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# Maternal Serum Soluble Endoglin at 30–33 Weeks in the Prediction of Preeclampsia

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**Key Words**
Third-trimester screening · Preeclampsia · Soluble endoglin · Pyramid of antenatal care

**Abstract**
***Objective:*** To investigate the potential value of maternal serum concentration of soluble endoglin (sEng) at 30–33 weeks' gestation in the prediction of preeclampsia (PE) developing at or after 34 weeks. ***Methods:*** Serum sEng was measured at 11–13 and at 30–33 weeks' gestation in a case-control study of 50 cases that developed PE at or after 34 weeks and 250 unaffected controls. Regression analysis was used to determine which of the factors amongst the maternal characteristics were significant predictors of first- and third-trimester $\log_{10}$ sEng in the control group. The measured values of sEng were converted into multiples of the unaffected median (MoM) and the MoM values in the PE and controls were compared. ***Results:*** The median sEng MoM at 30–33 weeks was significantly higher in the PE group (1.39, IQR 0.94–2.18) than in the controls (0.95, IQR 0.77–1.19), but at 11–13 weeks there was no significant difference between the groups. In screening by a combination of maternal characteristics and third-trimester sEng, the detection rates of intermediate- and late-PE, at a false-positive rate of 10%, were 64.3 and 50.0%, respectively. ***Conclusion:*** Screening by maternal characteristics and sEng at 30–33 weeks could identify most pregnancies that will subsequently develop PE.


## Introduction

Preeclampsia (PE), which affects 2–3% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality [1–3]. The condition has been subdivided into early-PE, requiring delivery before 34 weeks, intermediate-PE with delivery at 34–37 weeks, and late-PE delivering at or after 38 weeks [4]. We have recently proposed a two-stage strategy for identification of pregnancies at risk of PE [5]. The first stage, at 11–13 weeks, should be primarily aimed at effective prediction of early-PE because the prevalence of this condition can be potentially reduced substantially by the prophylactic use of low-dose aspirin started before 16 weeks' gestation [6, 7]. The sec-

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ond stage, at 30–33 weeks, should be aimed at effective prediction of intermediate- and late-PE because close monitoring of such pregnancies for earlier diagnosis of the clinical signs of the disease could potentially improve perinatal outcome through such interventions as the administration of antihypertensive medication and early delivery.

Endoglin is a homodimeric transmembrane glycoprotein and its soluble circulating form (sEng) is an anti-angiogenic factor implicated in the pathogenesis of PE [8]. Several studies have reported that in PE the maternal plasma concentration of sEng is increased [9–14] and there is also some evidence that the increase precedes the clinical onset of the disease and may be apparent from the first trimester of pregnancy [12, 15–19]. In vitro studies showed that in patients with PE there is upregulation in placental tissue expression of sEng and animal experiments demonstrated that administration of sEng induces a PE-like syndrome with hypertension and proteinuria [8, 11, 20].

The objective of this case-control study is to investigate the potential value of maternal serum concentration of sEng at 30–33 weeks' gestation in the prediction of intermediate- and late-PE.

## Methods

*Study Population*

This was a case-control study drawn from a prospective observational study for adverse pregnancy outcomes in women attending for their routine first- and third-trimester hospital visits in pregnancy at King's College Hospital London and Medway Maritime Hospital Kent between May 2011 and March 2012. In the first-trimester visit, at $11^{+0}$–$13^{+6}$ weeks' gestation, an ultrasound scan was carried out to, firstly, confirm gestational age from the measurement of the fetal crown-rump length [21], secondly, diagnose any major fetal abnormalities, and thirdly, measure fetal nuchal translucency thickness as part of screening for aneuploidies [22]. In addition, the maternal serum pregnancy-associated plasma protein-A and free β-human chorionic gonadotropin were determined and the results were combined with fetal nuchal translucency to estimate the patient-specific risk for aneuploidies [22]. The third-trimester visit, at $30^{+0}$–$33^{+6}$ weeks' gestation, included ultrasound examination for assessment of fetal growth and wellbeing. In both visits maternal blood was collected for research and the serum was stored 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 the ethics committee of each participating hospital.

The base cohort study population, wherein the present case-control study was nested, constituted 5,099 singleton pregnancies. We excluded 244 cases because they had missing outcome data (n = 156), they had PE at the time of screening or before 34 weeks (n = 25), the pregnancy resulted in delivery before 34 weeks' gestation (n = 37) or the birth of babies with major defects (n = 26). In the remaining 4,855 cases, there were 145 (3.0%) cases that developed PE, with 37 cases requiring delivery at 34–37 weeks (intermediate-PE) and 108 cases delivering at or after 38 weeks (late-PE), 161 (3.3%) cases developed gestational hypertension and 4,294 cases were unaffected by PE or gestational hypertension. There was available stored maternal blood from both the first- and third-trimester visits from 50 cases that developed PE and maternal serum sEng was measured in these 50 cases. Each case of PE was matched with 5 controls who had blood collected on the same day at both visits 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 were previously thawed and refrozen.

*Patient Characteristics*

Patient characteristics including maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian, and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs), cigarette smoking during pregnancy (yes or no), history of chronic hypertension (yes or no), family history of PE in the mother of the patient (yes or no) and obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks), previous pregnancy with PE (yes or no), maternal weight and height were recorded.

*Outcome Measures*

The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy [23]. The systolic blood pressure should be 140 mm Hg or more and/or the diastolic blood pressure should be 90 mm Hg or more which develops 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-hour 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*

Serum sEng was measured by enzyme-linked immunoassay (ELISA) technique (Quantikine Endoglin ELISA kit; R&D Systems Europe Ltd, Abingdon, UK). The lower limit of detection of the assays was 0.007 μg/l.

*Statistical Analysis*

Comparisons of maternal characteristics between outcome groups were done by $\chi^2$ or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables.

The values of first- and third-trimester maternal serum sEng were $\log_{10}$ transformed to make their distributions gaussian. Multiple regression analysis was used to determine which of the factors amongst the maternal characteristics and gestation were significant predictors of first- and third-trimester $\log_{10}$ sEng in the

control group. Gestational age at screening was centred by subtracting 12 from gestational age in weeks in the first trimester and 32 from gestational age in weeks in the third trimester. Maternal weight was centred by subtracting 65 kg in the first trimester and 75 kg in the third trimester and maternal height was centred by subtracting 165 cm. Each measured value in the outcome groups was expressed as a multiple of the normal median (MoM) after adjustment for those characteristics found to provide a substantial contribution to the $\log_{10}$-transformed value. Mann-Whitney U test with Bonferroni correction was used to compare the median value of first- and third-trimester sEng MoM between the outcome groups. Regression analysis was used to determine the significance of association between $\log_{10}$ sEng MoM and gestational age at delivery. Likelihood ratios for intermediate- and late-PE were calculated from the fitted bivariate gaussian distributions for sEng.

The a priori risks for intermediate- and late-PE based on maternal characteristics and obstetric history were determined as previously described [24]. Likelihood ratios for intermediate- and late-PE were calculated from the fitted bivariate gaussian distributions for sEng and these were combined with the a priori risks to produce a posteriori risks. The performance of screening for intermediate- and late-PE by maternal characteristics, sEng and their combination was determined by receiver operating characteristic (ROC) curve analysis.

The statistical software package SPSS 20.0 (SPSS, Inc., Chicago, Ill., USA) and MedCalc (MedCalc Software, Mariakerke, Belgium) were used for all data analyses.

**Table 1.** Maternal characteristics in outcome groups

| Characteristic | Control (n = 250) | PE (n = 50) |
|---|---|---|
| Maternal age, years | 31.2 (27.6–34.9) | 29.8 (24.2–33.8) |
| Maternal weight, kg | 76.9 (69.6–85.6) | 77.2 (67.9–90.1) |
| Maternal height, cm | 165 (161–169) | 163 (158–166) |
| Racial origin | | |
| Caucasian | 123 (49.2) | 24 (48.0) |
| Afro-Caribbean | 100 (40.0) | 20 (40.0) |
| South Asian | 9 (3.6) | 1 (2.0) |
| East Asian | 10 (4.0) | 2 (4.0) |
| Mixed | 8 (3.2) | 3 (6.0) |
| Parity | | |
| Nulliparous | 128 (51.2) | 30 (60.0) |
| Parous with no previous PE | 119 (47.6) | 16 (32.0)* |
| Parous with previous PE | 3 (1.2) | 4 (8.0)* |
| Cigarette smoker | 16 (6.4) | 4 (8.0) |
| Family history of PE | 10 (4.0) | 5 (10.0) |
| Conception | | |
| Spontaneous | 246 (98.4) | 48 (96.0) |
| Assisted | 4 (1.6) | 2 (4.0) |
| History of chronic hypertension | 0 | 5 (10.0)* |

Values are medians (interquartile range) or numbers (%). Comparisons between each outcome group with controls: $\chi^2$ test and Fisher's exact test for categorical variables and Mann-Whitney U test. * $p < 0.05$.

## Results

The maternal characteristics of the study population are given in table 1. In the PE group, compared to the control group, there was a higher prevalence of women with personal history of PE and chronic hypertension.

*Unaffected Group*

Multiple regression analysis in the unaffected group demonstrated that for first-trimester $\log_{10}$ sEng significant independent contributions were provided by maternal weight but not by age ($p = 0.658$), height ($p = 0.124$), racial origin ($p = 0.157$), parity ($p = 0.528$), smoking ($p = 0.398$), conception ($p = 0.919$) or gestational age ($p = 0.622$): first-trimester $\log_{10}$ expected sEng = $0.91865 - 0.0015940 \times$ (maternal weight – 65 kg); $R^2 = 0.043$, $p = 0.001$.

Multiple regression analysis in the unaffected group demonstrated that for third-trimester $\log_{10}$ sEng, significant independent contributions were provided by Afro-Caribbean racial origin and parity but not by maternal age ($p = 0.658$), weight ($p = 0.069$), height ($p = 0.406$), smoking ($p = 0.862$), conception ($p = 0.500$) or gestational age ($p = 0.260$): third-trimester $\log_{10}$ expected sEng = 1.015405 + (–0.052225 if Afro-Caribbean racial origin, 0 if other racial origin) + (0.052072 if nulliparous, 0 if parous); $R^2 = 0.057$, $p < 0.0001$.

In each patient we used these formulae to derive the first- and third-trimester expected $\log_{10}$ sEng and then expressed the observed values as MoM of the expected (table 2).

*PE Group*

At 11–13 weeks' gestation the median sEng MoM was not significantly different between the PE group and controls, whereas at 30–33 weeks the level was higher in the PE group than in the controls (table 2; fig. 1). In the PE group there was an inverse correlation between the $\log_{10}$ and MoM value of sEng with gestational age at delivery (intercept = 1.95270, SE = 0.65543, slope = –0.045956, SE = 0.016848, $p = 0.009$; fig. 2). The overlapping gaussian distributions of third-trimester $\log_{10}$ sEng MoM in the normal group and the intermediate- and late-PE groups were used to calculate likelihood ratios for intermediate- and late-PE (table 3).

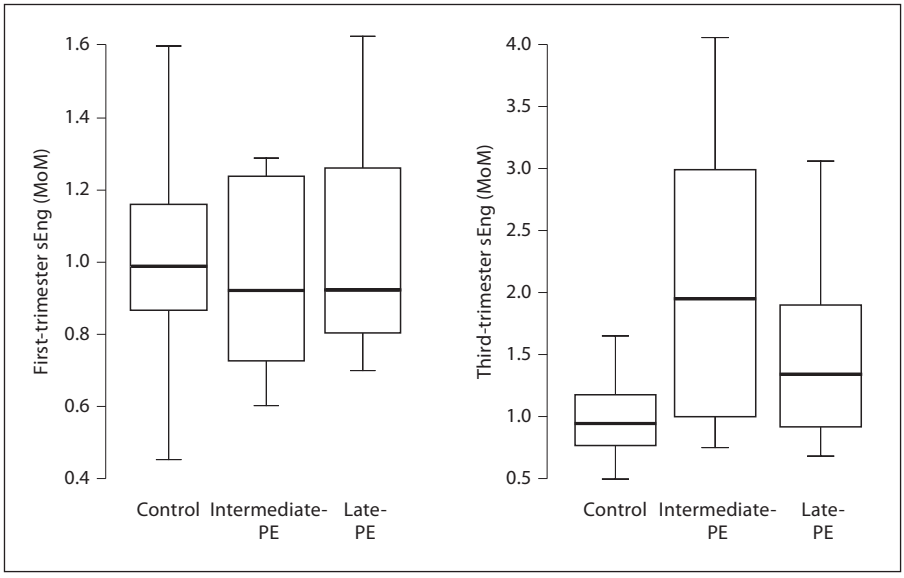


**Fig. 1.** Box-whisker plot of sEng MoM values in the outcome groups at 11–13 weeks' gestation (left) and at 30–33 weeks (right) of pregnancy.

**Table 2.** Median first- and third-trimester serum sEng in the outcome groups

| Outcome group | First-trimester sEng | | Third-trimester sEng | |
|---|---|---|---|---|
| | μg/ml | MoM | μg/ml | MoM |
| Control (n = 250) | 8.15 (7.02–9.47) | 0.99 (0.87–1.16) | 10.04 (8.11–12.64) | 0.95 (0.77–1.19) |
| PE | | | | |
| All cases (n = 50) | 7.53 (6.54–9.63) | 0.92 (0.80–1.25) | 13.87 (9.39–23.01) | 1.39 (0.94–2.18)* |
| Intermediate (n = 14) | 7.08 (5.93–10.18) | 0.92 (0.72–1.24) | 21.68 (10.73–32.05) | 1.99 (1.01–3.06)* |
| Late (n = 36) | 7.70 (6.65–9.54) | 0.92 (0.80–1.27) | 12.50 (8.88–22.15) | 1.35 (0.92–1.92)* |

Values in parentheses are IQR. Comparisons between outcome groups by Mann-Whitney U test with post hoc Bonferroni correction. * $p < 0.025$.

The a priori risks for intermediate- and late-PE based on maternal characteristics and obstetric history were determined as previously described [24]. The a posteriori risks for intermediate- and late-PE were derived by multiplying the a priori risks by the likelihood ratios for third-trimester sEng.

*Performance of Screening for PE*

The areas under ROC and the detection rates of intermediate- and late-PE for false-positive rates of 5 and 10% in screening by maternal characteristics, third-trimester sEng and their combination are given in table 4 and figure 3. In screening for PE by a combination of maternal characteristics and third-trimester sEng, the estimated detection rates of intermediate- and late-PE, at a false-positive rate of 10%, were 64.3 and 50.0%, respectively.

**Table 3.** Likelihood ratios for intermediate- and late-PE from third-trimester sEng MoM

| SEng MoM | Likelihood ratio (95% CI) | |
|---|---|---|
| | intermediate-PE | late-PE |
| 0.5 | 0.28 (0.27–0.30) | 0.53 (0.52–0.57) |
| 1.0 | 0.41 (0.37–0.43) | 0.66 (0.61–0.69) |
| 1.5 | 1.30 (1.25–1.43) | 1.52 (1.48–1.63) |
| 2.0 | 4.73 (4.30–4.98) | 3.92 (3.65–4.07) |
| 2.5 | 22.50 (17.58–27.42) | 12.29 (10.29–14.29) |

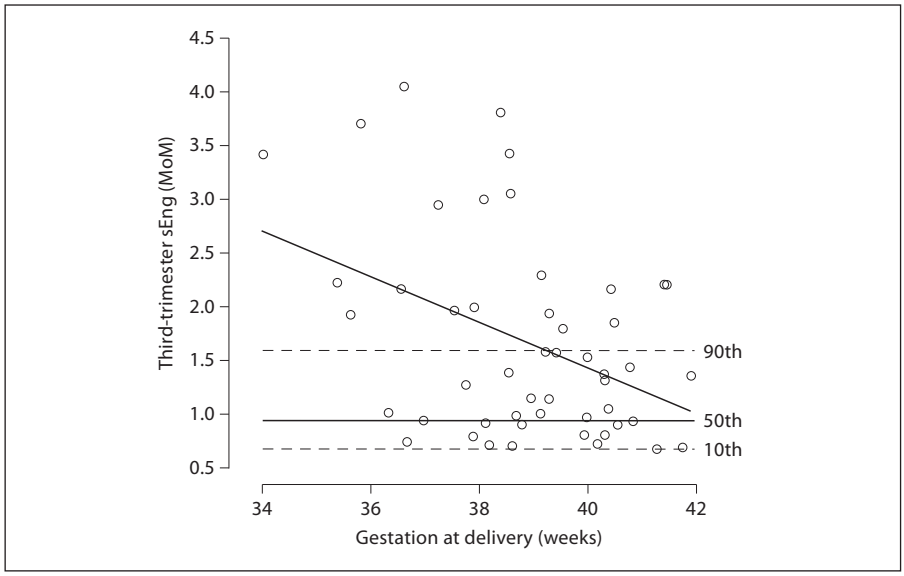


**Fig. 2.** Third-trimester sEng MoM with gestational age at delivery in pregnancies complicated by PE, plotted on the 10th, 50th and 90th percentile of the normal range.

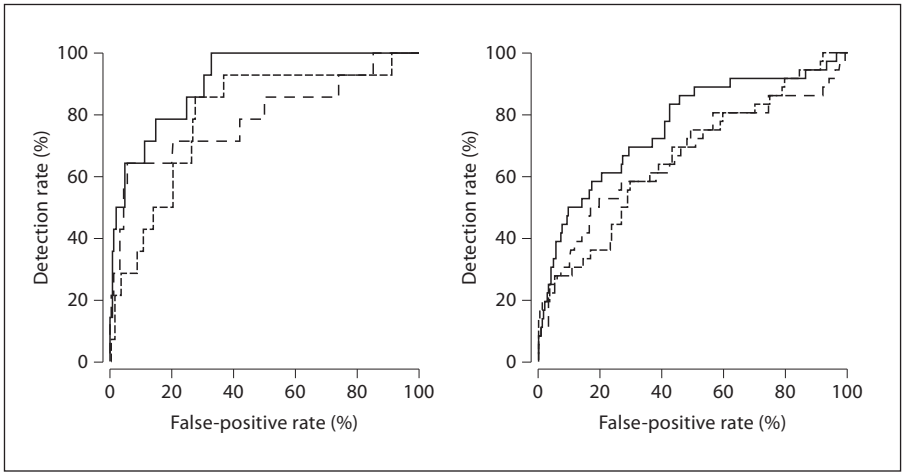


**Fig. 3.** ROC curves of maternal characteristics (-----), sEng at 30–33 weeks' gestation (––––) and their combination (——) in the prediction of intermediate-PE (left) and late-PE (right).

**Table 4.** Performance of screening for intermediate- and late-PE by maternal characteristics, third-trimester sEng and their combination

| Screening test | Intermediate-PE | | | Late-PE | | |
|---|---|---|---|---|---|---|
| | area under ROC (95% CI) | detection rate with (95% CI) for fixed false-positive rate of 5 and 10% | | area under ROC (95% CI) | detection rate with (95% CI) for fixed false-positive rate of 5 and 10% | |
| | | 5% | 10% | | 5% | 10% |
| Maternal history | 0.793 (0.739–0.840) | 21.4 (4.9–50.8) | 35.7 (12.9–64.8) | 0.663 (0.604–0.717) | 27.8 (14.2–45.2) | 30.6 (16.4–48.1) |
| SEng | 0.790 (0.736–0.838) | 57.1 (28.9–82.2) | 64.3 (35.2–87.1) | 0.663 (0.605–0.717) | 22.2 (10.1–39.2) | 33.3 (18.6–51.0) |
| Combined test | 0.908 (0.866–0.940) | 64.3 (35.2–87.1) | 64.3 (35.2–87.1) | 0.762 (0.708–0.810) | 33.3 (18.6–51.0) | 50.0 (32.9–67.1) |

## Discussion

This study has demonstrated that at 30–33 weeks' gestation in pregnancies which subsequently develop PE maternal serum sEng levels are increased and the increase in sEng is inversely related to the severity of the disease reflected in the gestational age at delivery. In screening for intermediate- and late-PE by a combination of maternal characteristics and serum sEng, the estimated detection rates, at a false-positive rate of 10%, were 64 and 50%, respectively.

At 11–13 weeks' gestation in pregnancies which subsequently develop PE requiring delivery at or after 34 weeks, serum sEng levels were not significantly different from those in controls. This finding is compatible with that of a previous study in which we found increased levels of serum sEng at 11–13 weeks in pregnancies that developed early-PE, but not so in those with intermediate- and late-PE [19]. A longitudinal study at 8–40 weeks' gestation demonstrated that patients destined to develop PE before 37 weeks and term PE had significantly higher concentrations of sEng than those with normal pregnancies at 23 and 30 weeks, respectively [25].

In the unaffected group, serum sEng at 30–33 weeks was higher than at 11–13 weeks. This is compatible with the results of a longitudinal study in 46 normotensive pregnancies at 8–40 weeks which reported that plasma sEng level remained relatively stable with gestational age until 25 weeks and then increased thereafter until term [25]. In the unaffected group, multiple regression analysis demonstrated that at 30–33 weeks serum sEng for third-trimester $\log_{10}$ sEng was significantly lower in women of Afro-Caribbean racial origin than in Caucasians and higher in nulliparous than in parous women. Consequently, the measured concentration of sEng must be adjusted for these variables before comparing with pathological pregnancies.

The higher level of serum sEng in women developing PE and the inverse relation between the level of sEng and gestational age at delivery for PE is compatible with the suggested role of this anti-angiogenic factor in inducing vascular endothelial cell injury and dysfunction before the clinical onset of the disease [8].

In our cross-sectional study the selected gestational ages for investigation were 11–13 and 30–33 weeks because these are widely used for assessment of risk for aneuploidies and fetal growth and wellbeing, respectively. In terms of screening for PE, the 11–13 weeks' assessment has been shown to effectively identify about 95% of the cases destined to develop early-PE, by a combination of maternal factors, serum placental growth factor, mean arterial pressure and uterine artery pulsatility index [26]. The importance of effective identification of pregnancies at high risk for early-PE in the first trimester is that the prevalence of the disease could be potentially reduced by prophylactic pharmacological intervention starting early in pregnancy [6, 7]. In the case of intermediate- and late-PE, effective identification of high-risk pregnancies could potentially be achieved by screening at 30–33 weeks. As shown in this study, a high proportion of such cases could be identified by a combination of maternal factors and measurement of serum sEng. Further studies will investigate the potential improvement of such prediction by the inclusion of additional biochemical and biophysical markers, as well as potential improvement in perinatal outcome by closer surveillance of the identified high-risk pregnancies.


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