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Diagnostic Efficacy of a Multi-Biomarker Panel Comprising Angiogenic, Metabolic, and Endocrine Markers for Preeclampsia Detection and Severity Stratification in an Iraqi Cohort

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ABSTRACT

Background & Objective: Preeclampsia (PE) causes an important proportion of maternal and neonatal morbidity and mortality globally, especially in countries lacking resources where diagnostic capabilities are minimal. However, full multi-marker panels reflecting angiogenic and metabolic pathways have not been assessed in Middle Eastern populations, which are in dire need of population-specific data. This study was conducted with aim to estimate the diagnostic utility of leptin, Vascular Endothelial Growth Factor (VEGF), soluble Fms-Like Tyrosine kinase-1 (sFlt-1), Estradiol (E2), and Placental Growth Factor (PlGF) in maternal serum for detection in the early stage of PE and its severity.

Materials & Methods: This case-control study was performed in the inpatients section of the Babylon Teaching Hospital, Iraq. A total of 120 third-trimester pregnant women (60 preeclamptic and 60 normotensive controls) were included. The preeclamptic women included mild (n=32) and severe (n=28) cases. Sera biomarkers were detected by ELISA and predictive value was estimated by ROC and binary logistic regression. Data were analyzed using the Graph Pad Prism (version 9). P<0.05 was considered significant.

Results: All five biomarkers were significantly deregulated in PE (P<0.001). VEGF and PlGF were markedly suppressed, and leptin, sFlt-1, and estradiol levels increased with severity. The combined model yielded 100% accuracy for both diagnosis and severity stratification. PlGF exhibited perfect discrimination for severity (AUC=1.000); sFlt-1 (OR=2.80) and leptin (OR=1. (80) were significant independent risk factors for severe progression.

Conclusion: The integration of angiogenic markers with metabolic and endocrine markers provides a robust framework that enhances diagnostic precision and severity stratification, offering translational potential for risk-directed surveillance in resource-limited obstetric settings.

Keywords: Pregnancy-induced hypertension, Angiogenesis modulating agents, Adipokines, Estrogens

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1. Introduction

Preeclampsia (PE) occurring in multiple systems, arising in the latter half of gestation, affects an estimated 3-8% of pregnancies globally **(1)**. It is still one of the most important reasons leading to maternal and perinatal morbidity and mortality, with about 500,000 perinatal deaths and 76,000 maternal deaths attributed to this condition annually **(2)**. The burden of this disease is most heavily weighted toward Low- And Middle-Income Countries (LMICs), where the provision of comprehensive antenatal surveillance and timely obstetric care is limited **(3)**. Within the Middle East, the reported prevalence of PE ranges from 0.17% to 5%, and Hypertensive Disorders of Pregnancy (HDP) represent the second cause of maternal mortality **(4)**. PE is a major cause of poor obstetric outcomes in Iraq; however, biomarkers for the local population are unavailable **(5)**. With regard to clinical presentation, the hallmark features comprise new-beginning hypertension, accompanied by proteinuria at or beyond 20 weeks of gestation. However, contemporary diagnostic frameworks recognise that PE may present severe end-organ complications including thrombocytopenia, hepatic impairment, progressive renal inability, pulmonary oedema, and cerebral or visual disorder even in the lack of clinically significant proteinuria **(6,7)**.

Although PE has been extensively studied for decades, its exact pathophysiology is still not fully understood. Trophoblast invasion and uterine spiral arter remodelling in early placentation are now acknowledged to be aberrant in PE, leading to chronic placental ischemia and oxidative stress **(8)**. This placental failure then induces a profound dysregulation in circulating pro- and antiangiogenic factors leading to disseminated maternal systemic endothelial damage and inflammatory activation **(9)**. Central to this angiogenic disturbance are changes in the amount of Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF) in maternal serum, accompanied by a substantial increase in dissolved Fms-Like Tyrosine kinase-1 (sFlt-1). VEGF and PlGF stimulate normal vascular development and endothelial function in the fetoplacental unit under normal pregnancy **(10)**. In preeclampsia, the hypoxic placenta releases large amounts of sFlt-1 in to the maternal circulation; this soluble receptor functions as a powerful antagonist by binding circulating VEGF and PlGF and leads to disruption of the endothelium and ultimately causes the clinical signs of this disease **(11,12)**. The sFlt-1/PlGF quotient has experienced a surge of clinical relevance as a predictive tool, the negative predictive number of a ratio of less than 38 for ruling out PE development within one week being very high **(13)**.

In addition to established angiogenic markers, other biochemical mediators have been implicated in the pathophysiology of PE. Leptin, an adipocyte-derived peptide hormone primarily known for its role in energy homeostasis, is also abundantly synthesized by the placenta **(14)**. Higher levels of leptin in maternal serum have been reported consistently in preeclamptic pregnancies regardless of body mass index and these leptin levels seem to be up-regulated by placental hypoxic condition **(15)**. Leptin promotes pathogenesis of PE through experimental studies by inducing endothelial dysfunction, increasing systemic inflammation and dysregulation of the immune system at maternal fetal interface **(16)**. In addition, the endocrine environment in pregnancy is subject to extreme changes in PE. Recent data reveal a marked decrease in circulating levels of estrogens, especially Estradiol (E2) **(17)**. Estradiol is important for vasodilation via the activation of eNOS and for sustaining vascular structure in pregnancy and its absence may contribute to the hypertensive and ischemic manifestations in PE **(18,19)**.

Although these single biomarkers play a specific part in the disease, there are major gaps in our knowledge. Most research has examined these biomarkers individually or in a handful of combinations, and usually among populations in Western or East Asian countries. A few studies have reported the use of a global multi-biomarker panel of angiogenic factors (VEGF, PlGF, sFlt-1) in conjunction with phenotypic markers of metabolic and endocrine functions (leptin, estradiol) to improve the diagnostic performance among Middle Eastern subjects **(20)**. Moreover, the ability of such a combined panel to distinguish between mild and severe PE has not been well addressed, particularly in the contexts with limited clinical means to closely monitor this disease **(21)**. Therefore, the present study was conducted with aim to investigate the diagnostic value and predictive potential of a multi-biomarker panel (maternal serum leptin, VEGF, sFlt-1, Estradiol (E2), and PlGF) to differentiate between preeclamptic women and normotensive pregnant controls in an Iraqi population.

2. Materials and Methods

The case-control study was conducted at the Babylon Teaching Hospital for Women and Children in Babylon Province, Central Iraq, from September 2025 to January 2026. Preeclampsia was defined based on the American College of Obstetricians and Gynecologists (ACOG) diagnostic criteria, characterized by new-onset hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) accompanied by proteinuria after 20 weeks of gestation **(7)**. A total of 120 third-trimester pregnant women (60 preeclamptic and 60 normotensive controls) were included. The preeclamptic cases were further stratified based on disease severity into mild (n=32) and severe PE groups (n=28). Severe PE was diagnosed based on the presence of one or more severe features, counting systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg, hepatic impairment, progressive pulmonary edema, renal impairment or cerebral disturbances **(7)**.

Stringent exclusion criteria were utilized to maintain the homogeneity of the study cohort. Women were eliminated if they presented with underlying medical comorbidities affecting the biochemical parameters, such as chronic high blood pressure, pre-existing renal or glomerular disease, chronic liver disease, heart failure, and gestational diabetes mellitus. Furthermore, multi fetal gestations, a history of PE in previous pregnancies, critically ill patients, and pregnancies complicated by fetal congenital anomalies incompatible with life (as verified by ultrasonography) were excluded from the study.

The sample size was determined based on the feasibility of subject recruitment and the availability of eligible participants who met the stringent inclusion criteria during the defined study period. Although this convenience-based approach limits the ability to make definitive claims regarding statistical power, the observed large effect sizes (Cohen's d>2.0 for most biomarkers) and the highly significant p-values obtained suggest that the sample size was adequate to detect the magnitude of biomarker differences in this cohort. Nevertheless, the absence of a formal power calculation is acknowledged as a limitation, and the findings should be validated in larger prospectively powered studies.

A detailed history of medical and obstetric conditions and a full clinical examination were recorded in each case. Baseline clinical and demographic characteristics, systolic blood pressure at attendance, and proteinuria, were extracted from official medical records. Maternal blood samples were obtained by venipuncture from all subjects for biochemical analysis. The samples were then processed immediately by centrifugation at 2500 rpm, for 5 minutes. Subsequently, the extracted serum was dispensed into selected microtubes and stored at -80°C until further analysis.

The quantitative determination of the selected maternal serum biomarkers was carried out using commercially available ELISA kits. The assays included E2 (catalog no. MBS580165), Human Leptin (catalog no. E1559Hu), VEGF (Catalog No. MBS3801531), sFlt-1 (Catalog No. MBS2601616) and Human PlGF (Catalog No. E-EL-H1555). All laboratory procedures and plate readings were performed in strict adherence to the respective manufacturers' protocols and standard operating procedures.

Data were analyzed using the Graph Pad Prism (version 9). The continuous clinical and biochemical data are presented as mean±SD with accompanying observed range (min-max). The Shapiro-Wilk test was employed to evaluate the distribution normality. Since many variables were not normally distributed, the three groups (Normal Control, Mild PE and Severe PE) were compared using the non-parametric Kruskal-Wallis test. For normal distribution variables, pairwise comparisons were performed using independent samples t-tests whenever appropriate. Post-hoc pairwise comparisons between particular groups were Bonferroni adjusted for multiple tests, and effect sizes were calculated using Cohen's d. ROC curves assessed the diagnostic and prognostic accuracy of the single biomarkers. The AUC was estimated with 95% CI using the DeLong method. The best cut-off points for diagnosis were determined by Youden index for each biomarker. Since the statistical data were non-parametric, Spearman's rank, correlation coefficients were used. In addition, binary logistic regression models were applied to predict PE and its severity classification based on the five most important biomarkers. All statistical tests were two-sided and $P<0.05$ was considered statistically significant.

3. Results

Table 1 presents the baseline clinical and general biochemical characteristics, as well as the concentrations of the analyzed maternal serum biomarkers in the three groups: normotensive pregnant women controls (n=60), mild PE (n=32), and severe PE (n=28). The following parameters significantly increased with disease severity: BMI, blood pressure parameters, leptin, sFlt-1, and E2 ($P<0.001$). Conversely, both VEGF and PlGF levels significantly declined in the preeclamptic groups compared with the normal controls.

Table 2 presents an in depth post-hoc pairwise statistical analysis detailing the specific differences between the groups. P-values have been modified for multiple comparisons using Bonferroni correction as a standard method to ensure control. I made a Type I error due to testing multiple hypotheses. The absolute sizes of changes observed (effect size) were also calculated with Cohen's d statistic, with an absolute value of >0.8 considered a large clinical effect. The data strongly implicates the changes in angiogenic factors (VEGF, PlGF, sFlt-1).

Individual serum biomarkers in differentiating women with PE from healthy normotensive pregnant controls are shown in Table 3. The diagnostic metrics (sensitivity, specificity, PPV and NPV) were obtained from optimal cut-off values calculated through the maximization of each ROC curve value via the Youden Index.

Table 4 further extends the diagnostic evaluation to assess the ability of the chosen biomarkers to stratify disease severity between mild and severe preeclampsia. The analysis is identical with Table 3 using the ROC curve and the Youden Index for the cutoff selection. PlGF is an excellent candidate for severity stratification showing a perfect discriminatory profile (AUC=1.000). In addition, sFlt-1 and VEGF also show very good performance (AUC=0.944 and 0.935, respectively).

Table 5 summarizes the Spearman's rank correlation for the relationships between the best serum biomarkers and key clinical variables to characterize these complex interactions in a manner that is nonparametric suitable with the data. The matrix reveals profound physiologic correlations: in particular, strong positive correlations ($|rho|>0.70$) are observed between pro-angiogenic elements (VEGF, PlGF), and negative correlations between these pro-angiogenic markers and the sFlt-1 as well as with clinical measures of disease severity, including systolic blood pressure and renal impairment.

Table 6 shows the results of full binary logistic regression analyses conducted to investigate the predictive power of a combined five-biomarker panel. Two models are shown: one for the overall prediction of preeclampsia (PE versus Normal) and one for the severity of disease (Severe versus Mild PE). The predictors were standardized and the 95% confidence intervals were obtained using 1000 bootstrap replications to guarantee the statistical soundness of the findings. Both models reached a perfect predictive accuracy of 100%. In the severity stratification model, high sFlt-1 (Odds Ratio [OR]=2.80) and leptin (OR=1.80) were identified as two most potent independent risk factors for severe disease development. In contrast, high levels of PlGF (OR=0.12) and VEGF (OR=0.43) are highly significant protective factors.

Table 1. Clinical Characteristics, Biochemical Parameters, and Biomarkers by Study Groups

Parameter / Biomarker	Normal Control (n=60)	Mild Preeclampsia (n=32)	Severe Preeclampsia (n=28)	P-value*
AGE (years)	25.93±1.15 (23.00-27.00)	25.56±1.90 (23.00-29.00)	26.36±1.57 (24.00-29.00)	0.09
BMI (kg/m²)	25.75±3.86 (20.80-35.20)	29.83±2.65 (25.90-36.10)	32.39±2.58 (27.00-37.90)	<0.001
Systolic BP (mmHg)	114.53±6.20 (105.00-128.00)	143.91±7.92 (130.00-165.00)	150.36±10.67 (130.00-169.00)	<0.001
Diastolic BP (mmHg)	74.75±3.79 (69.00-82.00)	91.31±5.06 (82.00-100.00)	93.93±6.82 (80.00-110.00)	<0.001
Platelets (x10^9/L)	276.57±34.02 (180.00-346.00)	218.66±38.59 (135.00-300.00)	170.36±58.43 (27.00-250.00)	<0.001
Urea (mmol/L)	2.68±1.14 (0.28-5.00)	3.87±1.05 (2.20-6.00)	6.46±4.39 (3.10-21.40)	<0.001
Creatinine (mg/dL)	0.58±0.12 (0.33-0.80)	0.67±0.12 (0.40-0.90)	1.15±1.18 (0.50-6.90)	<0.001
AST (U/L)	20.93±3.65 (14.00-30.00)	27.53±3.25 (21.00-35.00)	43.50±26.68 (23.00-155.00)	<0.001
ALT (U/L)	21.07±4.41 (10.00-30.00)	29.47±4.70 (21.00-41.00)	43.64±11.20 (24.00-62.00)	<0.001
LEPTIN (ng/ml)	20.35±1.41 (18.20-24.20)	47.16±7.91 (33.50-72.20)	59.61±10.98 (33.90-72.10)	<0.001
VEGF (pg/ml)	259.98±30.61 (210.30-319.10)	40.23±9.49 (18.10-56.30)	18.39±10.87 (5.30-57.20)	<0.001
sFlt-1 (ng/ml)	4.57±0.77 (3.08-5.90)	6.91±1.35 (4.40-10.10)	10.45±2.01 (7.50-13.90)	<0.001
E2 pg/ml	918.57±117.84 (15.30-988.60)	1049.27±25.40 (990.50-1110.20)	1064.24±25.18 (1019.20-1107.20)	<0.001
(PlGF)pg/ml	563.85±245.96 (177.30-980.20)	106.32±21.75 (68.30-144.10)	25.04±10.17 (10.50-44.20)	<0.001

*Kruskal-Wallis test. P-values<0.05 were considered statistically significant.

Table 2. Post-hoc Pairwise Comparisons and Cohen's d Effect Sizes

Variable	Normal vs. Mild PE (P-adj)	Normal vs. Mild (Cohen's d)	Normal vs. Severe PE (P-adj)	Normal vs. Severe (Cohen's d)	Mild vs. Severe PE (P-adj)	Mild vs. Severe (Cohen's d)
AGE	0.000003	0.774	0.000001	0.579	0.334083	-0.191
BMI	0.000000	-1.186	0.000000	-1.375	0.000570	-0.274
LEPTIN	0.000000	-4.956	0.000000	-6.112	0.000000	-1.244
VEGF	0.000000	8.488	0.000000	9.107	0.000000	2.138
sFlt-1	0.000000	-2.150	0.000000	-4.533	0.000000	-2.214
E2 pg/ml	0.000000	-1.114	0.000000	-1.484	0.054354	0.023
(PlGF)pg/ml	0.000000	2.333	0.000000	2.648	0.000000	4.210

Cohen's d values indicate effect sizes: |d|>0.8 is considered a large effect

Table 3. Diagnostic Performance of Biomarkers in Distinguishing Preeclampsia from Normal Controls

Biomarker	AUC	95% CI	Optimal Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden Index
LEPTIN	1.000	1.000-1.000	33.50 ng/ml	100.0%	100.0%	100.0%	100.0%	1.000
VEGF	1.000	1.000-1.000	56.30 pg/ml	100.0%	100.0%	100.0%	100.0%	1.000
sFlt-1	0.957	0.912-1.000	5.90 ng/ml	86.7%	98.3%	98.1%	88.1%	0.850
E2 pg/ml	1.000	1.000-1.000	990.50 pg/ml	100.0%	100.0%	100.0%	100.0%	1.000
(PlGF) pg/ml	1.000	1.000-1.000	144.10 pg/ml	100.0%	100.0%	100.0%	100.0%	1.000

Table 4. Diagnostic Performance of Biomarkers in Stratifying Preeclampsia Severity (Severe vs. Mild)

Biomarker	AUC	95% CI	Optimal Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden Index
LEPTIN	0.799	0.684-0.913	53.30 ng/ml	64.3%	90.6%	85.7%	74.4%	0.549
VEGF	0.935	0.875-0.996	30.10 pg/ml	96.4%	84.4%	84.4%	96.3%	0.808
sFlt-1	0.944	0.887-1.000	8.60 ng/ml	78.6%	93.8%	91.7%	83.3%	0.723
E2 pg/ml	0.704	0.569-0.839	1049.90 pg/ml	71.4%	62.5%	62.5%	71.4%	0.339
(PlGF) pg/ml	1.000	1.000-1.000	44.20 pg/ml	100.0%	100.0%	100.0%	100.0%	1.000

Performance metrics in stratifying severity (Severe vs. Mild). PlGF remains a perfect stratifying marker (AUC=1.000). sFlt-1 (AUC=0.944) and VEGF (AUC=0.935) also show excellent performance, whereas E2 and Leptin have moderate performance for severity stratification.

Table 5. Spearman Correlation Matrix of Main Biomarkers and Clinical Parameters

Parameter	LEPTIN	VEGF	sFlt-1	E2 pg/ml	(PlGF)pg/ml	systolic	diastolic	Platelets	Urea	creatinine	AST	ALT
LEPTIN	1.000	-0.801	0.770	0.812	-0.817	0.738	0.767	-0.344	0.292	-0.103	0.364	0.158
VEGF		1.000	-0.787	-0.793	0.813	-0.738	-0.731	0.291	-0.231	0.138	-0.388	-0.291
sFlt-1			1.000	0.772	-0.748	0.680	0.686	-0.222	0.176	-0.083	0.323	0.211
E2 pg/ml				1.000	-0.783	0.765	0.756	-0.333	0.255	-0.067	0.396	0.223
(PlGF) pg/ml					1.000	-0.726	-0.782	0.336	-0.268	0.118	-0.403	-0.168

Spearman's rank correlation coefficients (rho). All correlations with |rho|>0.18 are statistically significant (P<0.05). Strong correlations (|rho|>0.70).

Table 6. Binary Logistic Regression Analysis of the Multi-Biomarker Panel for Prediction of Preeclampsia and Severity Classification

Outcome Scenario	Predictor Biomarker	Coefficient (Beta)	Odds Ratio (OR)	95% Confidence Interval	P-value	Model Performance
PE vs Normal (Model Accuracy: 100.0% Nagelkerke R^2: 1.00)	LEPTIN	1.2819	3.6033	3.243-3.991	<0.001	
	VEGF	-1.8539	0.1566	0.140-0.185	<0.001	AUC=1.000
	sFlt-1	0.6910	1.9956	1.739-2.319	<0.001	Sensit*=100%
	E2 pg/ml	0.7385	2.0928	1.912-2.275	<0.001	Specifi*=100%
	(PlGF)pg/ml	-1.0927	0.3353	0.298-0.380	<0.001	
Severe vs Mild PE (Model Accuracy: 100.0%)	LEPTIN	0.5903	1.8045	1.200-2.796	<0.001	
	VEGF	-0.8387	0.4323	0.231-0.614	<0.001	AUC=1.000
	sFlt-1	1.0283	2.7963	2.264-3.559	<0.001	Sensit*=100%
	E2 pg/ml	0.1505	1.1624	0.935-1.553	0.124	Specifi*=100%
	(PlGF)pg/ml	-2.1168	0.1204	0.090-0.199	<0.001	

4. Discussion

The purpose of the present study was to evaluate the diagnostic efficacy and predictive capacity of a composite biomarker panel comprising maternal serum leptin, VEGF, sFlt-1, E2, and PlGF to distinguish preeclamptic women from normotensive pregnant controls in an Iraqi population. There were significant differences in all five biomarkers between normal pregnancies and those with preeclampsia, and the extent of perturbation corresponded closely to clinical severity. These data collectively support the unifying framework of PE as an angiogenic disorder characterized by systemic metabolic and endocrine dysfunction and highlight the future directions in which multi-marker approaches can provide novel opportunities for clinical management, especially in settings where advanced diagnostic platforms remain limited.

A cardinal observation of the present study was the dramatic suppression of circulating VEGF and PlGF concentrations, along with a accompanying increase in sFlt-1 levels in women with PE. The mean VEGF concentration in the severe PE group (18.39±10.87 pg/ml) represented a reduction exceeding 93% relative to normotensive controls (259.98±30.61 pg/ml), whereas PlGF levels fell by more than 95% in severe cases (25.04±10.17 pg/ml vs. 563.85±245.96 pg/ml in controls). Concentrations of the meantime of sFlt-1 were 10.45±2.01 ng/ml (cohort severe PE) vs. healthy gravidas women: 4.57±0.77 ng/ml, which was about more than double higher in patients with PE than controls at matched gestation **(3)**. These results are consistent with the two-stage model commonly accepted for PE pathophysiology, which suggest that abnormal placentation and resultant placental hypoxia leads to excess anti-angiogenic factors communicated to the maternal circulation capable of sequestering circulating pro-angiogenic factors perpetuating a vicious cycle with pronounced cytoprotective processes resulting in widespread endothelial injury **(8)**.

The ROC analysis in our cohort yielded remarkable discriminatory performances for these angiogenic markers. Both VEGF and PlGF achieved perfect diagnostic accuracy (AUC=1.000) when differentiating preeclamptic women from normotensive controls, whereas sFlt-1 demonstrated near-perfect performance (AUC=0.957). For severity stratification between mild and severe PE, PlGF retained its flawless discriminatory capacity (AUC=1.000), and sFlt-1 and VEGF exhibited excellent performance (AUC=0.944 and 0.935, respectively). These results compare favorably with and in several instances surpass the diagnostic metrics reported in other populations. Nikuei et al., in a case-control study in southern Iran reported an AUC of 0.90 for the sFlt-1/PlGF ratio in distinguishing preeclamptic patients from healthy controls, with a sensitivity of 84.2% and specificity of 85.0% at an optimal cut-off of 24.96 **(22)**. The superior performance observed in our study may partly reflect the more advanced gestational age at sampling (third trimester, >28 weeks) and stringent exclusion criteria applied, which reduced confounding from comorbid conditions.

In a South Asian population, Kumar et al., demonstrated that women experiencing adverse maternal outcomes had substantially higher sFlt-1 concentrations (11,500 pg/mL versus 3,051 pg/mL) and lower PlGF concentrations (44.88 pg/mL reverse 148.50 pg/mL) than those without complications, with the sFlt-1/PlGF ratio achieving an AUC of 0.81 for predicting adverse maternal outcomes **(23)**. Importantly, the absolute values of PlGF in our severe PE cohort (25.04±10.17 pg/ml) were lower than those presented by Kumar et al., **(23)** in their adverse outcome cohort, indicating that the Iraqi women in our sample may have displayed fairly extreme placental dysfunction. Unlike our results, a recent Iraqi study by Alshihabi and Alsaadi evaluating PlGF as a marker for

PE severity in 90 pregnant women, concluded that moderate and severe cases of PE had no significant differences in the levels of PlGF **(24)**. This discrepancy may be attributable to differences in disease classification criteria, gestational period at the time of sampling, and assay methodology. Our study employed a more granular severity stratification based on the ACOG criteria and measured PlGF using a different ELISA platform, which may account for the enhanced discriminatory capacity observed.

It was strengthened by the prognosis study that the perspiration sFlt-1/PlGF ratio<38 has a negative predictive value to exclude PE in one week, thereby confirming the limited clinical relevance of marginally altered values in the absence of corroborating clinical findings **(25)**. Our data extend these observations by demonstrating that PlGF alone at a cut_off of 144.10 pg/ml has 100% sensitivity and specificity for identification of PE in this cohort, a finding of particular importance in resource-limited clinical environments where simplified testing algorithms are desirable.

The second important result was a remarkable increase in maternal serum leptin levels in preeclamptic women, with concentrations gradually rising from normotensive controls (20.35±1.41 ng/ml) to mild PE (47.16±7.91 ng/ml), and severe PE (59.61±10.98 ng/ml). The effect sizes (Cohen's d) for these comparisons were huge, -1.244 for normal versus mild, and -6.112 for normal versus severe, indicating that the differences observed were of great clinical significance and not just statistical significance.

These findings are consistent with a growing body of evidence implicating leptin in the pathogenesis of PE. A comprehensive review by Elgazzaz et al., synthesized over two decades of clinical data and concluded that leptin is strongly associated with PE and its severity, independent of body mass index, in a big number of clinical studies **(26)**. The mechanistic basis for this association appears to involve placental hypoxia-driven overproduction of leptin, which subsequently promotes endothelial dysfunction through pathways involving endothelin-1, mineralocorticoid receptor activation, and mitochondrial dysfunction **(27)**. In a recent prospective controlled study, Toplu et al., reported mean serum leptin levels of 26.6±10.9 ng/ml in preeclamptic women compared with 10.8±5.3 ng/ml in controls, with an AUC of 0.928 for diagnostic prediction **(27)**. Our study observed considerably higher absolute leptin concentrations in the preeclamptic groups, which may reflect differences in gestational age at sampling, BMI distribution, or population-specific factors influencing the production of placental leptin.

A systematic review by Marcayata et al., confirmed remarkable increase in serum leptin levels among women with PE ($P \leq 0.00001$, $I^2=91\%$), providing robust evidence for the consistency of this association across diverse populations **(28)**. Our logistic regression analysis further identified leptin as a significant independent predictor of both the presence of PE (OR=3.60) and progression to severe disease (OR=1.80). The strong positive correlation between leptin and sFlt-1 (rho=0.770) observed in our correlation matrix suggests a mechanistic link between metabolic dysregulation and angiogenic imbalance, potentially mediated through shared upstream triggers such as placental hypoxia and oxidative stress **(16)**.

An intriguing observation in our dataset was the significantly elevated Estradiol (E2) levels in women with PE compared to normotensive controls. This observation should be interpreted with caution, considering the previous literature, which is paradoxical regarding the direction of estrogen alterations in PE. In this regard, Berkane et al., proposed that estrogen deficiency contributes to PE pathogenesis through impaired nitric oxide-mediated vasodilation **(18)**. Conversely, Hsieh et al., demonstrated that elevated serum E2 levels in early pregnancy were associated with an increased risk of PE, particularly following frozen embryo transfer cycles **(29)**. More recently, investigations into placental steroidogenesis have revealed that the relationship between estrogens and PE is complex and may vary according to gestational timing, disease subtype, and specific estrogen metabolite levels **(30)**.

In our cohort, E2 levels were elevated in women with preeclampsia (mild PE: 1049.27±25.40 pg/ml; severe PE: 1064.24±25.18 pg/ml) compared to those in controls (918.57±117.84 pg/ml). Although, the difference between the mild and severe groups was not statistically significant after Bonferroni correction and the ROC analysis for severity stratification yielded only moderate performance (AUC=0.704). Furthermore, E2 was not identified as a significant independent predictor of severe disease in the logistic regression model. These findings suggest that estradiol alterations are related to the preeclamptic condition, but these alterations might be the result of aberrant placental functioning and not the major determinant of disease severity. The elevated E2 levels in our group could reflect a compensatory upregulation of aromatase expression by the hypoxic placenta, a concept that needs to be substantiated by further mechanistic investigations.

The progressive deterioration in standard biochemical indices across the study groups provides an important clinical context for the interpretation of biomarker data. The significant decline in platelet counts from controls (276.57±34.02×10⁹/L) to severe PE (170.36±58.43×10⁹/L) reflects the consumptive coagulopathy and microangiopathic processes characteristic of severe disease **(31)**. Similarly, the escalation in hepatic transaminases (AST rising from 20.93±3.65 U/L in controls to 43.50±26.68 U/L in severe PE) and renal markers (creatinine increasing from 0.58±0.12 mg/dL to 1.15±1.18 mg/dL) corroborates the multi-organ involvement that defines

severe PE **(32)**. These traditional laboratory parameters are helpful in confirming end-organ damage, but they usually change only after the disease is well advanced. The biomarkers analyzed in our study, in particular PlGF and sFlt-1, have the additional benefit of reflecting upstream pathophysiological processes and may thus allow for earlier clinical intervention.

The significant higher BMI observed in preeclamptic women (mild PE: 29.83±2.65 kg/m²; severe PE: 32.39±2.58 kg/m²) compared with controls (25.75±3.86 kg/m²) is consistent with the well-established association between obesity and PE risk. Abraham et al., reviewed the mechanistic associations between high BMI and PE in detail, indicating that the pathways were common and involved chronic inflammation **(33)**. In the Middle Eastern setting, where there is notable rate of obesity among women of reproductive age, this finding holds significant clinical value for risk stratification protocols **(4)**.

A distinctive contribution of this study is the integration of five biomarkers spanning angiogenic, metabolic, and endocrine pathways into a unified predictive framework for CRC. The binary logistic regression models were 100% accurate in predicting both PE and its severity, surpassing the predictive performance of each biomarker individually. In the severity prediction model, sFlt1 was the most important risk variable (OR=2.80), followed by leptin (OR=1.80), whereas PlGF (OR=0.12) and VEGF (OR=0.43) were the two significant protective factors. This has a biological rationale: high levels of anti-angiogenic factors and metabolic stress markers promote disease progression, and sufficient availability of pro-angiogenic factors protects against severe disease.

The concept of multi-biomarker panels for PE prediction has gained traction in recent years. Ng et al., conducted a comprehensive review of biomarkers and point-of care examination methods, concluding that combinations of markers from distinct biological pathways consistently outperform single-marker strategies **(34)**. Similarly, Gupta et al., advocated for the integration of multi-biomarker panels incorporating cardiovascular, angiogenic, and inflammatory markers to improve the early identification of PE-associated cardiovascular risks **(20)**. Our results empirically validate this method for a Middle Eastern sample and suggest that the angiogenic factors in combination with leptin and estradiol provide diagnostic accuracy that is not easily attainable through usual clinical evaluation.

This study has several strengths, including simultaneous assessment of five biomarkers, strict use of ACOG diagnostic criteria for disease definition, and evaluation of biomarker performance by multiple methodologies, including ROC analysis, correlations, and logistic regression, thus providing a comprehensive evaluation of performance from unique statistical perspectives. The matching of controls for age rules out a possible confounding factor and the rigorous exclusion criteria increases the internal validity of the results.

However, the limitations of this study must be taken into account. The generalizability of the results is limited by the relatively small number of patients as well as single-centre study design. Since the samples were collected only once in the third trimester, the study's cross-sectional design prevents us from evaluating longitudinal biomarker trajectories, which is crucial for demonstrating their ability to predict illness before onset of symptoms. Furthermore, the very high AUC's determined might at least partly be due to an obvious clinical cleft between the groups at the time of sampling and not to early prediction. Subsequent prospective studies in which samples are taken serially from early pregnancy are needed to establish if these biomarkers can predict at-risk women prior to clinical manifestation.

The observation of perfect AUC values (1.000) with 100% sensitivity and 100% specificity for multiple individual biomarkers (Leptin, VEGF, E2, and PlGF) in distinguishing preeclamptic women from normotensive controls warrants explicit discussion. In real-world clinical biomarker research, achieving such absolute mathematical binary separation across four distinct biological markers simultaneously is exceedingly rare and raises important considerations regarding the generalizability of these findings. The perfect discriminatory performance observed in this study is most likely attributable to the study design, wherein all samples were collected during the third trimester from already hospitalized patients with clinically manifest disease. Consequently, the biomarker panel reflects fully established disease states with pronounced pathophysiological derangements rather than early or subclinical stages of the disease. The extreme separation between the groups at this advanced clinical stage does not necessarily translate to equivalent performance in early pregnancy screening scenarios, where biomarker differences may be subtler and overlapping distributions between groups are expected. Additionally, the possibility of model overfitting cannot be excluded given the relatively modest sample size (n=120) and the number of predictors employed. The absence of an independent external validation cohort further limits the ability to confirm the robustness of these perfect classification results. Future studies should incorporate prospective longitudinal designs with serial sampling from the first trimester, employ cross-validation techniques or split-sample approaches, and include larger multi-centre cohorts to determine whether these biomarkers retain their discriminatory capacity at earlier gestational timepoints when clinical intervention would be most beneficial.

5. Conclusion

The consideration of angiogenic factors with metabolic and endocrine components is a strong predictive basis that greatly improves the clinical evaluation of PE beyond traditional diagnosis. The parallel angiogenic suppression and metabolic abnormalities found across disease severity groups further support emerging models of PE as a systemic, multi-organ syndrome of intertwined vascular, placental, and metabolic failure, as opposed to a single endotheliopathy or mainly hypertensive disorder. From a translational standpoint, the remarkable discriminatory properties of PlGF and leptin, either alone or when combined in a multi-marker predictive model, provide a strong rationale for introducing these markers in simplified point-of-care algorithms adapted to low-resource obstetric settings in the Middle East. The findings that sFlt-1 and leptin are independent mediators of fatal disease allow for actionable targets for future risk-based surveillance regimens to funnel intensive monitoring resources to those women deemed most likely to deteriorate.

6. Declarations

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Ethical Considerations

The study strictly followed the moral principles of the Declaration of Helsinki 1964 and its later modification on human experimentation. The ethical approval of the study received from the University of Babylon, College of Medicine (Approval Number: 5-75; dated 18th of September 2025).

Authors' Contributions

E.H.A.A and A.J.A.A: Patient recruitment, sample collection, laboratory-analysis of biomarker panels, data-collation and statistical analysis, and drafting the manuscript. E.H.A.A carried out data interpretation, preparation of manuscript, and review of final version. All the authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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