Maternal plasma soluble endoglin at 11–13 weeks’ gestation in pre-eclampsia

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

ABSTRACT

Objectives To examine the performance of screening for pre-eclampsia (PE) by a combination of maternal factors, soluble endoglin (sEng), pregnancy associated plasma protein-A (PAPP-A), placental growth factor (PlGF) and uterine artery lowest pulsatility index (L-PI) at 11–13 weeks’ gestation.

Methods Uterine artery L-PI, sEng, PAPP-A and PlGF were measured at 11–13 weeks in 90 singleton pregnancies that subsequently developed PE, including 30 that required delivery before 34 weeks (early PE) and 60 with late PE, and 180 unaffected controls. Screening performance for PE by maternal factors, sEng, PAPP-A, PlGF and uterine artery L-PI and their combinations was determined.

Results In early PE, compared to controls, plasma sEng and uterine L-PI were significantly increased and serum PAPP-A and PlGF were decreased. In late PE, compared to controls, serum PlGF was decreased and uterine L-PI was increased but plasma sEng and serum PAPP-A were not significantly different. In screening for early PE, the detection rate for a 10% false-positive rate was 46.7% for sEng alone and 96.3% for a combination of maternal factors, sEng, PlGF and uterine artery L-PI and their combinations was determined.

Conclusions Effective screening for early PE can be provided by a combination of maternal factors, sEng, PlGF and uterine artery L-PI at 11–13 weeks’ gestation.

INTRODUCTION

Pre-eclampsia (PE) – a severe disorder unique to human pregnancy characterized by the onset of proteinuria and hypertension after the 20th week of pregnancy – is an important cause of maternal and perinatal morbidity and mortality. Etiologic factors are not fully characterized, although evidence supports the involvement of genetic, immune, angiogenic and other mechanisms. PE has been considered as a two-stage disease in which abnormal placentation precedes endothelial dysfunction. The impaired placentation is due to inadequate trophoblastic invasion of the maternal spiral arteries. Indeed, Doppler ultrasound studies have demonstrated increased pulsatility index (PI) in the uterine arteries from as early as 11–13 weeks’ gestation. There is evidence that pregnancy-associated plasma protein-A (PAPP-A), a placental protein used in first-trimester screening for chromosomal aneuploidies, also plays a role in placentation, and its relationship to uterine artery Doppler has been demonstrated in recent studies.

It has been hypothesized that endothelial dysfunction may represent an anti-angiogenic state mediated by high circulating levels of the anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) associated with low levels of the pro-angiogenic factors placental growth factor (PIGF) and vascular endothelial growth factor. The higher relative concentrations of anti-angiogenic factors are believed to trigger vascular endothelial cell injury in the liver, kidney and brain, as well as the placenta. Effective screening for PE can be provided by combining maternal characteristics and obstetric history with the uterine artery PI and maternal serum PlGF and PAPP-A levels at 11–13 weeks’ gestation. It is uncertain whether the performance of screening can be improved by addition of the measurement of maternal plasma sEng. Several studies have reported that in PE the maternal plasma concentration of sEng is increased (Table 1).
is also contradictory evidence that the increase in plasma sEng precedes the clinical onset of the disease and may be apparent from the first trimester of pregnancy17,20–23.

The aims of our study were, firstly, to investigate whether in pregnancies that subsequently develop PE the maternal plasma concentration of sEng at 11–13 weeks is increased and secondly, to determine whether the addition of sEng improves the performance of screening for PE provided by the combination of maternal factors, uterine artery PI, maternal serum PAPP-A and PlGF.

**METHODS**

**Study population**

This was a case–control study drawn from a large prospective observational 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 + 0 to 13 + 6 weeks of gestation, all women have an ultrasound scan, firstly to confirm gestational age from the measurement of the fetal crown–rump length (CRL); secondly to diagnose any major fetal abnormalities; and thirdly to measure fetal nuchal translucency (NT) thickness as part of screening for chromosomal abnormalities. In addition, the maternal serum PAPP-A and free β-human chorionic gonadotropin levels are determined and the results are combined with the fetal NT to calculate the patient-specific risk for trisomy 2124,25.

We recorded maternal characteristics and medical history, stored serum and plasma at −80°C for subsequent biochemical analysis and measured the PI by transabdominal pulsed Doppler in the left and right uterine arteries and recorded the lowest value (L-PI)6. We have previously reported that L-PI has a better performance in screening for PE than does mean PI6. Written informed consent was 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, in which the present case–control study was nested, was examined between March 2006 and March 2007 and comprised 8234 singleton pregnancies. In 147 (1.8%) cases there was subsequent development of PE, 135 (1.6%) cases developed gestational hypertension and 7922 cases were unaffected by PE or gestational hypertension. In addition, there were 30 (0.4%) pregnancies in which there was at least one episode of hypertension, but in which – on the basis of the available data – it was not possible to determine if the diagnosis was PE; these were excluded from further analysis. Stored maternal blood was available from 90 of the 147 cases who developed PE, and maternal plasma sEng was measured in all these 90 cases, 30 of whom required delivery before 34 weeks (early PE) while 60 delivered later (late PE). We also collected blood samples from 180 unaffected controls. 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 in the case–control study was previously thawed and refrozen.

This study is part of a research program on the early prediction of pregnancy complications, and the data from these patients on serum PlGF and uterine artery L-PI have been included in previous publications6,26. The values of serum PlGF were available in 87 patients in the PE group and 177 patients in the control group of the present study.

**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. A doctor together with the patient...
Outcome measures

The definition of PE used was that of the International Society for the Study of Hypertension in Pregnancy\textsuperscript{27}. The systolic blood pressure should be 140 mmHg or more and/or the diastolic blood pressure should be 90 mmHg or more, developing after 20 weeks' gestation, together with significant proteinuria in previously normotensive women. Significant proteinuria is defined as $\geq 300$ mg 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) for the diagnosis to be established.

Sample analysis

Plasma sEng and serum PI GF were measured by enzyme-linked immunoassay technique using DuoSet\textsuperscript{®} human sENG and Quantikine\textsuperscript{®} human PI GF immunoassay (R&D Systems Europe Ltd., Abington, UK). The lower limits of detection of the assays were 5 pg/mL for sEng and 7 pg/mL for PI GF. Samples whose coefficient of variation of the duplicates exceeded 15% were reanalyzed. Maternal serum PAPP-A was measured using the DELFIA XPRESS analyzer (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA). The intra-assay 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 distribution of maternal plasma sEng was made Gaussian using the following transformation: $Y = \log_{10}(sEng - 10,000)$. The data could not be made Gaussian by more standard transformations, such as log$_{10}$ or square root. The distribution of uterine artery L-PI and PI GF were made Gaussian after log$_{10}$ transformation. Distributions were confirmed to be Gaussian using the Kolmogorov–Smirnov test. Second, multiple regression analysis was used to determine which of the factors among the maternal characteristics and gestation were significant predictors of log(sEng) in the unaffected group. Then the distribution of log(sEng), expressed as multiples of the median (MoM) of the unaffected group, were determined in the PE group. The measured uterine artery L-PI was converted into MoM after adjustment for gestational age, maternal age, BMI and racial origin, as previously described\textsuperscript{6}. The measured concentration of PAPP-A was converted into MoM after adjustment for gestational age, maternal age, racial origin, weight, parity, cigarette smoking status and method of conception, as previously described\textsuperscript{28}. Similarly, the measured PI GF was converted into MoM after adjustment for fetal CRL, maternal weight, racial origin and cigarette smoking status, as previously described\textsuperscript{26}. Third, the Kruskal–Wallis test with post hoc Dunn’s procedure was used to compare median MoM of sEng, uterine artery L-PI, PAPP-A and PI GF between the outcome groups. Fourth, regression analysis was used to determine the significance of association between log(sEng) MoM, log(uterine artery L-PI) MoM log(PAPP-A) MoM and log(PI GF) MoM in the outcome groups. Fifth, the maternal factor-derived a priori risks for PE were determined as previously described and were then logarithmically transformed\textsuperscript{29}. Multivariate logistic regression analysis was used to determine whether the log transformed maternal factor-derived a priori risks, log(sEng) MoM, log(PAPP-A) MoM, log(PI GF) MoM and log(uterine artery L-PI) MoM had a significant contribution to 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 MoMs above given cut-offs. The performance of screening was determined by receiver–operating characteristics (ROC) curve 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, New York, NY, USA) were used for data analysis.

RESULTS

The maternal characteristics of each of the outcome groups are compared in Table 2. In the early-PE group compared to the unaffected group, there were more Black women, more women had PE in their previous pregnancy and were chronically hypertensive on antihypertensives medication, while in the late-PE group, women had a significantly higher BMI, there were more Black women, more women had PE in their previous pregnancy and were chronically hypertensive on antihypertensives medication.

Unaffected group

Multiple regression analysis in the unaffected group demonstrated that for log(sEng) significant independent contributions were provided by maternal weight but not by racial origin ($P = 0.602$), parity ($P = 0.757$), smoking ($P = 0.365$) or fetal CRL ($P = 0.675$):

$$\log(\text{expected sEng}) = 4.313 - (0.002 \times \text{maternal weight (kg)})$$

$$r^2 = 0.154, P = 0.013.$$ In each patient we used this formula to derive the expected log(sEng) and then
expressed the observed value as a MoM of the expected (Table 3).

Pre-eclampsia group
In early PE, compared to controls, plasma sEng and uterine L-PI were statistically significantly increased and serum PAPP-A and PlGF were decreased (Table 3, Figure 1). In late PE, compared to controls, serum PlGF was decreased and uterine L-PI was increased, but plasma sEng and serum PAPP-A were not significantly different. In the PE group there was a significant association between plasma sEng and serum PAPP-A (r = 0.212, P = 0.045) and serum PlGF (r = 0.236, P = 0.0001) but not serum PAPP-A (r = 0.253). In the unaffected group there was a significant association between plasma sEng and serum PAPP-A (r = 0.176, P = 0.019) but not serum PlGF (r = 0.236) or uterine artery L-PI (r = 0.726).

Screening for pre-eclampsia
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) 9.5, 95% CI, 2.6–34.6; P = 0.001), log(uterine artery L-PI) MoM (OR = 1.6e5, 95% CI, 353.0–8.1e7; P < 0.0001), log(PlGF) MoM (OR = 0.002, 95% CI, 0.0–0.10; P = 0.001) and log(sEng) MoM (OR = 465.2, 95%CI, 5.2–4.1e4; P = 0.007), but not from log(PAPP-A) MoM (P = 0.240).

The patient-specific risk for early PE is calculated from the formula: risk = odds/(1+odds), where odds = e\(^r\) and Y is derived from multivariate logistic regression analysis of the disease-specific log transformed maternal factor-derived a priori risk, log(uterine artery L-PI) MoM and log(serum PAPP-A) MoM and log(sEng) MoM: Y = 0.991 × (2.249 × log maternal factor-derived a priori risk) + (12.039 × log(uterine artery L-PI) MoM) − (6.032 × log(PlGF) MoM) + (6.142 × log(sEng) MoM); r\(^2\) = 0.674, P < 0.0001.

The estimated detection rates of early PE at fixed false-positive rates of 5 and 10% and their respective areas under the ROC curves in screening by maternal factor-derived a priori risk, sEng, PAPP-A, PlGF, uterine artery L-PI and by their combinations are shown in Table 4. The estimated detection rate of screening for early PE by sEng
Table 3  Median soluble endoglin (sEng), uterine artery lowest pulsatility index (L-PI), pregnancy associated plasma protein-A (PAPP-A) and placental growth factor (PIGF) in the outcome groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unaffected (n = 180)</th>
<th>Early pre-eclampsia (n = 30)</th>
<th>Late pre-eclampsia (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma sEng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MoM</td>
<td>0.98 (0.78–1.30)</td>
<td>1.38 (1.06–1.87)*</td>
<td>0.90 (0.73–1.21)</td>
</tr>
<tr>
<td>ng/mL</td>
<td>24.2 (21.5–29.2)</td>
<td>31.5 (25.6–38.0)</td>
<td>23.7 (20.2–27.4)</td>
</tr>
<tr>
<td>Uterine artery L-PI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MoM</td>
<td>1.00 (0.80–1.23)</td>
<td>1.65 (1.31–1.85)*</td>
<td>1.31 (1.13–1.55)*</td>
</tr>
<tr>
<td>Unit</td>
<td>1.33 (1.10–1.66)</td>
<td>2.29 (1.91–2.49)</td>
<td>1.88 (1.52–2.19)</td>
</tr>
<tr>
<td>Serum PAPP-A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MoM</td>
<td>1.01 (0.77–1.32)</td>
<td>0.56 (0.47–0.90)*</td>
<td>0.93 (0.57–1.18)</td>
</tr>
<tr>
<td>mU/L</td>
<td>3.04 (1.95–4.57)</td>
<td>2.35 (0.98–3.13)</td>
<td>2.66 (1.53–3.63)</td>
</tr>
<tr>
<td>Serum PIGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MoM</td>
<td>1.01 (0.83–1.33)</td>
<td>0.61 (0.46–0.84)*</td>
<td>0.82 (0.53–1.03)*</td>
</tr>
<tr>
<td>pg/mL</td>
<td>35.5 (27.6–48.6)</td>
<td>20.1 (14.0–33.1)</td>
<td>29.8 (21.5–33.1)</td>
</tr>
</tbody>
</table>

Data are given as median (interquartile range). Comparisons between outcome groups by Kruskal–Wallis test with post hoc Dunn’s procedure. *P < 0.0167. MoM, multiple of the unaffected median.

Figure 1  Box-and-whisker plots (median, interquartile range and range) of soluble endoglin multiples of the median (MoM), placental growth factor MoM and uterine artery lowest pulsatility index MoM in pregnancy outcome groups: unaffected control, early pre-eclampsia (PE), and late PE.

independently was 30.0 and 46.7% at false-positive rates of 5 and 10%, respectively. The combination of maternal factor-derived a priori risk, uterine artery L-PI, PIGF and sEng achieved detection rates of 77.8 and 96.3% for false-positive rates of 5 and 10%, respectively.

The maternal plasma sEng 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’ gestation, in women who subsequently develop early PE, the maternal plasma levels of sEng and uterine artery PI are increased and the maternal serum PIGF and PAPP-A levels are decreased. In late PE compared to unaffected controls the uterine artery PI was increased and serum PIGF was decreased but there was no significant difference in the maternal plasma concentration of sEng or serum PAPP-A. In a previous study we found that the anti-angiogenic protein sFlt-1, which is increased in PE both in the clinical phase of the disease and in the preceding few weeks, is not altered at 11–13 weeks\(^2\). In the unaffected controls, the measured concentration of maternal plasma sEng decreased with maternal weight but did not change significantly with other maternal or fetal characteristics. Consequently, as in the case of

Maternal plasma sEng in combination with factors from the maternal history, uterine artery PI and either serum PI GF or PAPP-A could provide effective first-trimester screening for the subsequent development of early PE. The performance of sEng in screening for PE has been examined in previous studies. De Vivo et al. examined 52 women at 24–28 weeks and reported that measurement of serum sEng would detect 84% of pregnancies destined to develop PE, at a false-positive rate of 20%. Similarly, Lim et al. examined 60 women at 14–19 weeks and reported that sEng could detect 62% of patients who subsequently developed PE, at a false-positive rate of 10%. There is no proven effective method for the prevention of PE. Nevertheless, routine prenatal care in the last 50 years has evolved with the aim of early identification of women at high risk for PE, which could potentially improve pregnancy outcome. 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. Early identification of the group at high risk for the development of PE is also

<table>
<thead>
<tr>
<th>Screening test</th>
<th>FPR 5%</th>
<th>FPR 10%</th>
<th>Area under ROC</th>
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</thead>
<tbody>
<tr>
<td>Maternal risk factor</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>sEng</td>
<td>29.6 (13.8–50.2)</td>
<td>40.0 (22.7–59.4)</td>
<td>0.751 (0.686–0.808)</td>
</tr>
<tr>
<td>PI GF</td>
<td>30.0 (14.8–49.4)</td>
<td>46.7 (28.4–65.7)</td>
<td>0.742 (0.677–0.800)</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>25.9 (11.2–46.3)</td>
<td>59.3 (38.8–77.6)</td>
<td>0.831 (0.772–0.880)</td>
</tr>
<tr>
<td>Uterine artery PI</td>
<td>2.0 (7.8–38.6)</td>
<td>43.4 (25.5–62.6)</td>
<td>0.741 (0.676–0.799)</td>
</tr>
<tr>
<td>Maternal risk factor plus:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sEng</td>
<td>33.3 (17.3–52.8)</td>
<td>56.7 (37.4–74.5)</td>
<td>0.865 (0.811–0.908)</td>
</tr>
<tr>
<td>PI GF</td>
<td>48.1 (28.7–68.0)</td>
<td>66.7 (46.0–83.4)</td>
<td>0.902 (0.852–0.939)</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>46.7 (28.4–65.7)</td>
<td>53.3 (34.3–71.6)</td>
<td>0.839 (0.782–0.886)</td>
</tr>
<tr>
<td>sEng and PI GF</td>
<td>55.6 (35.3–74.5)</td>
<td>74.1 (53.7–88.8)</td>
<td>0.923 (0.877–0.955)</td>
</tr>
<tr>
<td>sEng and PAPP-A</td>
<td>63.3 (43.9–80.0)</td>
<td>73.3 (54.1–87.7)</td>
<td>0.905 (0.857–0.941)</td>
</tr>
<tr>
<td>Uterine artery PI</td>
<td>63.3 (43.9–80.0)</td>
<td>76.7 (57.7–90.0)</td>
<td>0.908 (0.860–0.943)</td>
</tr>
<tr>
<td>Uterine artery PI and PAPP-A</td>
<td>60.0 (40.6–77.3)</td>
<td>83.3 (65.3–94.3)</td>
<td>0.923 (0.878–0.955)</td>
</tr>
<tr>
<td>Uterine artery PI and sEng</td>
<td>66.7 (47.2–82.7)</td>
<td>80.0 (61.4–91.2)</td>
<td>0.937 (0.895–0.966)</td>
</tr>
<tr>
<td>Uterine artery PI and PI GF</td>
<td>77.8 (57.7–91.3)</td>
<td>85.2 (66.3–95.7)</td>
<td>0.945 (0.904–0.972)</td>
</tr>
<tr>
<td>Uterine artery PI, PAPP-A and sEng</td>
<td>63.3 (43.9–80.0)</td>
<td>83.3 (65.3–94.3)</td>
<td>0.943 (0.903–0.971)</td>
</tr>
<tr>
<td>Uterine artery PI, PI GF and sEng</td>
<td>77.8 (57.7–91.3)</td>
<td>96.3 (81.0–99.4)</td>
<td>0.949 (0.909–0.975)</td>
</tr>
</tbody>
</table>

The findings that in pregnancies destined to develop early PE plasma sEng is increased and that there is a significant association between plasma sEng with uterine artery PI and an inverse relationship between sEng and PI GF provide supportive evidence for the hypothesis implicating this anti-angiogenic factor in the pathogenesis of PE. Several previous studies have reported that in established PE plasma sEng is increased by two to 10 times compared to normotensive controls. Three studies also reported that a mild increase in sEng is observed before the clinical onset of the disease and is apparent during the second trimester of pregnancy. Injury to the placental tissue expression of sEng is up-regulated in placentation. Animal experiments have shown that administration of sEng induces a PE-like syndrome with hypertension and proteinuria that may be mediated by inhibition of activation of nitric oxide synthase in endothelial cells, thereby disrupting key homeostatic mechanisms necessary for the maintenance of vascular health.

Animal experiments have shown that administration of sEng induces a PE-like syndrome with hypertension and proteinuria that may be mediated by inhibition of activation of nitric oxide synthase in endothelial cells, thereby disrupting key homeostatic mechanisms necessary for the maintenance of vascular health.
important for future studies investigating the potential role of pharmacological interventions starting from the first trimester to improve placentaion and reduce the prevalence of the disease.

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