of an infant with birth weight below the 5th percentile for gestation and sex²².

Statistical analysis

Normality of distribution of continuous data was examined with the Shapiro–Wilks test. Logarithmic transformation was performed for non-normally-distributed data. Descriptive data were expressed as mean \pm SD or as median (interquartile range) for normally and non-normally distributed data, respectively. Comparisons between groups were performed using unpaired *t*-test or one-way analysis of variance followed by the Bonferroni *post-hoc* test, as appropriate. The chi-square (χ^2) test was used to compare categorical variables among groups. Univariate linear regression analysis was performed where appropriate. Power analysis indicated that a sample of 12 cases of PE and 40 controls would have a power of more than 95%, with an alpha of 0.05 (2-tailed) for the detection of a mean difference of 65.4 pg/mL in the serum concentration of PlGF and a mean difference of 5% in maternal FMD between the two groups. The effect size was estimated from previous observations^{19,23}. The

statistical analyses were performed using the Statistical Package for Social Sciences (Version 12) (SPSS, Chicago, IL, USA).

RESULTS

Characteristics of the study participants

In Group A (normal Doppler findings, $n = 40$), none of the women developed PE and all of them delivered babies of appropriate size for gestation. The women in Group B (abnormal Doppler findings and normal fetal growth at presentation, $n = 43$) were sub-classified into three groups according to the outcome of pregnancy: those with no complications ($n = 16$ (37.2%)); those who delivered SGA infants ($n = 15$ (34.9%)); and those who developed PE ($n = 12$ (27.9%)). The group that developed PE included five women who had early-onset PE and had to be delivered prior to 34 weeks' gestation owing to the severity of the condition (median gestation at delivery, 30 weeks) and five who delivered an SGA infant along with the development of PE. The mean time interval between the Doppler examination of the uterine arteries and the development of PE was 10 weeks. The demographic and clinical characteristics of the groups, obtained on entry to the study, are presented in Table 1. There were no statistically significant differences between the groups in any of the baseline demographic characteristics, but systolic and diastolic blood pressure were significantly higher in women who eventually developed PE.

Levels of PlGF and sEng

The levels of PlGF and sEng, and sFlt-1 : PlGF and (sFlt-1 + sEng) : PlGF ratios in all the groups of women

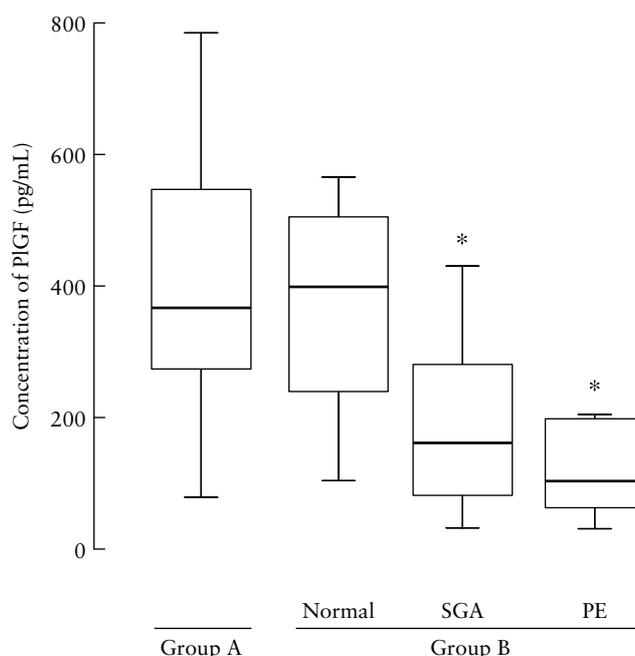


Figure 1 Box-and-whisker plot comparing placental growth factor (PlGF) concentrations at 23–25 weeks' gestation between the different patient groups: Group A ($n = 40$), women with normal Doppler and normal outcome and Group B ($n = 43$), with abnormal Doppler and either normal outcome ($n = 16$), or subsequent delivery of a small-for-gestational-age (SGA) infant ($n = 15$), or subsequent development of pre-eclampsia (PE) ($n = 12$). Boxes represent the interquartile range and lines represent the median. Whiskers represent the highest and lowest values. Significant differences from Group A are indicated ($*P < 0.05$).

are shown in Table 2, Figure 1 and Figure 2. Overall, the women in Group A had higher levels of PlGF and lower levels of sEng compared with those in Group B (PlGF: 423.3 ± 230.5 vs. 272 ± 214 pg/mL, $P = 0.003$; sEng: 6.5 (4.9–7.9) vs. 7.3 (5.8–10.6) ng/mL; $P = 0.01$).

Table 1 Demographic, clinical maternal and neonatal characteristics in each group of women, according to the outcome of pregnancy (Group A, women with normal Doppler examination and normal outcome; Group B, women with abnormal Doppler examination, with subgroups according to the pregnancy outcome)

Characteristic	Group A	Group B		
	Normal outcome ($n = 40$)	Normal outcome ($n = 16$)	Development of SGA ($n = 15$)	Development of PE ($n = 12$)
Maternal age (years)	28.7 ± 5.6	27 ± 6.3	28.1 ± 6.5	28.2 ± 5.8
Smoker	8 (20.0)	2 (12.5)	3 (20.0)	3 (25.0)
Ethnic group				
Caucasian	19 (47.5)	7 (44)	6 (40)	6 (50)
Afro-Caribbean	18 (45)	8 (50)	6 (40)	4 (33)
Other	3 (7.5)	1 (6)	3 (20)	2 (17)
Nulliparous	23 (57.5)	9 (56.2)	13 (86.7)	9 (75.0)
Body mass index (kg/m^2)	28 ± 5.6	27 ± 5	24.8 ± 3.6	28 ± 4.5
Systolic blood pressure (mmHg)	114.0 ± 8.2	114.3 ± 7.7	110.2 ± 7.1	$122.6 \pm 7.2^*$
Diastolic blood pressure (mmHg)	65.8 ± 6.6	65.1 ± 8.4	64.2 ± 4.7	$74.5 \pm 8.3^\ddagger$
Uterine artery pulsatility index	0.8 ± 0.2	1.5 ± 0.6	1.7 ± 0.6	1.6 ± 0.4
Gestational age at delivery (weeks)	39.4 ± 1.6	40.4 ± 1.6	38.7 ± 3	$34.5 \pm 3.3^\ddagger$
Birth weight (g)	3319.8 ± 516.3	3239.3 ± 405.1	$2301 \pm 612.8^\ddagger$	$1986 \pm 668.3^\ddagger$

Values are expressed as mean \pm SD or n (%). All comparisons were performed with Group A. $*P = 0.007$. $^\ddagger P = 0.002$. $^\ddagger P < 0.0001$. PE, pre-eclampsia; SGA, small-for-gestational age.

Table 2 Levels of placental growth factor (PlGF) and soluble endoglin (sEng), soluble fms-like tyrosine kinase 1 (sFlt-1):PlGF and (sFlt-1 + sEng):PlGF ratios and flow-mediated dilatation (FMD) of the brachial artery in each group of women, according to the outcome of pregnancy (Group A, women with normal Doppler examination and normal outcome; Group B, women with abnormal Doppler examination and four subgroups according to outcome)

Characteristic	Group B				
	Group A Normal outcome (n = 40)	Normal outcome (n = 16)	Development of SGA (n = 15)	Development of PE (n = 12)	
				Without SGA (n = 7)	With SGA (n = 5)
PlGF (pg/mL)	423.3 ± 230.5	405.2 ± 207.0	223.7 ± 197.5*	218.1 ± 172.1	65.2 ± 29.5*
sEng (ng/mL)	6.5 (4.9–7.9)	6.4 (5.7–7.6)	7.5 (5.9–12.1)*	7.6 (6.9–9.7)	15.3 (6.4–26.2)*
sFlt-1:PlGF ratio	1.2 (0.7–2.1)	0.9 (0.7–1.9)	4.1 (1.8–9.5)†	2.6 (0.9–4.5)	16.2 (8.7–48.2)†
(sFlt-1 + sEng):PlGF ratio	1.2 (0.7–2.1)	0.9 (0.7–1.9)	4.1 (1.8–9.6)*	2.7 (0.9–4.6)	16.3 (8.9–48.9)†
FMD (%)	8.4 ± 2.8	7.6 ± 4.5	6.1 ± 3.0	3.8 ± 3.2*	2.6 ± 2.3*

Values are expressed as mean ± SD or as median (interquartile range) for normally and non-normally distributed data, respectively. All comparisons were performed with Group A. * $P < 0.05$. † $P < 0.0001$. PE, pre-eclampsia; SGA, small-for-gestational age.

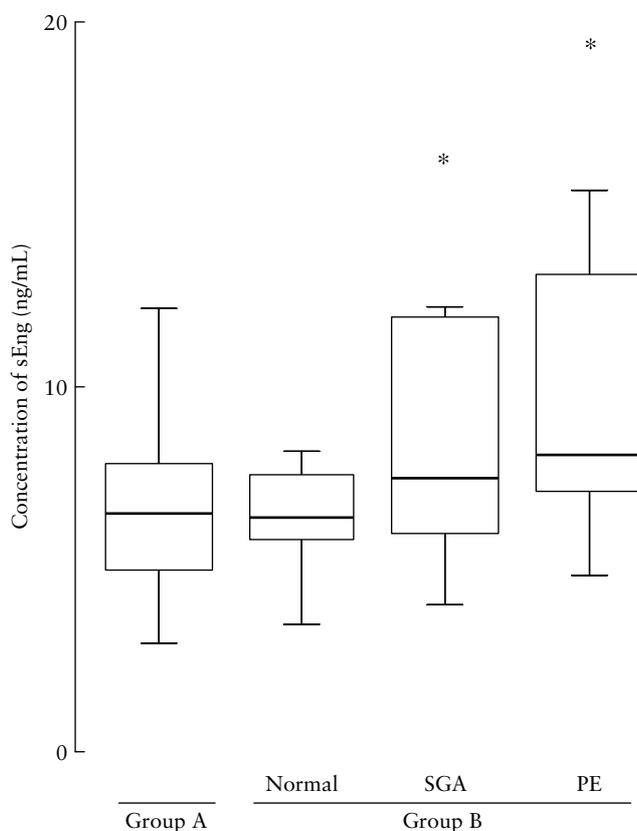


Figure 2 Box-and-whisker plot comparing soluble endoglin (sEng) concentrations at 23–25 weeks' gestation between the different patient groups: Group A ($n = 40$), women with normal Doppler and normal outcome and Group B ($n = 43$), with abnormal Doppler and either normal outcome ($n = 16$), or subsequent delivery of a small-for-gestational-age (SGA) infant ($n = 15$) or subsequent development of pre-eclampsia (PE) ($n = 12$). Boxes represent the interquartile range and lines represent the median. Whiskers represent the highest and lowest values. Significant differences from Group A are indicated (* $P < 0.05$).

Within Group B, women who subsequently developed PE, with or without an SGA infant, had lower levels of PlGF and higher levels of sEng compared to women with normal outcome (PlGF: 154.8 ± 150.8 vs. 423.3 ± 230.5 pg/mL,

$P < 0.001$; sEng: 8.1 (7.0–14.1) vs. 6.5 (4.9–7.9) ng/mL; $P < 0.05$), but this difference was more pronounced in women who developed PE and delivered an SGA infant (Table 2). In the five women who developed early-onset PE (requiring delivery before 34 weeks' gestation) the levels of PlGF and sEng tended to be lower and higher, respectively, compared to women who developed PE later on in pregnancy (PlGF: 141.2 ± 200.8 vs. 164.5 ± 120.4 pg/mL, $P = 0.8$, sEng: 10.7 (8.5–26.2) vs. 7.6 (5.0–8.2) ng/mL, $P = 0.05$), but this difference only reached a borderline significance, probably owing to the small number of patients.

FMD of the brachial artery

FMD of the maternal brachial artery was performed in 37 women from Group A and 36 from Group B (15 with normal outcome, 12 who subsequently developed SGA and nine who developed PE). The levels of FMD in the different subgroups of women are given in Table 2. The women who subsequently developed PE had significantly lower FMD compared to women with normal outcome, suggesting endothelial dysfunction and confirming previous reports¹⁹. In the same subgroup of women there was no correlation between the degree of endothelial dysfunction, as assessed by FMD, and concentrations of PlGF ($P = 0.95$, $r^2 = 0.001$), sEng ($P = 0.87$, $r^2 = 0.004$), sFlt-1:PlGF ratio ($P = 0.74$, $r^2 = 0.02$) and (sFlt-1 + sEng):PlGF ratio ($P = 0.78$, $r^2 = 0.01$).

DISCUSSION

This study has demonstrated that decreased levels of PlGF and increased levels of sEng precede the development of PE and the delivery of SGA infants in women at risk of these complications, as determined by Doppler examination of the uterine arteries during the second trimester of pregnancy. Similarly, elevated levels of the sFlt-1:PlGF and (sFlt-1 + sEng):PlGF ratios, used as a measure of

the balance between antiangiogenic and proangiogenic proteins, also predate the development of PE.

Our findings of alterations in PlGF in women with abnormal placentation are in accordance with previous studies, which have additionally demonstrated that the combined analysis of uterine Doppler examination and angiogenic factors (sFlt-1 and PlGF) during the second trimester of pregnancy substantially improves the sensitivity and specificity of the Doppler examination alone for adverse pregnancy outcome (PE and/or SGA) requiring iatrogenic preterm delivery before 34 weeks' gestation²³. However, sEng has not been previously measured in women with abnormal placentation. Our study indicates that this factor may also be of value in the assessment of this population.

Experimental studies in pregnant rats suggest that the maternal circulating levels of angiogenic and antiangiogenic factors play an important role in the pathogenesis of PE. Administration of sFlt-1 to pregnant rats induced hypertension, proteinuria and glomerular endotheliosis, the classic lesion of PE⁵. Consequently, it has been proposed that in PE the placenta produces elevated levels of the antiangiogenic factor sFlt-1, which captures the free VEGF and PlGF, inhibits their action and causes endothelial dysfunction. In pregnant rodents, over-expression of sEng led to increased vascular permeability, and induced modest hypertension without significant proteinuria¹⁰. Adenoviral-mediated over-expression of both sFlt-1 and sEng caused severe vascular damage, nephritic-range proteinuria and severe hypertension – a syndrome similar to the syndrome of hemolysis, elevated liver enzymes and low platelets – and SGA¹⁰. Thus, sEng and sFlt-1, two antiangiogenic proteins operating through separate mechanisms, may combine to produce endothelial dysfunction and severe PE. However, our findings provide evidence that this might not be the case in humans *in vivo*. In the present study, the lack of association between PlGF, sEng and maternal endothelial function, in women who subsequently developed PE, implies that there is no direct causal relationship between these factors and the endothelial dysfunction that these women demonstrate at this stage of pregnancy. This finding may cast doubt on the notion that the antiangiogenic factors are directly involved in the maternal endothelial dysfunction.

It is currently accepted that maternal endothelial dysfunction observed in women who develop PE persists for many years following delivery²⁴. It could be argued that the depressed FMD observed in the current study represents a baseline risk factor for the development of PE and not a cause of this complication of pregnancy. This could possibly explain the absence of association between maternal endothelial function and angiogenic factors. Moreover, our investigation was a small, cross-sectional study and selection bias cannot be excluded. Further longitudinal studies, even before conception, and studies of the direct effect of the angiogenic factors on endothelial function in human vessels is now required in

order to establish the exact mechanisms that underlie their role in pathological pregnancies.

In summary, we have shown that altered levels of PlGF and sEng predate the development of PE in women with abnormal placentation, as assessed by Doppler examination of the uterine arteries. However, it is possible that these factors are not directly responsible for the maternal endothelial dysfunction that characterizes the syndrome of PE. Further studies assessing the mode of action of these molecules are essential.

ACKNOWLEDGMENT

The study was supported by The Fetal Medicine Foundation (UK Registered Charity number: 1037116).

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