

First-Trimester Maternal Serum Biomarkers for Prediction of Pre-eclampsia: A Prospective Observational Study

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Abstract

Objective

To evaluate the association and predictive performance of first-trimester maternal serum biomarkers—alpha-fetoprotein (AFP), free β -human chorionic gonadotropin (f β -hCG), pregnancy-associated plasma protein-A (PAPP-A), and placental growth factor (PIGF)—for the prediction of pre-eclampsia (PE) and to assess their relationship with adverse maternal and perinatal outcomes.

Methods

This prospective observational study was conducted over 18 months at the Department of Obstetrics and Gynecology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, in collaboration with the Department of Pathology. Pregnant women with singleton pregnancies at 11 + 0 to 13 + 6 weeks of gestation were enrolled following written informed consent. Maternal characteristics and mean arterial pressure were recorded, and serum AFP, f β -hCG, PAPP-A, and PIGF were measured using DELFIA technology. Biomarker concentrations were expressed as multiples of the median (MoM), with adjustment for relevant maternal and pregnancy characteristics. Participants were followed prospectively until delivery. The primary outcome was development of PE. Receiver operating characteristic analysis was used to assess the discriminatory performance of individual biomarkers and predefined biomarker combinations. Multivariable logistic regression was performed to evaluate associations between biomarker abnormalities and subsequent PE.

Results

Among 300 enrolled women, 38 (12.7%) developed PE. Compared with women who did not develop PE, those who developed PE had higher mean AFP MoM (1.42 ± 0.24 vs. 1.01 ± 0.15 , $p < 0.001$) and lower mean PAPP-A MoM (0.72 ± 0.16 vs. 1.12 ± 0.20 , $p < 0.001$) and PIGF concentrations (98 ± 35 vs. 158 ± 42 pg/mL, $p < 0.001$). The discriminatory performance was highest for PIGF (AUC 0.90, 95% CI 0.84–0.95), followed by PAPP-A (AUC 0.86, 95% CI 0.79–0.91) and AFP (AUC 0.81, 95% CI 0.74–0.87). The combination of AFP, PAPP-A, and PIGF demonstrated an AUC of 0.94 (95% CI 0.89–0.97), with a sensitivity of 95.1% and specificity of 87.7%. Free β -hCG showed limited discriminatory performance (AUC 0.61, 95% CI 0.52–0.70). In multivariable analysis, low PAPP-A (< 0.85 MoM), low PIGF (< 120 pg/mL), and elevated AFP (> 1.25 MoM) were associated with increased odds of subsequent PE, with adjusted odds ratios of 3.72 (95% CI 1.87–7.41), 4.65 (95% CI 2.29–9.40), and 2.58 (95% CI 1.34–4.99), respectively.

Conclusion

In this single-center prospective cohort, first-trimester low PAPP-A and PIGF and elevated AFP were associated with subsequent development of PE. The combination of AFP, PAPP-A, and PIGF showed good discriminatory performance and may have potential for early risk stratification. However, the findings should be interpreted as internally derived estimates, and external validation in larger,

multicenter populations is required before routine clinical implementation. The influence of aspirin prophylaxis initiated following first-trimester risk assessment should also be considered when interpreting these findings.

Introduction

Pre-eclampsia (PE) is a multisystem disorder of pregnancy characterized by new-onset hypertension after 20 weeks of gestation, accompanied by proteinuria and/or maternal organ dysfunction or uteroplacental dysfunction. It affects approximately 2–5% of pregnancies worldwide and remains an important cause of maternal and perinatal morbidity and mortality, particularly when disease occurs at an early gestational age [1–4]. Early-onset and preterm PE are associated with increased risks of severe maternal complications, fetal growth restriction, preterm birth, neonatal morbidity, and perinatal mortality compared with PE occurring at term [5–7].

The clinical manifestations of PE usually become apparent after 20 weeks of gestation, whereas abnormalities in placentation and maternal vascular adaptation may begin much earlier in pregnancy. Consequently, first-trimester risk assessment provides an opportunity to identify women who may benefit from enhanced surveillance and, where clinically appropriate, preventive interventions. Maternal characteristics, including maternal age, body mass index (BMI), parity, previous history of PE, chronic hypertension, diabetes mellitus, smoking status, and mode of conception, are important components of early risk assessment [4, 8].

Maternal circulating biomarkers reflecting placental development and function have been investigated as potential early indicators of abnormal placentation. Pregnancy-associated plasma protein-A (PAPP-A) is involved in regulation of the insulin-like growth factor system and placental development. Reduced first-trimester PAPP-A concentrations have been associated with PE and other placenta-mediated complications, including fetal growth restriction [9–11]. Placental growth factor (PlGF), an angiogenic factor belonging to the vascular endothelial growth factor family, is involved in placental vascular development. Lower maternal PlGF concentrations have been associated with impaired placentation and subsequent PE [8, 12, 13].

Alpha-fetoprotein (AFP) and free β -human chorionic gonadotropin ($f\beta$ -hCG), traditionally used as serum markers in prenatal aneuploidy screening, have also been investigated in relation to adverse pregnancy outcomes. Abnormal AFP concentrations may reflect altered fetoplacental function, although its association with adverse pregnancy outcomes has been inconsistent across populations [14, 15]. Similarly, altered $f\beta$ -hCG concentrations have been reported in pregnancies subsequently complicated by PE, although its predictive performance appears to vary according to population characteristics, gestational age at sampling, and analytical methodology [16, 17].

The simultaneous assessment of maternal serum biomarkers during the first trimester may therefore provide information about placental function before the clinical onset of PE. Previous studies have demonstrated that combinations of PAPP-A, PlGF, AFP, $f\beta$ -hCG, maternal characteristics, mean arterial

pressure, and uterine artery Doppler indices may improve discrimination of pregnancies at increased risk of PE compared with individual biomarkers alone [17]. However, the performance of individual biomarkers and biomarker combinations may differ according to population characteristics, disease prevalence, laboratory methodology, and the definition of PE used.

First-trimester screening strategies have also been investigated in Indian populations, although evidence regarding the performance of individual maternal serum biomarkers and their combinations for PE prediction remains limited. Evaluation of these biomarkers in an Indian tertiary-care cohort may provide useful preliminary evidence regarding their discriminatory ability and their relationship with subsequent pregnancy outcomes.

Early identification of women at increased risk of PE is clinically relevant because preventive strategies, including low-dose aspirin in appropriately selected high-risk pregnancies, may reduce the risk of preterm PE when initiated early in pregnancy [21,22]. However, biomarker-based screening should be evaluated not only according to statistical discrimination but also in the context of subsequent clinical management and treatment.

Therefore, this prospective observational study evaluated first-trimester maternal serum AFP, f β -hCG, PAPP-A, and PIGF concentrations in relation to subsequent development of PE. The study also assessed the discriminatory performance of individual biomarkers and selected biomarker combinations and explored their association with adverse maternal and perinatal outcomes.

Materials and Methods

Study design and setting

This prospective observational cohort study was conducted over an 18-month period in the Department of Obstetrics and Gynecology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, in collaboration with the Department of Pathology. The study was conducted among pregnant women attending the antenatal care and Fetal Medicine outpatient clinics.

The study was approved by the Institutional Ethics Committee of Vardhman Mahavir Medical College and Safdarjung Hospital (Protocol No. IEC/VMMC/SJH/Thesis/2023/CC-184). Written informed consent was obtained from all participants before enrolment. The study was observational in design; subsequent clinical management, including aspirin prophylaxis, was provided according to the institutional risk-assessment protocol and clinical indications.

Study population

Women with ultrasound-confirmed singleton pregnancies between 11 + 0 and 13 + 6 weeks of gestation were assessed for eligibility. Gestational age was determined using the last menstrual period and

confirmed by ultrasound dating.

Women were eligible if they had a singleton pregnancy, reliable gestational dating, and were within the specified first-trimester gestational-age window. Women with multiple gestation, discrepant gestational dating, molar pregnancy, missed abortion, nuchal translucency greater than the 95th percentile, or known malignancy were excluded.

Sample-size estimation

The sample size was estimated using the Cochran formula with a 95% confidence level and a permissible absolute error of 0.06. A detection rate of 47% reported for a combination of first-trimester biochemical markers in a previous study was used for the initial estimation [5]. The calculated minimum sample size was 266 participants. To allow for potential exclusions and incomplete follow-up, the target sample size was increased to 300 participants.

Baseline clinical assessment

At enrolment, demographic and clinical information was recorded using a structured data collection form. Variables included maternal age, parity, gravidity, obstetric history, previous adverse pregnancy outcomes, pre-existing hypertension, diabetes mellitus, thyroid disease, other relevant medical conditions, smoking status, family history, obesity, and mode of conception, including assisted reproductive technology where applicable.

Maternal height and weight were measured at enrolment, and BMI was calculated as weight in kilograms divided by height in metres squared. Blood pressure was measured using a standardized procedure, and mean arterial pressure (MAP) was recorded for clinical risk assessment.

First-trimester biochemical assessment

A 3-mL maternal venous blood sample was collected between 11 + 0 and 13 + 6 weeks of gestation. Serum AFP, f β -hCG, PAPP-A, and PIGF were measured using DELFIA technology on the PerkinElmer VICTOR 2D platform.

The four biomarkers were selected because they represent different aspects of fetoplacental and placental biology and have previously been investigated in first-trimester screening for PE and other pregnancy complications [14–17,25,29,30].

Aneuploidy risk assessment was performed using LifeCycle 7.0 software as part of the routine first-trimester screening process. The present analysis focused on the relationship between maternal serum biomarkers and subsequent PE rather than on aneuploidy screening performance.

Biomarker standardization and MoM calculation

Serum concentrations of AFP, f β -hCG, and PAPP-A were converted to multiples of the median (MoM) using gestational-age-specific reference medians. Biomarker values were adjusted according to relevant maternal characteristics and pregnancy factors incorporated into the laboratory screening system, including maternal age, maternal weight, ethnicity, smoking status, mode of conception, and gestational age.

PAPP-A and AFP were analyzed primarily using MoM values. PIGF was analyzed using its measured serum concentration in pg/mL. The predefined analytical thresholds used in the present dataset were PAPP-A < 0.85 MoM, AFP > 1.25 MoM, and PIGF < 120 pg/mL. These thresholds were used for exploratory classification of biomarker abnormalities and were also evaluated in the diagnostic analyses.

Because cut-offs were evaluated within the study cohort, their diagnostic performance was considered internally derived and was not interpreted as externally validated clinical thresholds.

Follow-up and ascertainment of pre-eclampsia

Participants were followed prospectively from enrolment until delivery. Antenatal records were reviewed to document blood pressure measurements, development of hypertensive disorders, laboratory investigations, fetal growth, Doppler findings, gestational age at delivery, and neonatal outcomes.

Pre-eclampsia was diagnosed according to institutional clinical criteria based on new-onset hypertension after 20 weeks of gestation accompanied by proteinuria and/or clinically relevant maternal organ dysfunction or uteroplacental dysfunction, as applicable. Gestational hypertension was defined separately in women who developed new-onset hypertension without the diagnostic features required for PE.

For analysis, PE was categorized according to gestational age at diagnosis as early-onset PE and later-onset PE, with preterm PE defined as PE occurring before 37 + 0 weeks of gestation. The exact gestational age at diagnosis was recorded for all PE cases.

Aspirin prophylaxis

Women identified as being at increased risk during first-trimester assessment were advised to receive low-dose aspirin (Aspilet, 150 mg once daily at bedtime), in accordance with institutional clinical practice. Aspirin was initiated before 16 weeks of gestation when indicated.

The indication for aspirin, date of initiation, and available information regarding continuation/adherence were recorded. Because aspirin exposure could influence the subsequent occurrence and severity of PE,

aspirin use was considered a potential treatment-related confounder when interpreting the association between first-trimester biomarkers and PE.

The study therefore evaluates biomarker discrimination within the context of the clinical screening-and-management pathway rather than untreated natural history.

Outcome measures

The primary outcome was the development of PE during the index pregnancy.

Secondary maternal outcomes included gestational hypertension, preterm labor, mode of delivery, placental abruption, maternal complications related to hypertensive disease, and other clinically relevant pregnancy complications.

Secondary fetal and neonatal outcomes included fetal growth restriction, birth weight, preterm birth, 5-minute Apgar score, NICU admission, stillbirth, early neonatal death, and major fetal or neonatal abnormalities identified during pregnancy or after delivery. Aneuploidy and neural tube defects were recorded when diagnosed as part of routine antenatal evaluation.

The denominator for each outcome was defined according to the number of participants for whom that outcome was ascertainable. Missing outcome data were documented and were not assumed to represent absence of the outcome.

Statistical analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 21. Continuous variables were assessed for distribution using the Shapiro–Wilk test. Normally distributed variables were summarized as mean $\pm$ standard deviation (SD), whereas non-normally distributed variables were summarized as median with interquartile range (IQR). Categorical variables were presented as frequencies and percentages.

Continuous biomarker concentrations were compared between participants who developed PE and those who did not using the independent-samples t-test for normally distributed variables or the Mann–Whitney U test for non-normally distributed variables. Comparisons involving more than two groups were performed using one-way analysis of variance or the Kruskal–Wallis test, as appropriate. Categorical variables were compared using the chi-square test or Fisher's exact test where appropriate.

Receiver operating characteristic (ROC) curve analysis was performed to assess the discriminatory ability of individual biomarkers and selected biomarker combinations for subsequent PE. The area under the ROC curve (AUC) with 95% confidence intervals (CIs) was reported. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for the evaluated thresholds. Because the biomarker thresholds were evaluated using the study cohort, these estimates were considered internally derived and were not regarded as external validation.

Multivariable logistic regression was used to examine the association between predefined biomarker abnormalities and subsequent PE after adjustment for clinically relevant maternal characteristics. Candidate covariates included maternal age, BMI, nulliparity, and other prespecified clinical factors considered relevant to PE risk. Adjusted odds ratios (aORs) with 95% CIs were reported.

Given the relatively small number of PE events, the multivariable model was kept parsimonious to reduce the risk of model overfitting. The number of events contributing to each model was reported, and associations were interpreted as exploratory rather than as evidence of a fully validated prediction model.

Correlation analyses were performed using Pearson or Spearman correlation coefficients according to the distribution of the variables. All statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant.

Handling of missing data and reporting of denominators

The number of participants available for each analysis was reported explicitly. Where outcome data were unavailable, the denominator used for the corresponding analysis was stated. Complete-case analysis was used for analyses requiring variables that were unavailable for individual participants, and the extent of missing data was documented.

Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. No additional invasive procedure was performed solely for research purposes beyond the blood sampling required for the first-trimester biochemical assessment.

All pregnant women between 11 to 13 + 6 weeks attending ANC and fetal medicine OPD, meeting eligibility criteria were included

Figure 1: FLOW CHART OF METHODOLOGY

Results

Study population and baseline characteristics

A total of 300 pregnant women with singleton pregnancies who met the eligibility criteria were included in the final analysis. The participants underwent first-trimester assessment between 11 + 0 and 13 + 6 weeks of gestation, including maternal clinical assessment and measurement of serum AFP, free β -hCG, PAPP-A, and PIGF.

The mean maternal age was 26.7 ± 4.8 years (range, 18–39 years). Of the 300 participants, 146 (48.7%) were nulliparous and 154 (51.3%) were gravida ≥ 2 . Pre-existing hypothyroidism was present in 27 (9.0%)

women, chronic hypertension in 12 (4.0%), pre-existing diabetes mellitus in 6 (2.0%), and a history of tuberculosis in 14 (4.7%). The reported maternal BMI ranged from 13.5 to 54.5 kg/m² and should be verified against the original clinical records because of the unusually low lower value.

During follow-up, pre-eclampsia (PE) developed in 38 women (12.7%), while 12 (4.0%) developed gestational hypertension. Preterm birth occurred in 29 women (9.7%), low birth weight was recorded in 24 pregnancies (8.1%), 52 neonates (17.2%) required NICU admission, and one stillbirth (0.3%) was documented.

Table 1
Baseline maternal characteristics and pregnancy outcomes

Characteristic	Overall cohort (n = 300)
Maternal age, mean ± SD, years	26.7 ± 4.8
Maternal age range, years	18–39
Nulliparous, n (%)	146 (48.7)
Gravida ≥ 2, n (%)	154 (51.3)
Hypothyroidism, n (%)	27 (9.0)
Pre-existing hypertension, n (%)	12 (4.0)
Pre-existing diabetes mellitus, n (%)	6 (2.0)
History of tuberculosis, n (%)	14 (4.7)
Pre-eclampsia, n (%)	38 (12.7)
Gestational hypertension, n (%)	12 (4.0)
Preterm birth, n (%)	29 (9.7)
Low birth weight, n (%)	24 (8.1)
NICU admission, n (%)	52 (17.2)
Stillbirth, n (%)	1 (0.3)

SD, standard deviation; NICU, neonatal intensive care unit.

First-trimester biomarker concentrations

First-trimester biomarker values differed between women who subsequently developed PE and those who did not. Mean AFP MoM was significantly higher among women who developed PE than among those without PE (1.42 ± 0.24 vs 1.01 ± 0.15, $p < 0.001$). PAPP-A was significantly lower in the PE group (0.72 ± 0.16 vs 1.12 ± 0.20 MoM, $p < 0.001$), as was PIGF concentration (98 ± 35 vs 158 ± 42 pg/mL, $p < 0.001$).

The difference in free β -hCG was not statistically significant (1.24 ± 0.22 vs 1.15 ± 0.18 MoM, $p = 0.08$).

Table 2
First-trimester biomarker measurements according to subsequent development of pre-eclampsia

Biomarker	No PE	PE	p-value
AFP, MoM	1.01 ± 0.15	1.42 ± 0.24	< 0.001
Free β -hCG, MoM	1.15 ± 0.18	1.24 ± 0.22	0.08
PAPP-A, MoM	1.12 ± 0.20	0.72 ± 0.16	< 0.001
PIGF, pg/mL	158 ± 42	98 ± 35	< 0.001

AFP, alpha-fetoprotein; free β -hCG, free beta-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; PIGF, placental growth factor; MoM, multiples of the median.

Diagnostic performance of individual biomarkers

Receiver operating characteristic analysis demonstrated variable discriminatory performance among the individual biomarkers. PIGF showed the highest AUC at 0.90 (95% CI, 0.84–0.95), followed by PAPP-A with an AUC of 0.86 (95% CI, 0.79–0.91) and AFP with an AUC of 0.81 (95% CI, 0.74–0.87). Free β -hCG demonstrated limited discrimination, with an AUC of 0.61 (95% CI, 0.52–0.70).

Using the study-derived thresholds, PIGF < 120 pg/mL provided a sensitivity of 89.4% and specificity of 83.0%, while PAPP-A < 0.85 MoM showed a sensitivity of 84.2% and specificity of 78.5%. AFP > 1.25 MoM showed a sensitivity of 76.3% and specificity of 72.5%. Free β -hCG > 1.20 MoM showed the lowest diagnostic performance.

Table 3
Diagnostic performance of individual first-trimester biomarkers for pre-eclampsia

Biomarker	Threshold	Sensitivity (%)	Specificity (%)	AUC (95% CI)
AFP	> 1.25 MoM	76.3	72.5	0.81 (0.74–0.87)
Free β -hCG	> 1.20 MoM	58.0	66.1	0.61 (0.52–0.70)
PAPP-A	< 0.85 MoM	84.2	78.5	0.86 (0.79–0.91)
PIGF	< 120 pg/mL	89.4	83.0	0.90 (0.84–0.95)

AUC, area under the receiver operating characteristic curve; CI, confidence interval; AFP, alpha-fetoprotein; free β -hCG, free beta-human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; PIGF, placental growth factor; MoM, multiples of the median.

Performance of biomarker combinations

Combinations of biomarkers demonstrated greater discriminatory performance than most individual biomarkers. The combination of PAPP-A and PIGF yielded an AUC of 0.92, with sensitivity of 92.1% and specificity of 85.4%. The three-marker combination of AFP, PAPP-A, and PIGF demonstrated the highest reported discrimination, with an AUC of 0.94, sensitivity of 95.1%, and specificity of 87.7%.

Because the analysis did not demonstrate a clear incremental contribution from free β -hCG, the three-marker combination rather than all four biomarkers provided the best-performing model in this dataset. Therefore, these findings should be interpreted as evaluation of biomarker combinations rather than validation of a complete four-marker “quadruple test” model.

Table 4
Diagnostic performance of selected biomarker combinations

Biomarker combination	Sensitivity (%)	Specificity (%)	AUC
AFP + PAPP-A	85.5	78.1	0.88
PAPP-A + PIGF	92.1	85.4	0.92
AFP + PIGF	90.2	80.0	0.89
AFP + PAPP-A + PIGF	95.1	87.7	0.94

AUC, area under the receiver operating characteristic curve; AFP, alpha-fetoprotein; PAPP-A, pregnancy-associated plasma protein-A; PIGF, placental growth factor.

Association of abnormal biomarker values with pregnancy outcomes among women with pre-eclampsia

Among women who developed PE, abnormal biomarker values were frequently observed in pregnancies complicated by low birth weight, preterm delivery, and NICU admission. Low PIGF and low PAPP-A were particularly common among pregnancies with these adverse outcomes.

However, these analyses were based on the PE subgroup and the available outcome counts were small. The findings therefore represent descriptive associations and should not be interpreted as demonstrating independent prediction of adverse outcomes.

Table 5
Adverse pregnancy outcomes according to abnormal biomarker status among women with pre-eclampsia

Biomarker abnormality	Low birth weight, n (%)	Preterm delivery, n (%)	NICU admission, n (%)	Stillbirth, n (%)
AFP > 1.25 MoM	17 (70.8)	14 (58.3)	15 (62.5)	1 (4.2)
PAPP-A < 0.85 MoM	20 (83.3)	15 (62.5)	19 (79.1)	1 (4.2)
PIGF < 120 pg/mL	21 (87.5)	16 (66.7)	19 (81.2)	1 (4.2)

NICU, neonatal intensive care unit; MoM, multiples of the median.

Percentages in this table require verification against the underlying denominators because the table is based on biomarker-positive subgroups rather than the entire cohort. The single stillbirth represents one event and should not be interpreted as evidence of a biomarker-specific association.

Multivariable analysis

Multivariable logistic regression was performed to examine the association between selected first-trimester characteristics and subsequent PE. Low PAPP-A, low PIGF, and elevated AFP were significantly associated with PE. The adjusted odds ratios were 3.72 (95% CI, 1.87–7.41) for low PAPP-A, 4.65 (95% CI, 2.29–9.40) for low PIGF, and 2.58 (95% CI, 1.34–4.99) for elevated AFP.

Maternal age > 30 years and nulliparity were not significantly associated with PE. BMI > 30 kg/m² showed a borderline association (adjusted OR, 1.93; 95% CI, 1.00–3.72; p = 0.05).

Given that only 38 PE events occurred, the regression findings should be considered exploratory because the number of outcome events was relatively small for a model containing several predictors.

Table 6
Multivariable logistic regression for factors associated with pre-eclampsia

Predictor	Adjusted OR	95% CI	p-value
Low PAPP-A	3.72	1.87–7.41	< 0.001
Low PIGF	4.65	2.29–9.40	< 0.001
High AFP	2.58	1.34–4.99	0.004
Maternal age > 30 years	1.42	0.75–2.69	0.27
BMI > 30 kg/m ²	1.93	1.00–3.72	0.05
Nulliparity	1.14	0.61–2.13	0.67

OR, odds ratio; CI, confidence interval; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; AFP, alpha-fetoprotein; BMI, body mass index.

Overall, the results indicate that first-trimester PAPP-A and PlGF were the strongest individual biomarkers associated with subsequent PE in this cohort, while the combination of AFP, PAPP-A, and PlGF demonstrated the highest apparent discrimination. These performance estimates were derived from the same study population in which the biomarker thresholds were evaluated and therefore require external validation before clinical implementation.

Discussion

In this prospective observational study of 300 pregnant women undergoing first-trimester assessment, maternal serum AFP, PAPP-A, and PlGF were significantly associated with subsequent development of pre-eclampsia (PE). Women who developed PE had higher AFP and lower PAPP-A and PlGF concentrations than those who did not develop PE. Among the individual biomarkers, PlGF demonstrated the highest discriminatory performance, followed by PAPP-A and AFP, whereas free β -hCG showed limited discrimination. The combination of AFP, PAPP-A, and PlGF demonstrated the highest apparent discriminatory performance in the present cohort.

The findings for PAPP-A and PlGF are biologically plausible and consistent with previous evidence linking abnormal placentation and impaired angiogenic function with the development of PE [1–4]. PE is a heterogeneous pregnancy disorder in which abnormal placentation, maternal endothelial dysfunction, and altered angiogenic balance contribute to the clinical phenotype. First-trimester assessment of maternal characteristics and biochemical markers may therefore provide an opportunity to identify women at increased risk before clinical manifestations become apparent [3, 4].

PAPP-A is involved in the regulation of insulin-like growth factor bioavailability and has an important role in placental development. Reduced first-trimester PAPP-A concentrations have been associated with impaired placental development and subsequent adverse pregnancy outcomes [5–7]. In the present study, mean PAPP-A was substantially lower among women who subsequently developed PE than among those without PE, and PAPP-A demonstrated an AUC of 0.86. The adjusted analysis also showed a significant association between low PAPP-A and PE. These findings support the potential contribution of PAPP-A to early risk stratification.

PlGF showed the strongest individual discriminatory performance in this study, with an AUC of 0.90. PlGF is a placental angiogenic factor involved in vascular development and maternal–placental vascular adaptation. Reduced PlGF concentrations have been associated with impaired placental angiogenesis and PE [8, 9]. The markedly lower PlGF concentration observed in women who developed PE is therefore consistent with the proposed role of angiogenic imbalance in PE pathophysiology. The adjusted odds ratio for low PlGF was also higher than that observed for the other biomarkers, although this association should be interpreted in the context of the relatively small number of PE events.

AFP demonstrated moderate-to-good discrimination, with an AUC of 0.81, and was significantly higher among women who subsequently developed PE. Previous studies have investigated AFP as a marker of placental dysfunction and adverse pregnancy outcomes, although its association with PE has not been as consistent as that reported for PAPP-A and PIGF [10, 11]. The present findings therefore suggest that AFP may provide complementary information when incorporated into a multivariable biomarker approach, rather than serving as a standalone screening marker.

Free β -hCG demonstrated relatively limited discriminatory ability, with an AUC of 0.61. Alterations in free β -hCG have previously been investigated in relation to placental dysfunction and pregnancy complications, but its predictive contribution appears to vary across populations and study designs [10, 12]. In the current study, the difference in free β -hCG between women with and without PE was not statistically significant. This may explain why the best-performing biomarker combination did not include free β -hCG.

The improved performance observed with biomarker combinations is consistent with the multifactorial nature of PE. Previous studies have suggested that combinations of first-trimester maternal characteristics and biochemical markers may provide better discrimination than individual markers alone [4, 13]. In the present study, the combination of PAPP-A and PIGF produced an AUC of 0.92, while addition of AFP resulted in an AUC of 0.94, with a sensitivity of 95.1% and specificity of 87.7%. These findings suggest that biomarkers reflecting different aspects of placental development and function may provide complementary information.

However, the reported performance of the three-marker combination requires cautious interpretation. The thresholds and combination were evaluated in the same dataset from which the model was derived. Consequently, the reported sensitivity, specificity, and AUC may be higher than would be observed in an independent population. Similar concerns regarding internal optimism and the need for external validation apply to predictive models developed from relatively small datasets [13, 14]. Therefore, the present findings should be considered preliminary and internally derived rather than externally validated.

The prevalence of PE in the study population was 12.7%. This is important when interpreting predictive values because positive and negative predictive values are influenced by the underlying prevalence of the outcome. The study was conducted at a tertiary-care hospital, where the obstetric population may have a higher baseline risk than a general community population. Consequently, the observed performance should not automatically be extrapolated to low-risk populations or community-based screening programmes.

The multivariable analysis demonstrated significant associations between low PAPP-A, low PIGF, and elevated AFP and subsequent PE. Low PIGF showed the strongest adjusted association, followed by low PAPP-A and elevated AFP. Maternal age > 30 years and nulliparity were not significantly associated with PE after adjustment, while BMI > 30 kg/m² showed a borderline association. These findings are broadly compatible with the concept that maternal characteristics and biochemical markers contribute complementary information to first-trimester risk assessment [3, 4, 15].

Nevertheless, the regression model should be interpreted as exploratory. Only 38 PE events occurred among the 300 participants, while several predictors were included in the multivariable analysis. A relatively high predictor-to-event ratio can lead to model instability and overfitting. Future studies with substantially larger samples and independent validation cohorts should therefore confirm the magnitude and stability of the observed associations before the model is considered suitable for clinical prediction.

The findings may be particularly relevant because early identification of women at increased risk of PE can facilitate timely preventive strategies. Low-dose aspirin initiated early in pregnancy has been investigated extensively for prevention of preterm PE in women identified as being at increased risk [16, 17]. In the present study, aspirin was administered to women classified as high risk during first-trimester screening. Therefore, the observed PE incidence reflects a screening-and-treatment pathway rather than the untreated natural history of PE. Aspirin exposure, timing of initiation, dose, and adherence should consequently be considered when interpreting the observed associations and should be incorporated into future prediction models.

The study also has relevance to settings where first-trimester biochemical screening infrastructure is already available. The biomarkers evaluated in this study can be measured using platforms routinely used for prenatal biochemical assessment, potentially allowing PE risk assessment to be integrated into existing screening pathways. Previous work has demonstrated the potential usefulness of first-trimester biochemical markers for risk assessment in different populations, including Asian and Indian cohorts [5, 8, 14, 15]. However, the present study did not undertake a formal health-economic evaluation. Therefore, claims regarding cost-effectiveness, cost-efficiency, or suitability for national screening programmes cannot be established from the current data.

An important distinction is required regarding the term "quadruple test." Although four biomarkers—AFP, free β -hCG, PAPP-A, and PIGF—were measured, the highest-performing combination in the present analysis consisted of AFP, PAPP-A, and PIGF. Free β -hCG showed limited discriminatory performance and was not included in the best-performing combination. Therefore, the findings should be described as an evaluation of first-trimester maternal serum biomarkers and selected biomarker combinations rather than as validation of a complete four-marker quadruple-test algorithm unless a prespecified four-marker model is formally analysed.

The study has several strengths. It used a prospective design and assessed biomarkers during the clinically relevant first-trimester window. Multiple biomarkers reflecting different biological pathways were evaluated within the same cohort, allowing their individual and combined discriminatory performance to be compared. The use of standardized biochemical assessment and adjustment of biomarker values for relevant maternal and gestational characteristics also strengthens the internal consistency of the analysis.

Several limitations should be acknowledged. First, the single-centre design and recruitment from a tertiary-care hospital may limit generalizability. Second, the number of PE events was relatively small, limiting the precision and stability of multivariable estimates. Third, the biomarker thresholds and

predictive performance were assessed within the same dataset without an independent validation cohort. Fourth, the current analysis does not provide sufficient evidence to establish clinical superiority of one biomarker combination over another without formal statistical comparison of the corresponding ROC curves. Fifth, the study did not include a formal cost-effectiveness analysis. Finally, the analysis did not evaluate a fully specified and externally validated four-marker quadruple-test algorithm.

Future multicentre prospective studies should validate these findings in larger and more diverse populations, including women from lower-risk and community-based settings. Such studies should prespecify biomarker thresholds, incorporate maternal clinical characteristics and aspirin exposure, assess model calibration in addition to discrimination, and use independent validation cohorts. Evaluation of clinically relevant PE phenotypes, particularly preterm and early-onset PE, would further determine the clinical utility of first-trimester biomarker-based risk stratification.

Conclusion

In this prospective cohort, first-trimester maternal serum PAPP-A, PIGF, and AFP were significantly associated with subsequent development of PE. PIGF and PAPP-A demonstrated the strongest individual discriminatory performance, while the combination of AFP, PAPP-A, and PIGF showed the highest apparent discrimination in the study population.

These findings support the potential value of combining first-trimester placental biomarkers for early risk stratification of PE. However, the reported thresholds and model performance were derived and evaluated in the same cohort and therefore require independent external validation. The findings should not be interpreted as validation of a complete quadruple-test algorithm or as evidence of cost-effectiveness. Larger multicentre studies with independent validation are required before this biomarker combination can be recommended for routine clinical screening.

Declarations

Ethics Approval and Consent to Participate

The study was conducted in accordance with the principles of the Declaration of Helsinki and received approval from the Institutional Ethics Committee of VMMC & Safdarjung Hospital, New Delhi (IEC/VMMC/SJH/Thesis/2023/CC-184). Written informed consent was obtained from all participants before enrolment.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request, subject to applicable institutional and ethical restrictions.

Competing Interests

The authors declare that they have no competing interests.

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No specific funding was received for this study.

Authors' Contributions

VM contributed to participant recruitment, data collection, data analysis, and manuscript preparation. US contributed to study conception, supervision, clinical management, and critical revision of the manuscript. KP contributed to participant recruitment, clinical assessment, and data collection. NKM contributed to laboratory investigations and interpretation of biochemical results. DKT contributed to data analysis and manuscript review. AL contributed to study coordination, data analysis, interpretation of results, and manuscript preparation and critical revision. All authors reviewed and approved the final manuscript.

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References

1. Leite DF, Cecatti JG, Krupa FG et al. Prediction of preeclampsia: current perspectives and future directions. *Pregnancy Hypertens.* 2019.
2. MacDonald TM, Walker SP, Hannan NJ, Tong S, Kaitu'u-Lino TJ. Clinical tools and biomarkers for prediction of preeclampsia. *Pregnancy Hypertens.* 2022.
3. Poon LC, Magee LA, Verlohren S et al. A literature review and best practice advice for screening and diagnosis of pre-eclampsia. *Int J Gynecol Obstet.* 2021.
4. Chaemsaihong P, Pooh RK, Zheng M et al. First-trimester prediction of pre-eclampsia using maternal characteristics and biomarkers: current evidence and clinical implications. *Am J Obstet Gynecol.* 2022.
5. Tan MY, Wright D, Syngelaki A et al. Comparison of diagnostic accuracy of early pregnancy biomarkers for prediction of pre-eclampsia. *Ultrasound Obstet Gynecol.* 2018.
6. Luewan S, Teja-Intr M, Jatavan P, Tongsong T. Low maternal serum pregnancy-associated plasma protein-A as a predictor of preeclampsia and adverse pregnancy outcomes. *J Obstet Gynecol Res.* 2018.

7. Conover CA, Oxvig C. The pregnancy-associated plasma protein-A family: biological functions and clinical implications. *Endocr Rev.* 2023.
8. Verma S et al. First-trimester placental growth factor and its association with pre-eclampsia and adverse pregnancy outcomes. *J Maternal-Fetal Neonatal Med.* 2022.
9. Ontario Health. Placental growth factor testing for suspected pre-eclampsia: evidence review. Toronto: Ontario Health; 2023.
10. Johnson RJ et al. First-trimester maternal serum biochemical markers and prediction of pregnancy complications. *Prenat Diagn.* 2013.
11. Huang T et al. Maternal serum alpha-fetoprotein and free β -human chorionic gonadotropin in the prediction of adverse pregnancy outcomes. *Eur J Obstet Gynecol Reproductive Biology.* 2015.
12. Yliniemi A et al. First-trimester biochemical markers and subsequent development of pre-eclampsia: prospective cohort study. *Acta Obstet Gynecol Scand.* 2015.
13. Nevalainen J et al. First-trimester maternal serum biomarkers for prediction of pre-eclampsia. *Hypertens Pregnancy.* 2017.
14. Suresh S et al. First-trimester maternal serum biochemical markers and risk of pre-eclampsia in an Indian population. *J Obstet Gynecol Res.* [Year].
15. Tanbo TG et al. Maternal characteristics and biochemical markers for first-trimester prediction of pre-eclampsia. *Ultrasound in Obstetrics & Gynecology.* [Year].
16. Ma'ayeh M, Costantine MM. Prevention of preeclampsia: role of low-dose aspirin. *Curr Opin Obstet Gynecol.* 2020.
17. Allard S et al. Aspirin for prevention of pre-eclampsia and associated adverse pregnancy outcomes. *Cochrane Database Syst Reviews.* 2024.

Figures

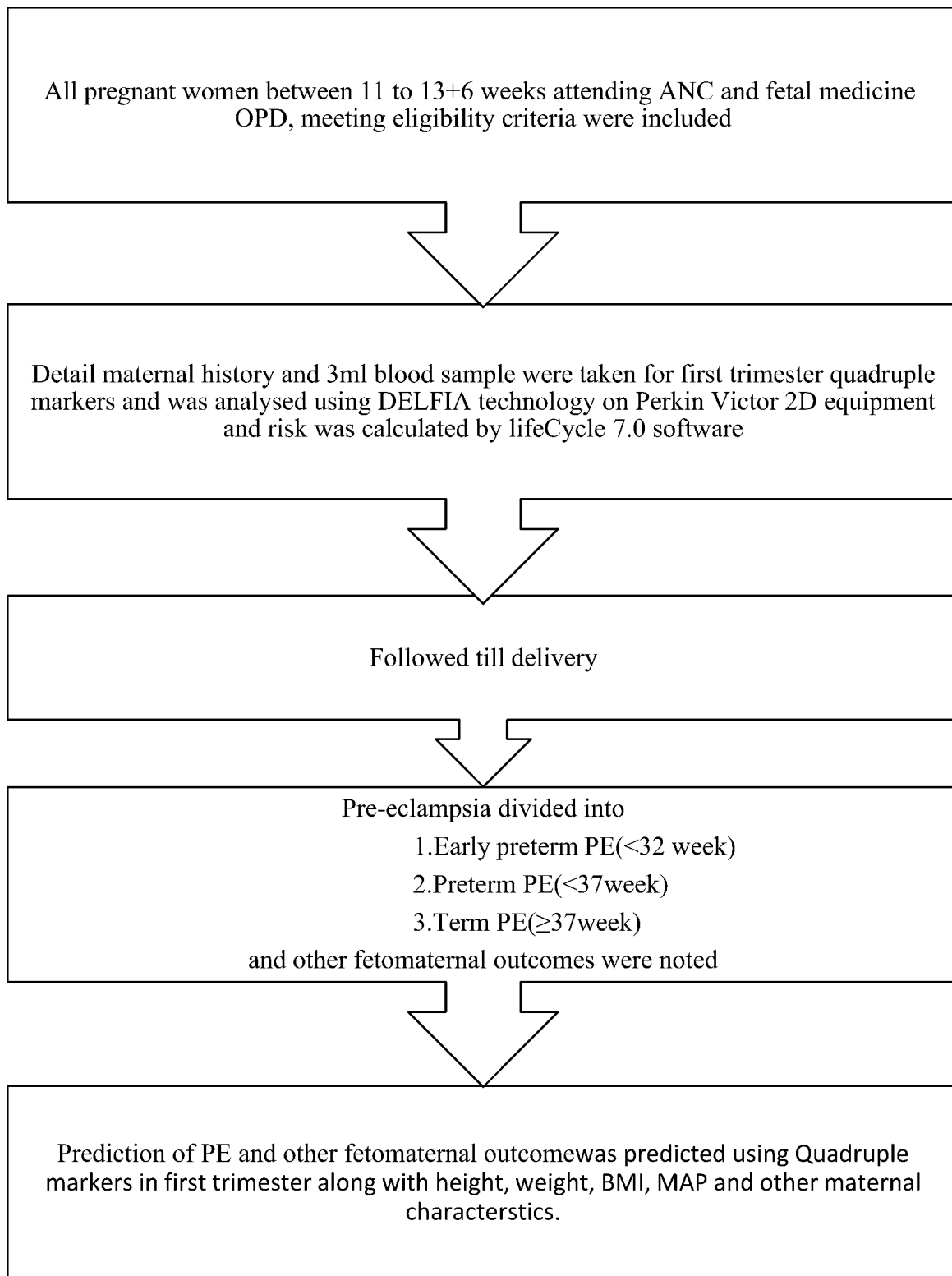


Figure 1

Flow Chart Of Methodology