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Evaluation of Maternal Serum Placental Protein 13 and Soluble Endoglin as Predictive Biomarkers for Preeclampsia

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ABSTRACT

Background: *Preeclampsia involves placental dysfunction and maternal endothelial injury that may be reflected by altered placental protein 13 (PP13) and soluble endoglin (sEng).* ***Objective:*** *To evaluate serum PP13 and sEng, individually and jointly, for distinguishing established preeclampsia from healthy pregnancy.* ***Materials and methods:*** *This hospital-based case-control study included 50 women with preeclampsia and 50 healthy pregnant controls; 27 cases were coded mild and 23 severe. Groups were compared using Welch's t, Mann–Whitney U, or Kruskal–Wallis tests with Holm-adjusted pairwise comparisons. ROC curves quantified discrimination, and repeated five-fold cross-validation assessed internal model performance.* ***Results:*** *PP13 was lower in preeclampsia than controls (84.28 ± 17.97 versus 154.24 ± 25.76; P<0.001; Hedges' g=−3.13), whereas sEng was higher (17.02 ± 4.42 versus 8.61 ± 3.39; P<0.001; rank-biserial r=0.887). sEng increased across controls, mild, and severe preeclampsia (8.61 ± 3.39, 14.20 ± 2.51, and 20.34 ± 3.85; P<0.001). PP13 remained below control values in both case subgroups but was higher in severe than mild disease. PP13 yielded an AUC of 0.996 (95% CI 0.987–1.000) at ≤112.7, with 96% sensitivity and 98% specificity. sEng yielded an AUC of 0.943 (95% CI 0.898–0.976) at ≥11.2, with 100% sensitivity and 72% specificity. The PP13–sEng model had an apparent and mean cross-validated AUC of 1.000.* ***Conclusion:*** *Lower PP13 and higher sEng strongly distinguished preeclampsia in this sample. Because sampling time and assay specifications were unavailable and thresholds came from a small case-control dataset, the results establish biomarker candidacy rather than prospective prediction. Independent gestational-age-standardized validation is required.*

Keywords: Gestational Hypertension; Angiogenic Imbalance; Receiver Operating Characteristic; Case-Control Studies.

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INTRODUCTION

Preeclampsia is a hypertensive disorder of pregnancy with multisystem involvement that continues to be a leading cause of maternal and perinatal morbidity and mortality. Contemporary international consensus management guidelines describe the condition as new-onset hypertension after 20 weeks of gestation, with proteinuria, maternal organ dysfunction, or uteroplacental dysfunction [1] [21]. It may have a clinical course that is more benign or it may progress rapidly, with severe hypertension, neurological impairment, hepatic or renal injury, fetal growth restriction (FGR), placental abruption, and iatrogenic preterm delivery [15] [21]. A 2025 systematic review reported a pooled global prevalence of 4.43%, with differences by geographical region, income level, and diagnostic criteria [2]. Such a burden should make accurate risk stratification and informative biomarkers crucially important even in those settings where maternal-fetal surveillance advanced level is perhaps less widely available.

While the pathogenesis of preeclampsia is complex, abnormal placentation, deficient spiral-artery remodeling, placental ischemic stress, angiogenic imbalance, inflammation, and systemic maternal endothelial dysfunction

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are described as key features [14] [15] [23] [24]. Clinical blood pressure and proteinuria detect established disease but do not fully reflect the antecedent placental disturbance or risk of progression. Therefore, when released from the placenta or induced by endothelial damage, such biomarkers may also be useful in supporting the clinical evaluation [3] [23]. Nevertheless, a useful screening marker should be reproducible across gestational ages, disease phenotypes, assay platforms, and populations—not just have the ability to separate a small number of cases from healthy controls. Hence, current screening strategies do not rely on a single analyte but on a combination of maternal characteristics, mean arterial blood pressure, uterine-artery Doppler, and biochemical markers that has been validated [16]. Placental protein 13 (PP13) is also known as galectin-13 and is encoded by LGALS13. It contributes to maternal–fetal immune tolerance, trophoblast invasion, uteroplacental vascular remodeling, and endothelial function [3][17]. Reduced levels of maternal PP13 early in pregnancy have been linked with subsequent preeclampsia, especially with preterm and early-onset form of the disease [4]. In a meta-analysis of 17 observational studies with 40,474 pregnancies, first-trimester PP13 alone exhibited a pooled sensitivity of 62%, specificity of 84% and a summary AUC of 0.84 [4]. Another meta-analysis involving 14 studies and 8,239 women found a sensitivity of 53% and a specificity of 83%, confirming that PP13 holds biological and screening promise, but does not result in a definitive stand-alone prediction [19].

Soluble endoglin (sEng) is a form of endoglin that is found in the circulation, and a transforming growth factor-β co-receptor that plays a role in vascular development and homeostasis. Strong evidence exists that sEng disrupts proangiogenic signaling and is linked to endothelial dysfunction, vasoconstriction, increased vascular permeability, inflammation, and aberrant placental angiogenesis [3] [22] [24]. A meta-analysis of 20 studies, which was also a systematic review, revealed significantly elevated maternal sEng levels in preeclampsia in the second and third trimesters, but heterogeneity was very high and alterations in the first trimester were not statistically significant [5]. Longitudinal data suggest sEng may increase weeks prior to disease manifestation, particularly in the context of other antiangiogenic perturbations, and elevated levels have been associated with blood pressure, proteinuria, disease severity and poor prognosis [6] [20]. Due to its dependence on gestational age and large between-study variability, however, its universal application as a target concentration is limited.

PP13 and sEng are complementary facets of preeclampsia biology: PP13 is a marker of placental development and immune–vascular modification, and sEng is a marker of antiangiogenic and endothelial dysfunction [17] [22]. Combined evaluation might presumably enhance discrimination compared with each marker separately; precedent from age-adjusted multimarker models and longitudinal studies of angiogenic factors favour testing of biologically complementary combinations [16] [20]. However, direct external validation of the exact PP13–sEng combination remains limited. Furthermore, regional data are required because biomarker distributions may vary with population characteristics, timing, phenotype, sample handling, and assay calibration. The aim of study to compare maternal serum PP13 and sEng in women with preeclampsia and normal pregnant controls in a tertiary care centre in Middle Iraq and to study their pattern over workbook-coded mild and severe disease and to evaluate the discriminative performance of the individual and combined biomarkers to discriminate the study populations.

MATERIALS AND METHODS

Study design, setting, and period

This case-control study was conducted at Babylon Teaching Hospital for Women and Children, in the central Iraq Babylon Gouvernate from September 2025 to January 2026. The practical side of the study was performed at the laboratory of chemistry and Biochemistry Department/ College of Medicine.

The analysis mentioned above was performed solely utilizing the provided electronic workbook. The data consist of 100

individuals, with 50 women classified as having preeclampsia, and 50 healthy pregnant controls. Within the preeclampsia group, 27 records were mild and 23 severe.

Clinical classification and eligibility information

Subjects were categorized as having preeclampsia or a healthy normotensive pregnancy based on the clinical diagnoses at study entry, and preeclampsia participants were further divided into mild or severe subgroups as per the definitions in the source data set. The analysis dataset contains information about maternal-organ malfunction, uteroplacental malfunction, medicine use, fetal results, or the original inclusion and exclusion criteria. The guidelines of the International Society for the Study of Hypertension in Pregnancy and the American College of Obstetricians and Gynecologists were applied solely to offer interpretive clinical context [1] [21].

Data and specimen collection

Full medical and obstetric histories were taken, and clinical examination performed on all women. Standard demographic and clinical data, such as maternal age, body mass index (BMI), were collected from the study participant's formal hospital record.

Peripheral venous whole blood sample was drawn from each subject under aseptic condition for biochemical estimations. To separate the serum, blood samples were centrifuged at 2,500 revolutions per minute for 5 minutes immediately. The obtained serum was then aliquoted in labeled microcentrifuge tubes and stored at −80°C until further use for biochemical analysis in order to avoid degradation and to maintain biomarker stability.

Maternal serum biomarker levels were measured by using commercially available enzyme linked immunosorbent assay (ELISA) kits. The following were the analytes and the related catalogue number: estradiol (E2; Catalogue No. MBS580165), PP13 (Catalogue No. MBS729520), sEng; Catalogue No. E-EL-H6172), and human placental growth factor (PlGF; Catalogue No. E-EL-H1555)

All the tests, such as preparation of reagents, incubation of samples, washing steps, and reading of microplates, were conducted in compliance with the manufacturers' instructions and standard operating procedures of the laboratory.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) with range. Normality of the data was evaluated using Shapiro–Wilk tests, skewness and visual inspection. Preeclampsia and control groups were analyzed with the use of Welch's independent-samples T test for normal or nearly normal variables and Mann–Whitney U test for variables with skewed distribution. Effect size was presented as Hedges' g for parametric comparisons, rank-biserial correlation for nonparametric comparisons and nonparametric bootstrap 95% confidence intervals based on 3,000 resamples for both. Due to the fact that at least one subgroup violated the normality assumption or showed significant heteroscedasticity, Kruskal–Wallis test was used to compare healthy controls, mild preeclampsia and severe preeclampsia. Pairwise Mann–Whitney tests were conducted following a significant global finding and were adjusted with the Holm method. Associations among PP13, sEng, PlGF, BMI, and an ordinal binary severity code were examined in the preeclampsia group using Spearman rank correlation. The discrimination between preeclampsia and healthy controls was evaluated by ROC curves. As lower PP13 was indicative of disease, its directionality was reversed with respect to ROC analyses. AUCs were accompanied by 95% bootstrap confidence intervals with 3,000 replications. All tests were two-sided, and a $P<0.05$ was considered significant; exact P values are reported unless $P<0.001$.

RESULTS

The results appear in Table 1. The analysis included 100 participants, equally divided between the preeclampsia and healthy-control groups. Women with preeclampsia were modestly younger than controls (25.34 ± 1.64 versus 26.38 ± 1.87 years; $P=0.006$), whereas BMI was significantly higher (30.63 ± 3.53 versus 26.18 ± 3.49 kg/m²; $P<0.001$).

PP13 was substantially lower in preeclampsia than in controls (84.28 ± 17.97 versus 154.24

± 25.76; Welch's t=−15.75, P<0.001). The standardized difference was very large (Hedges' g=−3.13, 95% CI −3.70 to −2.72). In contrast, sEng was significantly higher in preeclampsia (17.02 ± 4.42 versus 8.61 ± 3.39; Mann–Whitney U=2358.5, P<0.001), with a rank-biserial correlation of 0.887 (95% CI 0.801–0.954

Table 1. Case-control comparison of maternal characteristics and serum measurements.

Variable	Healthy controls (n=50), mean ± SD (range)	Preeclampsia (n=50), mean ± SD (range)	*P* value	Effect size (95% CI)
Age, years	26.38 ± 1.87 (24.0–29.0)	25.34 ± 1.64 (23.0–28.0)	0.006	*r*rb=−0.31 (−0.51 to −0.089)
BMI, kg/m²	26.18 ± 3.49 (20.8–35.2)	30.63 ± 3.53 (23.7–37.9)	<0.001	*r*rb=0.61 (0.46–0.77)
PP13 pg/mL	154.24 ± 25.76 (109.6–198.3)	84.28 ± 17.97 (55.4–119.2)	<0.001	Hedges' g=−3.12 (−3.7 to −2.7)
sEng ng/mL	8.61 ± 3.39 (3.1–14.2)	17.02 ± 4.42 (11.2–27.2)	<0.001	*r*rb=0.88 (0.801–0.954)
E2, pg/mL	928.93 ± 19.95 (900.6–988.5)	1051.63 ± 30.30 (990.5–1110.2)	<0.001	*r*rb=1.00 (1.00–1.00)
PlGF, pg/mL	555.44 ± 244.08 (177.3–980.2)	64.87 ± 40.84 (10.5–144.1)	<0.001	*r*rb=−1.00 (−1.00 to −1.00)

†PP13 and sEng units were not specified in the supplied workbook and are reported in source-workbook units. Effect direction is preeclampsia minus control. *r*rb, rank-biserial correlation.

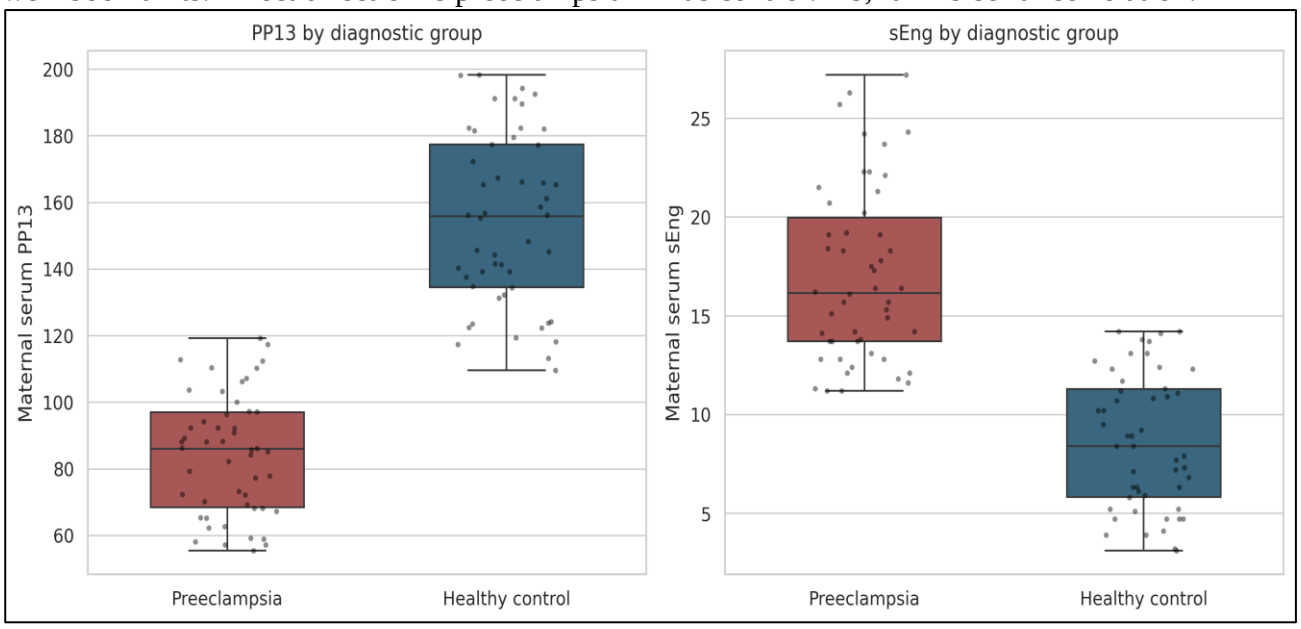

Figure 1. Maternal PP13 and sEng distributions by case-control group. Points show individual observations; central distribution summaries demonstrate markedly lower PP13 and higher sEng in preeclampsi

The results appear in Table 2. PP13 and sEng differed significantly across healthy controls, mild preeclampsia, and severe preeclampsia (both global P<0.001). PP13 was lower in both preeclampsia subgroups than in controls; however, the severe group had a higher mean PP13 than the mild group (96.14 ± 14.08 versus 74.18 ± 14.51; Holm-adjusted P<0.001). Conversely, sEng showed a monotonic increase from controls through mild to severe disease (8.61 ± 3.39, 14.20 ± 2.51, and 20.34 ± 3.85, respectively), and every pairwise comparison remained significant after Holm adjustment (P<0.001). E2 increased and PlGF decreased from mild to severe preeclampsia, while BMI did not differ

significantly between the two preeclampsia subgroups (adjusted *P*=0.199).

Table 2. Comparison across healthy-control, mild-preeclampsia, and severe-preeclampsia groups.

Variable	Healthy controls (n=50), mean ± SD (range)	Mild PE (n=27), mean ± SD (range)	Severe PE (n=23), mean ± SD (range)	*P* value
Age, years	26.38 ± 1.87 (24.00–29.00)	25.11 ± 1.50 (23.00–28.00)	25.61 ± 1.78 (23.00–28.00)	0.015
BMI, kg/m²	26.18 ± 3.49 (20.80–35.20)	30.10 ± 2.76 (26.30–35.40)	31.26 ± 4.25 (23.70–37.90)	<0.001
PP13 pg/mL	154.24 ± 25.76 (109.60–198.30)	74.18 ± 14.51 (55.40–112.70)	96.14 ± 14.08 (57.20–119.20)	<0.001
sEng ng/mL	8.61 ± 3.39 (3.10–14.20)	14.20 ± 2.51 (11.20–21.30)	20.34 ± 3.85 (13.10–27.20)	<0.001
E2, pg/mL	928.93 ± 19.95 (900.06–988.50)	1039.45 ± 27.78 (990.50–1085.30)	1065.93 ± 27.15 (1022.70–1110.20)	<0.001
PlGF, pg/mL	555.44 ± 244.08 (177.30–980.20)	98.30 ± 22.52 (66.40–144.10)	25.61 ± 11.50 (10.50–44.20)	<0.001

†Reported in source-workbook units. PE, preeclampsia. After significant global tests, Holm-adjusted pairwise Mann–Whitney comparisons showed significant PP13 and sEng differences for control versus mild, control versus severe, and mild versus severe groups (all adjusted *P*<0.001).

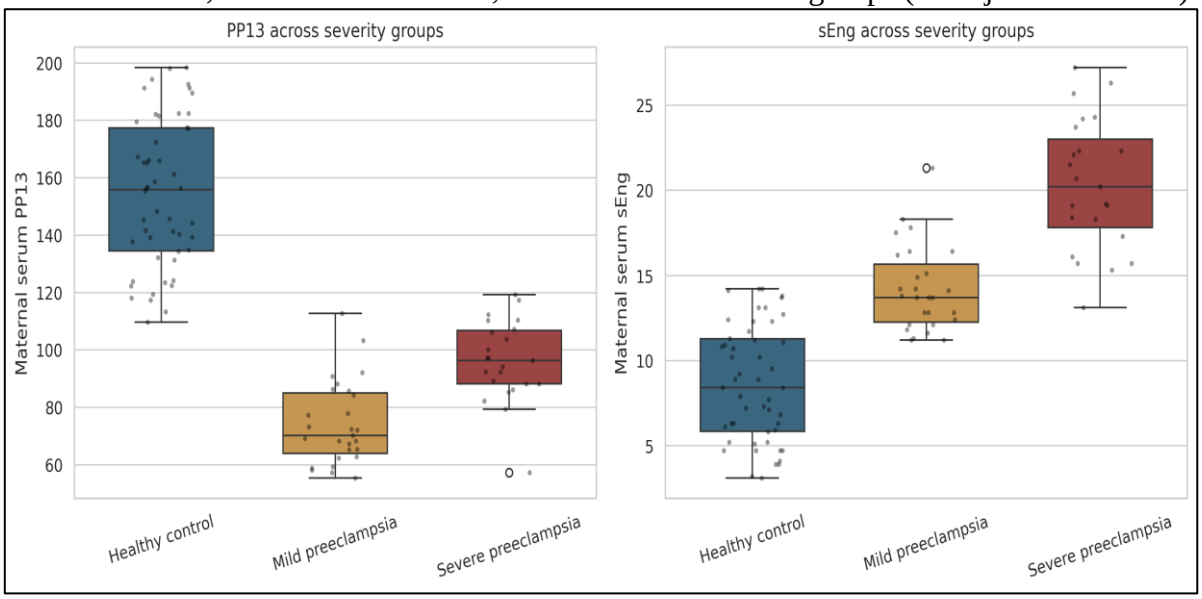

Figure 2. Severity-stratified PP13 and sEng distributions. PP13 was lower in both preeclampsia groups than in controls but higher in severe than mild disease; sEng increased stepwise across the three groups.

The ROC results appear in Table 3 and Figure 3. PP13 provided near-perfect case-control discrimination, with an AUC of 0.996 (95% CI 0.987–1.000). At the data-derived threshold ≤112.7, sensitivity was 96%, specificity 98%, PPV 98.0%, NPV 96.1%, and accuracy 97%. sEng produced an AUC of 0.943 (95% CI 0.898–0.976). At ≥11.2, sEng identified all cases (100% sensitivity and 100% NPV) but had 72% specificity, yielding 86% accuracy.

A model combining PP13 and sEng achieved an apparent AUC of 1.000 with 100% sensitivity and specificity in the development data. Its mean AUC across 100 repeated cross-validation folds was also 1.000 (SD 0.000). Adding age and BMI did not improve apparent performance; that model had an apparent AUC of 1.000 and a mean cross-validated AUC of

0.999 (SD 0.003; range 0.990–1.000). Because thresholds and model coefficients were derived in the same small case-control sample, these estimates are internal and not externally validated.

Table 3. ROC performance for distinguishing preeclampsia from healthy pregnancy

Marker/model	AUC (95% CI)	Data-derived decision rule	Sensitivity	Specificity	PPV	NPV	Accuracy
PP13	0.996 (0.987–1.000)	≤112.7†	96%	98%	98.0%	96.1%	97%
sEng	0.943 (0.898–0.976)	≥11.2†	100%	72%	78.1%	100%	86%
PP13 + sEng	1.000 (1.000–1.000)	Model probability ≥0.974	100%	100%	100%	100%	100%
PP13 + sEng + age + BMI	1.000 (1.000–1.000)	Model probability ≥0.986	100%	100%	100%	100%	100%

†Threshold is expressed in source-workbook units. AUC confidence intervals were estimated by 3,000 bootstrap resamples. PPV and NPV are conditional on the study's artificially balanced 50% case prevalence and should not be transferred to clinical populations with different prevalence.

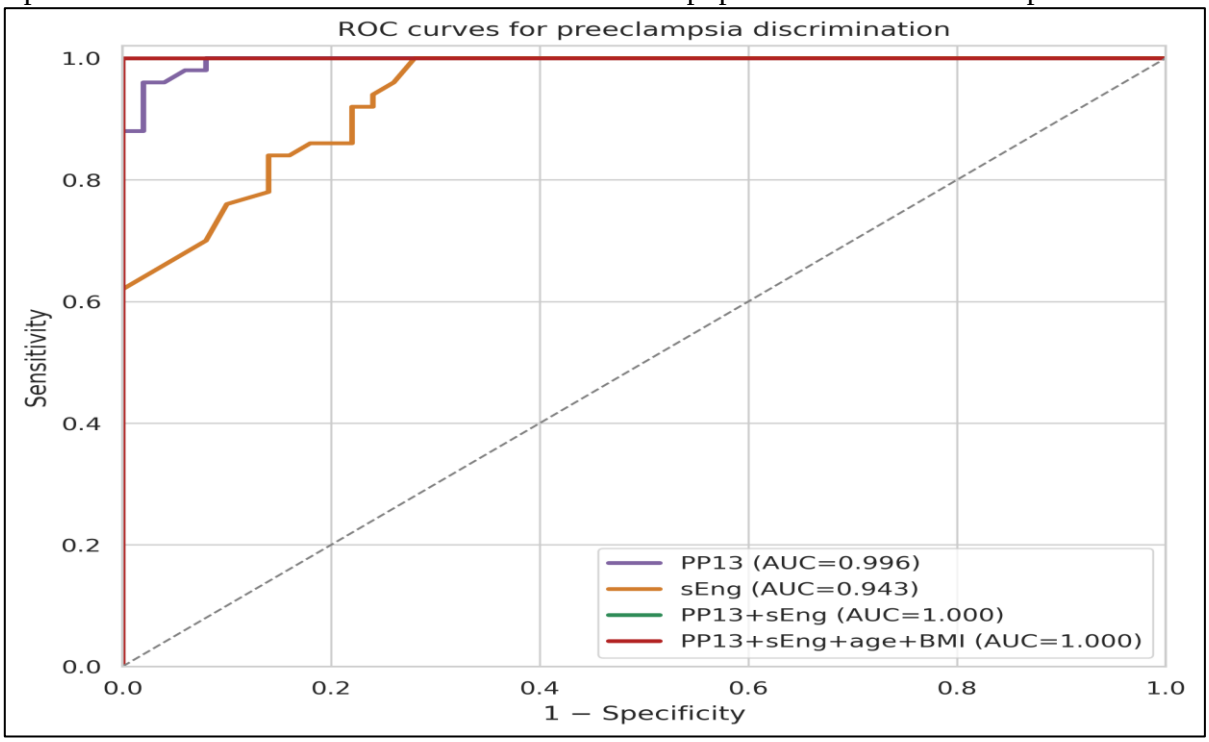

Figure 3. ROC curves for PP13, sEng, and combined models. The perfect apparent performance of the combined models should be interpreted cautiously because evaluation occurred in the development case-control sample.

Within the preeclampsia group, sEng correlated positively with severe disease (Spearman ρ=0.709, *P*<0.001), and PP13 also showed a positive correlation with the workbook-coded severe category (ρ=0.622, *P*<0.001), reflecting the higher PP13 values in

severe than mild cases. PP13 and sEng were moderately positively correlated with each other (ρ=0.430, *P*=0.002). Both PP13 and sEng were inversely correlated with PlGF (ρ=−0.687 and −0.580, respectively; both *P*<0.001). Neither biomarker was significantly correlated with BMI among cases.

Table 4. Selected Spearman correlations within the preeclampsia group.

Variables	Spearman ρ	*P* value
PP13 and sEng	0.430	0.002
PP13 and severe-category code	0.622	<0.001
sEng and severe-category code	0.709	<0.001
PP13 and PlGF	−0.687	<0.001
sEng and PlGF	−0.580	<0.001
PP13 and BMI	0.256	0.073
sEng and BMI	0.191	0.184

DISCUSSION

This study demonstrated marked, directionally opposite alterations in the two principal biomarkers: maternal PP13 was substantially lower, whereas sEng was substantially higher, in women classified with preeclampsia than in healthy pregnant controls. The magnitude of both differences was large, and each biomarker showed excellent case-control discrimination. PP13 yielded an AUC of 0.996 with 96% sensitivity and 98% specificity at a cutoff of ≤112.7 source-workbook units; sEng yielded an AUC of 0.943 with 100% sensitivity and 72% specificity at a cutoff of ≥11.2 units. There was also a progressive increase in sEng from control to mild to severe groups, indicating a potential link with severity of the disease. The PP13 severity profile was more nuanced: levels continued to be lower than controls in both preeclamptic groups but merely higher in severe than mild cases. Combining PP13 and sEng gave perfect apparent and internally cross-validated discrimination, but this finding should be considered as hypothesis-generating rather than proven clinical predictions.

The decreased level of PP13 in the preeclampsia group is consistent with the known biological and epidemiological literature. PP13 promotes implantation, placental vascular remodelling and maternal–fetal immune tolerance and decreased circulating levels, particularly in early gestation, have been associated with later preeclampsia [3] [17]. Wu, Liu and Ding, analyzed 17 studies including 40,474 pregnancies, and found the summary AUC of the first-trimester PP13 alone was 0.84, with sensitivity of 62% and specificity of 84% [4]. Vasilache et al. isolated 14 studies and resulted in decreased sensitivity (53%) with comparable specificity (83%), confirming its insufficient standalone utility [19]. Romero and associates also reported that low PP13 at 8–13 weeks was most predictive of early-onset and preterm preeclampsia but was a poor performer for mild disease at term [7]. These data support PP13 as a marker for aberrant placental development, while highlighting gestational age and phenotype-dependence.

The present AUC of 0.996 is substantially higher than the pooled first-trimester estimate and should not be interpreted as superior prospective screening performance. This investigation compared already classified hospital cases with healthy controls, whereas longitudinal screening studies measure PP13 before disease onset in a more heterogeneous population. Such case-control spectrum selection tends to increase apparent discrimination. A recent third-trimester case-control study by Palalioglu and Erbiyik reported lower PP13 in preeclampsia but more modest accuracy: a cut-off ≤107.03 pg/mL gave 72.5% sensitivity and 77.5% specificity [8]. Although their threshold is numerically near the present value of 112.7, direct equivalence cannot be claimed because units and assay details were unavailable in the current workbook.

Regional evidence also supports lower PP13 in established disease. Nasser, Majid, and Baban studied Iraqi women in the third trimester and reported mean PP13 of approximately 54.29 pg/mL in preeclampsia versus 67.06 pg/mL in controls [9]. They found similar concentrations in mild and severe disease, unlike the higher PP13 observed in the present severe subgroup. Importantly, PP13 behavior can reverse with disease stage: Than et al. reported reduced placental expression but increased maternal serum PP13 in preterm preeclampsia and HELLP syndrome, plausibly because of enhanced syncytiotrophoblast shedding [18]. The current within-case increase may therefore reflect gestational-age differences, disease-onset phenotype, treatment, sample timing relative to clinical deterioration, placental release dynamics, assay variation, or severity misclassification. Because the workbook did not contain gestational age or diagnostic components, this unexpected gradient cannot be resolved. It should not be interpreted as evidence that higher PP13 causes or reliably marks severe disease.

The sEng findings are strongly concordant with prior evidence. A meta-analysis of 20 studies involving 1,146 women with preeclampsia and 1,675 normotensive pregnant women found significantly elevated sEng in the second and third trimesters, with pooled mean differences of 5.554 and 31.006 ng/mL, respectively [5]. Longitudinal work by Levine et al. further showed that sEng can rise two to three months before clinical onset, with earlier elevation in pregnancies that subsequently develop preterm disease [20]. The very high heterogeneity in the meta-analysis (I^2 97–98%) demonstrates why universal absolute thresholds are difficult to justify. The present stepwise rise from 8.61 in controls to 14.20 in mild and 20.34 in severe preeclampsia is nevertheless biologically coherent with increasing antiangiogenic stress [22] [24].

The present sEng AUC of 0.943 closely matches the AUC of 0.94 reported by Nikuei and colleagues, who observed means of 13.58 ng/mL in controls, 24.08 ng/mL in mild disease, and 26.34 ng/mL in severe disease; their 20.4-ng/mL cut-off produced 92.1% sensitivity and 90.0% specificity [10]. Leaños-Miranda et al., in a much larger study of 1,002 women with preeclampsia, found progressively higher sEng across disease without severe features, disease with severe features, and HELLP/eclampsia, together with associations with blood pressure, proteinuria, organ injury, adverse outcomes, and earlier delivery [6]. Sachan et al. likewise reported increasing sEng from healthy pregnancy through nonsevere, severe, and eclamptic groups, with an AUC of 1.00 in a strongly selected case-control sample [11]. These studies support the severity relationship while illustrating how selected clinical groups can generate exceptionally high apparent accuracy. The current findings are also consistent with Iraqi studies. Muhammed, Ali, and Hameed reported increasing sEng from normotensive controls to nonsevere and severe preeclampsia and an AUC of 1.00 [12]. More recently, Al-Khalidi, Al Ghazali, and Kermasha observed higher serum sEng in Iraqi cases than controls and an increase from mild to severe disease [13]. Absolute values differed considerably among these studies and the present dataset. Differences in gestational timing, specimen handling, ELISA calibration, population characteristics, and units are plausible explanations and underscore that the threshold of 11.2 found here is sample- and assay-specific.

The opposite case-control direction of PP13 and sEng offers a biologically plausible complementary signal. Low PP13 may reflect impaired syncytiotrophoblast function, abnormal trophoblast invasion, deficient immune tolerance, and inadequate uteroplacental remodeling [17]. Elevated sEng represents an antiangiogenic response that disrupts transforming growth factor-β and endothelial nitric-oxide signaling, promotes vascular permeability and hypertension, and can act synergistically with sFlt-1 [22]. Combining a placentation-related marker with an antiangiogenic endothelial marker could therefore capture different components of the heterogeneous preeclampsia pathway [14] [23].

Nevertheless, the combined AUC of 1.000 must be interpreted with substantial caution. The study contained only 50 cases and 50

controls; thresholds and coefficients were selected in the same dataset used to describe apparent accuracy; cases and controls were strongly separated by several variables; and conventional multivariable logistic regression failed because of separation. Repeated cross-validation assesses reproducibility across partitions of the same source sample but cannot test transportability to another hospital, assay lot, gestational window, or unselected obstetric population. Moreover, no established external validation study of the exact PP13–sEng pair was identified. The appropriate interpretation is therefore that the combination merits prospective evaluation, not that it currently provides perfect clinical prediction.

The distinction between discrimination of established disease and prediction before disease onset is fundamental. To establish predictive utility, biomarkers must be measured at a prespecified gestational window among women who are free of preeclampsia, and participants must then be followed for standardized outcomes. PP13 should ideally be expressed as gestational-age-adjusted multiples of the median, while sEng thresholds should be calibrated to the assay and sampling period. This design principle is consistent with first-trimester multimarker guidance and with longitudinal evidence that the timing of angiogenic-marker changes depends on disease phenotype [16] [20]. External validation should report calibration, decision-curve utility, and performance separately for early-onset, preterm, term, and severe disease [23].

BMI was significantly higher in the preeclampsia group, consistent with obesity as a recognized clinical risk factor in clinical guidance and contemporary reviews, while the modest age difference favored slightly younger cases in this sample [15] [21]. Adjustment could not be estimated reliably by unpenalized logistic regression because the biomarker distributions produced quasi-complete separation. The combined age/BMI model did not materially improve cross-validated discrimination, but this should not be taken to exclude confounding. A larger study with adequate overlap and prespecified modeling is necessary to estimate adjusted associations.

E2 was higher and PlGF lower in preeclampsia, with complete separation of observed ranges. Low PlGF is consistent with the established angiogenic-imbalance model, in which excess placental antiangiogenic factors reduce free proangiogenic signaling [23] [24]. These secondary results also demonstrate the selected spectrum of the dataset. Within cases, both PP13 and sEng were inversely related to PlGF. The moderate positive correlation between PP13 and sEng, despite their opposite case-control directions, reflects the unusual increase of PP13 from mild to severe cases and reinforces that cross-group and within-group associations need not have the same interpretation.

Strengths and limitations

The study has strengths including numbers of cases and controls (n=82 and 79, respectively), a priori separation of workbook categories mild and severe, no missing data for any of the continuous variables recorded, effect size reporting, pairwise tests adjusted for multiplicity, bootstrap confidence intervals for AUC, and repeated cross-validation. All analysis was reproducible, with every headline statistic and generated output confirmed by independent validation.

Inferences are substantially limited by several factors. First, case-control design does not yield temporal prediction despite the use of term “predictive biomarkers” in the study title. Second, the sample size was small, single-center and artificially balanced; thus, PPV and NPV do not mirror obstetric prevalence in routine practice. Third, the workbook did not include gestational age, parity, blood pressure, proteinuria, onset phenotype, treatment, maternal comorbidities or pregnancy outcomes. Fourth, the diagnostic and severity criteria were not codified, so the case definitions could not be independently verified. Fifth, laboratory techniques, units of assay, manufacturer, accuracy, sample storage and drawing time information were unavailable, which affected the reproducibility and comparability of the determined thresholds. Sixth, the same source sample was used for model development and performance estimation, and complete separation precluded stable multivariable odds-ratio estimates.

Finally, the unexpected PP13 severity pattern may be proxying for unmeasured timing or phenotype differences and must be replicated

Clinical and research implications

These data provide strong local evidence that low PP13 and high sEng characterize women with established preeclampsia and that sEng may reflect workbook-coded severity. Before either marker is adopted for screening, an adequately powered prospective study should enroll consecutive pregnant women before disease onset, measure biomarkers at standardized gestational ages, document assay quality control, and apply prespecified thresholds. The PP13–sEng model should then be externally validated against clinical maternal factors and established strategies incorporating PlGF, mean arterial pressure, and uterine-artery Doppler, with assessment of calibration and incremental clinical utility [16] [24]. Longitudinal analyses should also determine whether PP13 adds information beyond the combined antiangiogenic profile that precedes clinical disease [20]. Such work could determine whether the complementary biological information observed here translates into useful prediction in the Babylon obstetric population.

CONCLUSION

Maternal serum PP13 was markedly lower and sEng markedly higher in women with preeclampsia than in healthy pregnant controls. sEng increased stepwise from mild to severe disease, whereas PP13 showed a non-monotonic severity pattern despite remaining below control values. Each biomarker demonstrated excellent case-control discrimination, and their combination produced perfect apparent discrimination in this dataset. These findings support PP13 and sEng as promising complementary biomarkers of established preeclampsia, but they do not yet establish prospective predictive performance. Standardized assays, complete clinical phenotyping, prespecified gestational sampling, and independent prospective validation are required before clinical implementation.

Declarations

Ethics approval:

The protocol of this study was approved by the ethics committee of University of Babylon/College of Medicine (Code of approval: 5-75; Dated: 18 September 2025) and the study protocol was in compliance with the committee's standards and following the principles of the Declaration of Helsinki (1964) along with its later amendments.

Competing interests:

The authors declare no competing interests.

Funding: None.

Data availability:

Sequence alignments and supporting molecular data are available from the corresponding author upon reasonable request.

Author contributions:

Saba Muhammed Jassim: study design, laboratory work, data analysis, and manuscript writing, manuscript review, and editing.

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