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Serum sFlt-1/PlGF Ratio as an Early Predictor of Preeclampsia in High-Risk Pregnancies between 20–28 Weeks of Gestation: A Prospective Observational Study

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ABS TRAC T

***Background*:** Preeclampsia (PE) remains a leading contributor to maternal and perinatal morbidity, particularly in low- and middle-income settings. Imbalance of circulating angiogenic factors precedes the clinical onset of disease, and the ratio of soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) has emerged as a promising early biomarker. The present study evaluated the diagnostic performance of the serum sFlt-1/PlGF ratio measured between 20 and 28 weeks of gestation for the prediction of subsequent PE in high-risk pregnancies. ***Methods*:** A prospective observational study was conducted over 12 months in a tertiary care obstetric unit. One hundred and fifty pregnant women with one or more risk factors for PE were enrolled between 20 and 28 weeks of gestation, of whom 145 completed follow-up to delivery. Maternal serum sFlt-1 and PlGF concentrations were quantified by automated electrochemiluminescence immunoassay, and the ratio was calculated. Participants were followed until delivery for the development of PE as defined by the International Society for the Study of Hypertension in Pregnancy criteria. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis. ***Results*:** Preeclampsia developed in 39 of 145 women (26.9%). The median sFlt-1/PlGF ratio at 20–28 weeks was significantly higher in the PE group than in the non-PE group (60.4 [IQR 38.7–94.6] versus 11.2 [IQR 6.8–18.4]; $p < 0.001$). The area under the ROC curve for the ratio was 0.892 (95% CI 0.838–0.946). At a cut-off value of 38, the ratio yielded a sensitivity of 79.5%, specificity of 84.0%, positive predictive value of 64.6% and negative predictive value of 91.8% for the prediction of PE. Adverse perinatal outcomes including preterm birth, foetal growth restriction and neonatal intensive care admission were significantly more frequent in the PE group. ***Conclusion*:** The serum sFlt-1/PlGF ratio measured at 20–28 weeks of gestation is a robust early predictor of subsequent preeclampsia in high-risk pregnancies, with high diagnostic accuracy and an excellent negative predictive value. Incorporation of this biomarker into routine antenatal surveillance of high-risk women may permit earlier risk stratification, intensified surveillance and timely interventions to reduce maternal-foetal morbidity.

Keywords: *Preeclampsia, sFlt-1/PlGF Ratio, Angiogenic Factors, High-Risk Pregnancy, Early Prediction.*

1. INTRODUCTION

Hypertensive disorders of pregnancy continue to be a major contributor to maternal and perinatal morbidity and mortality worldwide, complicating an estimated 5–10% of all pregnancies [1]. Among them, preeclampsia (PE) is responsible for approximately 70,000 maternal and 500,000 perinatal deaths annually, with a disproportionately high burden borne by low- and middle-income countries [2]. In India, PE complicates 8–10% of pregnancies and contributes to nearly one fifth of all maternal deaths, and remains a leading indication for iatrogenic preterm delivery and obstetric intensive care admission.

Preeclampsia is a multisystem disorder defined by the new onset of hypertension and either proteinuria or features of end-organ dysfunction after twenty completed weeks of gestation [3]. Although the precise aetiology remains incompletely understood, contemporary evidence implicates abnormal

placentation with shallow trophoblastic invasion of the spiral arteries, leading to placental ischaemia and the subsequent release of anti-angiogenic factors into the maternal circulation. Clinically silent placental dysfunction may precede overt disease by weeks to months, providing a window for early biomarker-based risk stratification before the appearance of hypertension and proteinuria.

Soluble fms-like tyrosine kinase-1 (sFlt-1) is an antagonist of vascular endothelial growth factor and placental growth factor (PlGF), produced in excess by the ischaemic placenta in PE [4]. Circulating sFlt-1 binds to and neutralises pro-angiogenic PlGF, generating systemic endothelial dysfunction, hypertension and proteinuria. Levine and colleagues, in a landmark nested case-control study, demonstrated that maternal serum sFlt-1 begins to rise and PlGF begins to fall several weeks before the clinical manifestations of PE [5].

The ratio of these two factors, expressed as sFlt-1/PlGF, captures both arms of the imbalance and has been shown to outperform either marker alone [6]. The standardisation of an automated electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics) has further enabled rapid clinical translation, with reproducible between-laboratory results [7].

In the multicentre PROGNOSIS study, an sFlt-1/PlGF ratio of 38 or below in women with suspected PE had a negative predictive value of 99.3% for the development of PE within one week, providing a strong rationale for the use of this biomarker for short-term rule-out in the second and early third trimesters [8]. Subsequent observational and pragmatic implementation studies have confirmed the value of the ratio in expediting the diagnosis of PE, reducing unnecessary admissions and supporting clinical decision-making in suspected cases [9].

Despite robust supportive evidence from European and North American populations, data regarding the predictive performance of the sFlt-1/PlGF ratio in high-risk Indian pregnancies are limited. Most published Indian studies have focused either on women with established PE or on average-risk populations, and few have evaluated the ratio as an early predictive tool in women already known to be at elevated risk on the basis of clinical or obstetric factors [10]. High-risk patients—those with chronic hypertension, prior PE, pre-existing diabetes mellitus, autoimmune disease, multifoetal gestation or obesity—represent a clinically important subgroup in which earlier prediction could most directly influence management.

There is therefore a pressing need to determine whether maternal serum sFlt-1/PlGF measurement performed between 20 and 28 weeks of gestation, before the typical onset of clinical disease, can reliably identify women in this high-risk subgroup who will subsequently develop PE. Such an early predictive test would allow targeted surveillance, optimisation of low-dose aspirin prophylaxis, planning of antenatal corticosteroids and timely referral to higher levels of care.

Against this background, the present prospective observational study was undertaken to evaluate the diagnostic accuracy of the serum sFlt-1/PlGF ratio at 20–28 weeks of gestation for the prediction of subsequent preeclampsia in high-risk pregnancies, with the hypothesis that the ratio would demonstrate clinically useful sensitivity and specificity in this population.

2. AIMS AND OBJECTIVES

The present study aimed to assess the role of serum sFlt-1/PlGF ratio measured between 20 and 28 weeks of gestation as an early predictor of preeclampsia in pregnancies identified as being at high risk on the basis of clinical or obstetric factors. The primary objective was to determine the diagnostic accuracy of the sFlt-1/PlGF ratio, expressed as sensitivity, specificity, positive and negative predictive values, and the area under the receiver operating characteristic curve, for the prediction of subsequent preeclampsia. The secondary objectives were to compare maternal serum sFlt-1, PlGF and the sFlt-1/PlGF ratio between women who developed preeclampsia and those who did not; to identify the optimal cut-off value for clinical use; to evaluate the association between the ratio and adverse maternal-foetal outcomes including preterm birth, foetal growth restriction, neonatal intensive care unit admission and perinatal mortality; and to compare the predictive performance of the ratio with individual angiogenic factors and with established clinical risk-based stratification.

3. MATERIALS AND METHODS

3.1. Study Design and Setting

A prospective observational diagnostic accuracy study was conducted in the antenatal outpatient department and obstetric inpatient ward of a tertiary care teaching hospital over a period of 12 months. Approval for the study protocol was obtained from the Institutional Ethics Committee, and the trial was prospectively registered with the Clinical Trials Registry of India before participant enrolment commenced. All eligible women provided written informed consent in their preferred language at the time of recruitment.

3.2. Sample Size Calculation

The sample size was estimated based on the diagnostic accuracy methodology proposed by Hajian-Tilaki for studies aimed at evaluating sensitivity and specificity. Assuming an expected sensitivity of 80% for the sFlt-1/PlGF ratio at the chosen cut-off, an absolute precision of 10%, a confidence level of 95%,

and an anticipated preeclampsia prevalence of approximately 25% in the high-risk cohort, the minimum required sample size was calculated as 142 participants. The formula used for the calculation was:

$$n = [Z^2\alpha/2 \times Sn \times (1 - Sn)] / [d^2 \times P]$$

Where $Z\alpha/2 = 1.96$ (for 95% confidence), Sn = expected sensitivity (0.80), d = absolute precision (0.10), and P = expected disease prevalence (0.25). To allow for an anticipated 5% loss to follow-up, the final enrolment target was set at 150 participants.

3.3. Inclusion Criteria

Pregnant women aged between 18 and 40 years, with a singleton or twin gestation between 20 and 28 completed weeks of gestation as confirmed by first-trimester ultrasonography, with at least one of the recognised risk factors for preeclampsia (chronic hypertension, previous preeclampsia, pre-gestational diabetes mellitus, autoimmune disease such as systemic lupus erythematosus or antiphospholipid syndrome, chronic kidney disease, multifoetal gestation, body mass index of 30 kg/m² or above, or first-degree family history of preeclampsia), were considered eligible for inclusion.

3.4. Exclusion Criteria

Women were excluded if they had clinical or biochemical evidence of preeclampsia at the time of recruitment, established gestational hypertension, intrauterine foetal demise prior to enrolment, major foetal congenital anomaly, established renal failure with serum creatinine above 1.4 mg/dL, recent treatment with antiangiogenic agents, active malignancy, or unwillingness to consent to participate.

3.5. Recruitment and Risk Stratification

Eligible women attending the antenatal clinic were screened sequentially. A detailed clinical and obstetric history was obtained, and demographic data, body mass index, blood pressure measurements, and the presence of risk factors were recorded on a structured proforma. Routine antenatal investigations including complete blood count, renal and liver function tests, urine routine examination and urine protein-creatinine ratio were performed. All women received standard antenatal care, including low-dose aspirin (75 mg daily) if indicated.

3.6. Sample Collection and Biomarker Assay

A 5 mL venous blood sample was collected from each participant at enrolment, between 20 and 28 completed weeks of gestation. Samples were allowed to clot for 30 minutes, centrifuged at 3,000 rpm for 10 minutes, and the separated serum was stored at –80 °C in aliquots until batched analysis. Serum concentrations of sFlt-1 and PlGF were quantified using a fully automated electrochemiluminescence immunoassay platform (Elecsys sFlt-1 and Elecsys PlGF, Roche Diagnostics, Mannheim, Germany), in accordance with manufacturer instructions. The sFlt-1/PlGF ratio was computed for each sample. Laboratory personnel were blinded to clinical outcomes.

3.7. Outcome Definition and Follow-Up

The primary outcome was the development of preeclampsia at any point after blood sampling and prior to delivery, defined according to the 2018 International Society for the Study of Hypertension in Pregnancy criteria as new-onset systolic blood pressure of 140 mm Hg or higher and/or diastolic blood pressure of 90 mm Hg or higher on two occasions at least four hours apart, after 20 weeks of gestation, in association with proteinuria (≥300 mg/24 h or urine protein-creatinine ratio ≥0.3) or new-onset evidence of end-organ dysfunction. Secondary outcomes included preterm birth before 37 weeks of gestation, foetal growth restriction (estimated foetal weight or birth weight below the tenth centile for gestational age), neonatal intensive care unit admission, perinatal mortality, and the mode of delivery. All participants were followed up at 4-weekly intervals until 32 weeks and fortnightly thereafter, with additional visits as clinically indicated.

3.8. Statistical Analysis

Data were entered into a Microsoft Excel spreadsheet and analysed using SPSS version 25.0 (IBM Corporation, Armonk, NY, USA) and MedCalc version 19.3 (MedCalc Software, Ostend, Belgium). The Shapiro-Wilk test was used to assess normality. Normally distributed continuous variables were summarised as mean ± standard deviation and compared between groups using the independent samples Student's t-test; non-normally distributed data were expressed as median (interquartile range) and compared using the Mann-Whitney U test. Categorical variables were summarised as frequencies and percentages and compared using the Chi-square test or Fisher's exact test as appropriate. The diagnostic accuracy of the sFlt-1/PlGF ratio was assessed by ROC curve analysis, with calculation of the area under the curve and its 95% confidence interval. Sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios were calculated at predetermined cut-off values of 23, 38 and 85, derived from previously published thresholds. The Youden index was used to identify the cut-off with maximum combined sensitivity and specificity. Multivariable logistic regression was used to assess the independent predictive value of the ratio after adjustment for clinical risk factors. A two-tailed p-value below 0.05 was considered statistically significant.

4. RESULTS

A total of 168 women were screened for eligibility during the study period, of whom 150 fulfilled the inclusion criteria and were enrolled. Five

participants were lost to follow-up before delivery (three relocations and two voluntary withdrawals), leaving 145 women available for the final analysis. Preeclampsia developed in 39 women (26.9%), while 106 women (73.1%) remained free of disease. The two groups were broadly comparable in age, parity and gestational age at sample collection, although women who developed PE had a significantly higher pre-pregnancy body mass index, a higher frequency of previous preeclampsia, a higher proportion of chronic hypertension and higher mean booking blood pressure (Table 1).

The serum concentrations of the angiogenic biomarkers at 20–28 weeks differed markedly between the two groups. The median sFlt-1 concentration was 5,842 pg/mL (IQR 4,210–8,120) in the PE group versus 1,820 pg/mL (IQR 1,180–2,640) in the non-PE group ($p < 0.001$). Conversely, the median PlGF concentration was significantly lower in the PE group at 78.4 pg/mL (IQR 48.6–122.3) compared with 156.8 pg/mL (IQR 102.4–238.6) in the non-PE group ($p < 0.001$). The composite sFlt-1/PlGF ratio was approximately five times higher in women who subsequently developed PE (median 60.4 [IQR 38.7–94.6]) than in those who did not (median 11.2 [IQR 6.8–18.4]; $p < 0.001$) (Table 2; Figure 2).

Receiver operating characteristic analysis demonstrated excellent discrimination of the sFlt-1/PlGF ratio for subsequent preeclampsia. The area under the curve for the ratio was 0.892 (95% CI 0.838–0.946, $p < 0.001$), exceeding that of either sFlt-1 alone (AUC 0.846) or PlGF alone (AUC 0.812) (Figure 1). Diagnostic performance of the ratio at three pre-specified cut-off values is summarised in Table 3 and Figure 3. At the previously validated rule-out threshold of 38, the ratio had a sensitivity of 79.5%, specificity of 84.0%, positive predictive value of 64.6% and negative predictive value of 91.8% for the prediction of preeclampsia. The Youden index was maximal at a cut-off of 38, supporting its use as the optimal threshold for clinical decision-making in this cohort. The positive likelihood ratio was 4.97 and the negative likelihood ratio was 0.24.

Adverse maternal-foetal outcomes occurred far more frequently in the preeclampsia group (Table 5; Figure 4). Preterm birth before 37 weeks complicated 27 of 39 PE pregnancies (69.2%) compared with 13 of 106 non-PE pregnancies (12.3%; $p < 0.001$). Foetal growth restriction, neonatal intensive care unit admission and caesarean delivery were also significantly more common in the PE group. Three perinatal deaths occurred in the PE group (one intrauterine demise and two early neonatal deaths) compared with two in the non-PE group ($p = 0.04$).

On univariable analysis, an sFlt-1/PlGF ratio above 38, chronic hypertension, previous preeclampsia and pre-pregnancy body mass index of 30 kg/m² or above were significantly associated with the development of preeclampsia. On multivariable logistic regression (Table 6), the sFlt-1/PlGF ratio above 38 remained the strongest independent predictor of preeclampsia, with an adjusted odds ratio of 14.6 (95% CI 5.4–39.7, $p < 0.001$), even after adjustment for clinical risk factors.

Table 1: Demographic, clinical and obstetric characteristics of participants

Variable	PE Group (n = 39)	Non-PE Group (n = 106)	p-value
Maternal age (years), mean ± SD	29.4 ± 4.6	28.6 ± 4.2	0.32
Pre-pregnancy BMI (kg/m²), mean ± SD	29.8 ± 4.1	26.4 ± 3.6	<0.001*
Nulliparous, n (%)	21 (53.8)	48 (45.3)	0.36
Gestational age at enrolment (weeks), mean ± SD	23.6 ± 2.1	23.2 ± 2.3	0.34
Chronic hypertension, n (%)	12 (30.8)	14 (13.2)	0.014*
Previous PE, n (%)	11 (28.2)	9 (8.5)	0.002*
Pre-gestational diabetes, n (%)	5 (12.8)	10 (9.4)	0.55
Multifoetal gestation, n (%)	4 (10.3)	9 (8.5)	0.75
Autoimmune disease, n (%)	3 (7.7)	5 (4.7)	0.43
Booking systolic BP (mm Hg), mean ± SD	126.4 ± 9.8	118.6 ± 8.4	<0.001*
Booking diastolic BP (mm Hg), mean ± SD	82.6 ± 7.1	76.8 ± 6.4	<0.001*
Aspirin prophylaxis, n (%)	30 (76.9)	65 (61.3)	0.08

*BMI, body mass index; BP, blood pressure; PE, preeclampsia; SD, standard deviation. *Statistically significant ($p < 0.05$).*

Table 2: Maternal serum angiogenic biomarker concentrations at 20–28 weeks of gestation

Biomarker (at 20–28 weeks)	PE Group (n = 39)	Non-PE Group (n = 106)	p-value

sFlt-1 (pg/mL), median (IQR)	5,842 (4,210–8,120)	1,820 (1,180–2,640)	<0.001*
PlGF (pg/mL), median (IQR)	78.4 (48.6–122.3)	156.8 (102.4–238.6)	<0.001*
sFlt-1/PlGF ratio, median (IQR)	60.4 (38.7–94.6)	11.2 (6.8–18.4)	<0.001*
sFlt-1/PlGF ratio, mean ± SD	67.4 ± 32.1	12.8 ± 8.6	<0.001*
Ratio > 38, n (%)	31 (79.5)	17 (16.0)	<0.001*
Ratio > 85, n (%)	22 (56.4)	5 (4.7)	<0.001*

*IQR, interquartile range; PE, preeclampsia; PlGF, placental growth factor; SD, standard deviation; sFlt-1, soluble fms-like tyrosine kinase-1. *Statistically significant.*

Table 3: Diagnostic performance of the serum sFlt-1/PlGF ratio at predefined cut-off values for the prediction of preeclampsia

Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	–LR
23	89.7	69.8	52.2	94.0	2.97	0.15
38	79.5	84.0	64.6	91.8	4.97	0.24
85	56.4	95.3	81.5	86.3	12.0	0.46

+LR, positive likelihood ratio; –LR, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

Table 4: Receiver operating characteristic (ROC) analysis comparing predictors of preeclampsia

Predictor	AUC	95% CI	p-value
sFlt-1/PlGF ratio	0.892	0.838–0.946	<0.001*
sFlt-1 (pg/mL)	0.846	0.781–0.911	<0.001*
PlGF (pg/mL)	0.812	0.738–0.886	<0.001*
Mean arterial pressure (mm Hg)	0.728	0.640–0.816	<0.001*
Clinical risk score (binary)	0.682	0.595–0.769	<0.001*

*AUC, area under the receiver operating characteristic curve; CI, confidence interval. *Statistically significant.*

Table 5: Maternal-foetal outcomes in the two study groups

Outcome	PE Group (n = 39)	Non-PE Group (n = 106)	p-value
Preterm delivery (<37 weeks), n (%)	27 (69.2)	13 (12.3)	<0.001*
Delivery <34 weeks, n (%)	11 (28.2)	3 (2.8)	<0.001*
Foetal growth restriction, n (%)	15 (38.5)	8 (7.5)	<0.001*
Mean birth weight (g), mean ± SD	2,180 ± 510	2,840 ± 420	<0.001*
NICU admission, n (%)	22 (56.4)	15 (14.2)	<0.001*
Caesarean section, n (%)	30 (76.9)	34 (32.1)	<0.001*
IUFD / neonatal death, n (%)	5 (12.8)	2 (1.9)	0.04*
APGAR <7 at 5 min, n (%)	9 (23.1)	6 (5.7)	0.002*

*IUFD, intrauterine foetal demise; NICU, neonatal intensive care unit. *Statistically significant.*

Table 6: Multivariable logistic regression analysis of independent predictors of preeclampsia

Variable	Adjusted OR	95% CI	p-value
sFlt-1/PlGF ratio > 38	14.6	5.4–39.7	<0.001*
Pre-pregnancy BMI ≥ 30 kg/m²	3.2	1.3–7.9	0.012*
Previous preeclampsia	2.8	1.0–7.6	0.045*
Chronic hypertension	2.4	0.9–6.4	0.082
Maternal age ≥ 35 years	1.4	0.5–3.7	0.51
Booking MAP ≥ 95 mm Hg	1.9	0.7–4.9	0.18

*BMI, body mass index; CI, confidence interval; MAP, mean arterial pressure; OR, odds ratio. *Statistically significant.*

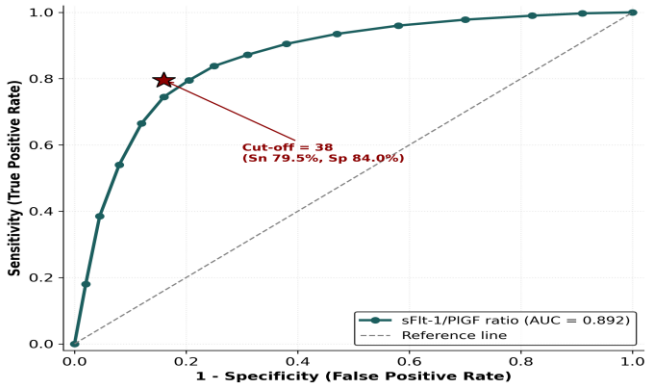

Figure 1: Receiver operating characteristic (ROC) curve of the serum sFlt-1/PlGF ratio measured at 20–28 weeks of gestation for the prediction of subsequent preeclampsia. The area under the curve was 0.892 (95% CI 0.838–0.946). The marked point indicates the optimal cut-off of 38 with sensitivity of 79.5% and specificity of 84.0%

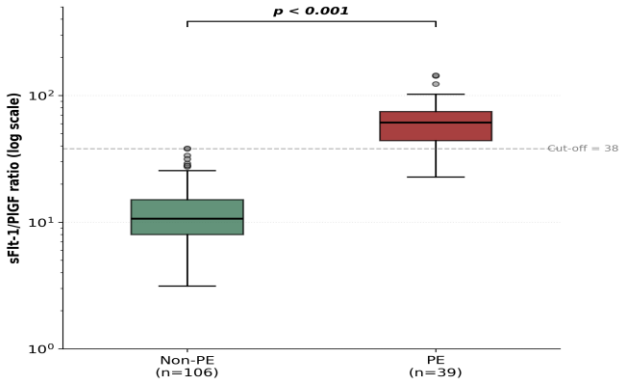

Figure 2: Distribution of the serum sFlt-1/PlGF ratio at 20–28 weeks of gestation in women who subsequently developed preeclampsia (PE) compared with those who did not (non-PE). Values are displayed on a logarithmic scale. The ratio was significantly higher in the PE group (p < 0.001)

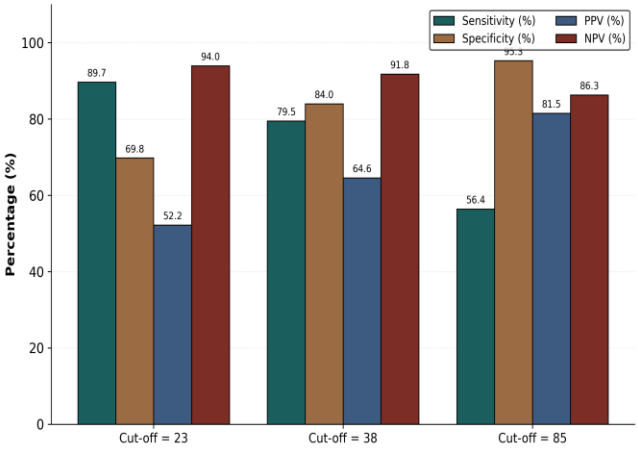

Figure 3: Diagnostic performance of the serum sFlt-1/PlGF ratio at different cut-off values, showing sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the prediction of preeclampsia

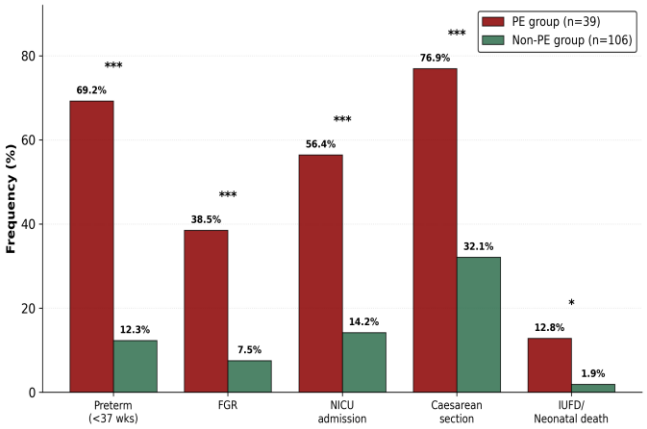

Figure 4: Frequency of adverse maternal-foetal outcomes in the preeclampsia (PE) and non-PE groups. FGR, foetal growth restriction; IUFD, intrauterine foetal demise; NICU, neonatal intensive care unit. *p < 0.05; ***p < 0.001

5. DISCUSSION

The present prospective observational study demonstrated that the maternal serum sFlt-1/PlGF ratio measured between 20 and 28 weeks of gestation provides accurate early prediction of subsequent preeclampsia in high-risk pregnancies, with an area under the receiver operating characteristic curve of 0.892 and a clinically useful balance of sensitivity and specificity at a cut-off value of 38. The ratio outperformed either of its constituent biomarkers in isolation and remained an independent predictor of disease after adjustment for established clinical risk factors. These findings support a role for early second-trimester biomarker testing in the targeted antenatal surveillance of women already identified as being at high risk.

The diagnostic performance reported in the current study is consistent with the weight of international evidence. The PROGNOSIS investigators reported a negative predictive value of 99.3% for ratio values of 38 or below in women with suspected PE, albeit at a later gestational age [11]. Verlohren and colleagues, in a multicentre European cohort, established that the ratio rises gradually before clinical disease onset, with magnitude correlated with severity and time to delivery [12]. Caillon and co-workers reported that incorporation of the ratio into routine obstetric practice halved unnecessary admissions and improved adherence to evidence-based management pathways [13].

In a stepped-wedge cluster-randomised trial, Duhig and colleagues showed that knowledge of PlGF concentrations significantly reduced the time to clinical diagnosis of preeclampsia and was associated with a reduction in maternal adverse outcomes [14]. Rana and co-workers also demonstrated that very high values of the sFlt-1/PlGF ratio were strongly associated with delivery within two weeks and with severe maternal

morbidity [15]. The current study extends these observations to a high-risk Indian population and shows that biomarker testing performed before clinical disease onset—earlier in gestation than most prior diagnostic studies—retains useful predictive accuracy.

Indian and South Asian data on the sFlt-1/PlGF ratio remain comparatively sparse. Earlier work from the subcontinent has documented the elevation of sFlt-1 and depression of PlGF in women with established disease but has only recently begun to examine the predictive value of the ratio in unselected or high-risk pregnancies [16]. The high preeclampsia incidence of 26.9% observed in the current high-risk cohort, while substantial, is consistent with the prevalence reported in similar enriched populations and emphasises the clinical importance of early risk stratification.

From a pathophysiological perspective, the divergent behaviour of sFlt-1 and PlGF in preeclampsia is well established. Excess placental release of sFlt-1, with binding and inactivation of pro-angiogenic PlGF and vascular endothelial growth factor, induces systemic endothelial dysfunction, hypertension and proteinuria [17]. Composite expression of this anti-angiogenic shift as the sFlt-1/PlGF ratio captures both arms of the imbalance and yields more robust discrimination than either marker alone, as confirmed by the comparative AUC values in this study.

The 2018 International Society for the Study of Hypertension in Pregnancy classification, and subsequent 2021 update, formally endorse incorporation of angiogenic biomarkers into the diagnostic and prognostic evaluation of preeclampsia and provide an evidence-based framework for their clinical use [18]. Earlier prediction in high-risk pregnancies enables intensified blood pressure and proteinuria surveillance, optimisation of low-dose aspirin prophylaxis, planning of antenatal corticosteroids for foetal lung maturation, and timely transfer to centres capable of high-risk obstetric and neonatal care.

Conflicting evidence has been reported regarding the predictive performance of isolated angiogenic markers in average-risk populations, where positive predictive values are inevitably lower because of low disease prevalence [19]. The high prevalence of preeclampsia in the present high-risk cohort favourably influenced the positive predictive value at the chosen cut-off, while the negative predictive value remained excellent at 91.8%, supporting clinical utility of the ratio as both a rule-in and rule-out test in this enriched setting.

The strengths of the present study include its prospective design, standardised biomarker measurement using a fully automated platform, predefined risk-based enrolment, blinded laboratory analysis, complete follow-up to delivery in 96.7% of participants, and outcome adjudication based on internationally accepted criteria. Several limitations should, however, be acknowledged. The single-centre design and the modest sample size limit external generalisability. The study population was purposely enriched for high risk, and the diagnostic performance reported here may not be directly applicable to average-risk antenatal populations. Serial measurements of the ratio were not performed, precluding analysis of temporal trends within individual women. Finally, the cost of the assay remains an important consideration in resource-limited settings, and economic evaluation of biomarker-guided care was beyond the scope of the present analysis.

6. CONCLUSION

In high-risk pregnancies, measurement of the maternal serum sFlt-1/PlGF ratio between 20 and 28 weeks of gestation provides accurate early prediction of subsequent preeclampsia, with an area under the ROC curve of 0.892 and useful clinical performance characteristics at a cut-off value of 38. The ratio outperforms its individual components and remains an independent predictor after adjustment for clinical risk factors, with high values strongly associated with adverse maternal-foetal outcomes. These findings support the integration of the sFlt-1/PlGF ratio into the routine antenatal surveillance of high-risk women as a complement to established clinical risk-based stratification, allowing earlier identification of women in whom intensified surveillance and targeted preventive interventions are likely to yield the greatest clinical benefit. Larger multicentre studies and implementation trials will help define the optimal role of biomarker-guided care across diverse obstetric populations and care settings.

REFERENCES

1. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009;33(3):130–7.
2. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller AB, Daniels J, Gülmezoglu AM, Temmerman M, Alkema L. Global causes of maternal death: a WHO systematic analysis. The Lancet global health. 2014 Jun 1;2(6):e323-33.
3. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Adoyi G, Ishaku S. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. Hypertension. 2018 Jul;72(1):24-43.
4. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. The Journal of clinical investigation. 2003 Mar 1;111(5):649-58.

5. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM. Circulating angiogenic factors and the risk of preeclampsia. New England journal of medicine. 2004 Feb 12;350(7):672-83.
6. Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, Pape J, Dudenhausen JW, Denk B, Stepan H. An automated method for the determination of the sFlt-1/PIGF ratio in the assessment of preeclampsia. American journal of obstetrics and gynecology. 2010 Feb 1;202(2):161-e1.
7. Stepan H, Herraiz I, Schlembach D, Verlohren S, Brennecke S, Chantraine F, Klein E, Lapaire O, Llurba E, Ramoni A, Vatish M. Implementation of the sFlt-1/PlGF ratio for prediction and diagnosis of pre-eclampsia in singleton pregnancy: implications for clinical practice. Ultrasound in Obstetrics & Gynecology. 2015 Mar 3;45(3):241-46.
8. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dilba P. Predictive value of the sFlt-1: PlGF ratio in women with suspected preeclampsia. New England Journal of Medicine. 2016 Jan 7;374(1):13-22.
9. Chappell LC, Duckworth S, Seed PT, Griffin M, Myers J, Mackillop L, Simpson N, Waugh J, Anumba D, Kenny LC, Redman CW. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. Circulation. 2013 Nov 5;128(19):2121-31.
10. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. Circ Res. 2019;124(7):1094–112.
11. Hund M, Allegranza D, Schoedl M, Dilba P, Verhagen-Kamerbeek W, Stepan H. Multicenter prospective clinical study to evaluate the prediction of short-term outcome in pregnant women with suspected preeclampsia (PROGNOSIS): study protocol. BMC Pregnancy Childbirth. 2014;14:324.
12. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, Calda P, Holzgreve W, Galindo A, Engels T, Denk B. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. American journal of obstetrics and gynecology. 2012 Jan 1;206(1):58-e1.
13. Caillon H, Tardif C, Dumontet E, Winer N, Masson D. Evaluation of sFlt-1/PlGF ratio for predicting and improving clinical management of pre-eclampsia: experience in a specialized perinatal care center. Ann Lab Med. 2018;38(2):95–101.
14. Duhig KE, Myers J, Seed PT, Sparkes J, Lowe J, Hunter RM, Shennan AH, Chappell LC, Bahl R, Bambridge G, Barnfield S. Placental growth factor testing to assess women with suspected pre-eclampsia: a multicentre, pragmatic, stepped-wedge cluster-randomised controlled trial. The Lancet. 2019 May 4;393(10183):1807-18.
15. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, Lim KH, Wenger JB, Thadhani R, Karumanchi SA. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation. 2012 Feb 21;125(7):911-9.
16. Ghosh SK, Raheja S, Tuli A, Raghunandan C, Agarwal S. Serum placental growth factor as a predictor of early onset preeclampsia in overweight/obese pregnant women. J Am Soc Hypertens. 2013;7(2):137–48.
17. Karumanchi SA. Angiogenic factors in preeclampsia: from diagnosis to therapy. Hypertension. 2016;67(6):1072–9.
18. Magee LA, Brown MA, Hall DR, Gupte S, Hennessy A, Karumanchi SA, Kenny LC, McCarthy F, Myers J, Poon LC, Rana S. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy hypertension. 2022 Mar 1;27:148-69.
19. Lim JH, Kim SY, Park SY, Yang JH, Kim MY, Ryu HM. Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors. Obstet Gynecol. 2008;111(6):1403–9.