Research Article

Linking Glomerular Endothelial Dysfunction with Urinary KIM-1, sFlt-1, Serum IL-10, and Regulatory T Cells in Preeclampsia

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Article Info

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Keywords

Regulatory T cells, Serum sFlt[1], IL-10, Urinary KIM-1, Preeclampsia

Abstract

Background: Preeclampsia (PE) usually presents after 20 weeks of pregnancy with high blood pressure and protein levels in the urine. An imbalance between the body's pro-inflammatory and anti-inflammatory responses has been suggested to be a key issue in the pathophysiology of the disease. Some important factors, such as soluble fms like tyrosine kinase -1 (sFlt-1), T regulatory cells (Tregs), and Interleukin-10 (IL-10) molecules are thought to be involved as mediators in a systematic response affecting the blood vessel lining. Proteinuria is an essential feature of preeclampsia suggesting the involvement of the kidneys in the disease.

Objective: Our study aimed to explore how Tregs, IL-10 and sFlt-1 correlate with Kidney Injury Molecule-1(KIM-1) protein levels in urine to better understand preeclampsia-induced renal endothelial dysfunction in better way.

Methodology: 36 normal pregnant women and 29 women with preeclampsia were enrolled in this cross-sectional study. Tregs, IL-10, sFlt-1 and KIM-1 levels were analysed and correlated between both the groups.

Results: Our findings revealed that the levels of CD4+FOXP3+ Treg cells and serum IL-10 were much higher and the levels of serum sFlt-1 and urinary KIM-1 were lower in normal pregnant women than in those with preeclampsia. ROC curve showed that serum sFlt-1 was a strong marker for diagnosing preeclampsia with a sensitivity of 93% and specificity of 92%, followed by urinary KIM-1 with a sensitivity of 76% and specificity of 58%, implying at ongoing kidney injury in preeclampsia.

Conclusion: Our study elucidates preeclampsia and supports better biomarker use and treatments, aiming to improve health outcomes for mothers and babies.

Introduction

Preeclampsia (PE) is a hypertensive disorder of pregnancy characterized by new-onset hypertension after 20 weeks of gestation, defined as a systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two occasions at least 4 hours apart, in a previously normotensive woman, accompanied by either proteinuria (≥300 mg per 24 hours or a protein-to-creatinine ratio ≥ 0.3) or, in the absence of proteinuria, new-onset maternal organ dysfunction such as thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, or cerebral or visual symptoms [1]. This condition affects approximately 3-5% of pregnant women worldwide and it's one of the key causes of perinatal mortality and morbidity.

If not properly managed, preeclampsia can lead to severe complications such as eclampsia, pulmonary edema, or kidney failure in the mother. Additionally, the baby may face risks like premature birth or impaired growth (fetal growth restriction). Due to these risks, it is crucial to promptly detect and manage preeclampsia to ensure the well-being of both the mother and the baby. At present, there are no specific treatments available for this condition. The primary aim is to control symptoms and delay childbirth until approximately 34 weeks to enhance outcomes for both mothers and their infants [2].

Despite extensive research, the exact etiology of PE remains incompletely elucidated and is likely multifactorial [3,4]. Preeclampsia is often observed in two phases. The first phase, early placental phase, involves problems with how the placenta develops and is due to poor trophoblast invasion into the spiral arteries, resulting in reduced blood flow to the fetus. In the later phase of preeclampsia, the clinical manifestations arise from the maternal syndrome, which reflects the maternal response to placental dysfunction rather than a distinct late-onset stage of disease. The ischemic placenta releases excess anti-angiogenic factors, particularly soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), into the maternal circulation. These molecules antagonize the effects of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), leading to widespread endothelial dysfunction, vasoconstriction, and increased vascular permeability. The resulting systemic effects manifest as hypertension, proteinuria, and multi-organ involvement, which together constitute the maternal syndrome of preeclampsia [1,5]. One of these substances is sFlt-1. This protein can block the actions of important growth factors such as Vascular Endothelial Growth factor (VEGF) and Placental Growth Factor (PlGF)-thereby preventing their binding to the endothelial lining of blood vessels in the placenta ultimately causing an increase in maternal blood pressure during preeclampsia [6]. Moreover, increased sFlt-1 levels have been shown to accelerate proteinuria by lowering the expression of the podocyte protein nephrin as demonstrated in rat models of crescentic glomerulonephritis [7]. Thus, findings like these point out the importance of antiangiogenic proteins like sFlt-1 in understanding preeclampsia.

From early pregnancy, the maternal immune system undergoes adaptive changes to promote tolerance toward the semi-allogenic fetus. A critical component of this immunological adaptation involves regulatory T cells (Tregs), which actively suppress excessive T cell–mediated immune responses and maintain maternal–fetal immune tolerance. These cells help prevent the maternal immune system from recognizing and attacking fetal antigens. In healthy pregnancies, the balance between pro-inflammatory and anti-inflammatory responses is tightly regulated. However, in preeclampsia, impaired Treg function and an increase in inflammatory T helper (Th1 and Th17) responses disturb this delicate equilibrium, contributing to abnormal placentation and endothelial dysfunction[8,9]. Studies in mice have shown that having sufficient healthy Tregs is important for supporting changes in blood vessels during pregnancy thereby preventing the development of inflammatory problems related to the placenta[10].

Interleukin-10 (IL-10) is another key player; -it helps to reduce inflammation by inhibiting pro-inflammatory T helper 1 (Th1) cell activities and promoting healthy development of the placenta and its blood vessels-making IL-10 a crucial factor for successful pregnancy [11]. The lack of IL-10 can lead to serious problems such as premature birth or even miscarriage [12,13].

KIM-1 is a special trans-membrane glycoprotein mainly synthesized by proximal tubular cells in the renal system; when it is found in blood or urine, it usually means that kidney tubular damage is occurring [14].Therefore high levels of KIM-1 could provide an early warning about potential kidney injury related to preeclampsia.

Therefore, Regulatory T cells, serum IL-10, sFlt-1, and urinary KIM-1 which have shown the potential to be key clinical mediators in the pathogenesis of glomerular endothelial injury in preeclampsia, have been studied. Dysregulated levels of these markers contribute to immune imbalance, angiogenic disruption, and renal glomerular damage, thereby exacerbating disease severity. Current study hypothesised that these markers act through interrelated pathways, and their interactions could provide deeper insights into the mechanisms underlying preeclampsia. This hypothesis further suggests that a comprehensive understanding of these biomarkers could facilitate the development of advanced diagnostic tools and targeted therapeutic strategies for effective management of preeclampsia.

The current study was conducted to examine how Tregs, IL-10, sFlt-1 correlate with KIM-1 protein levels found in the urine of patients with preeclampsia compared with normal pregnancies. This comparison could help us to better understand the relationship between kidney damage and preeclampsia a lot better.

Materials and methods

It is a cross-sectional study done at All India Institute of Medical Sciences, Bhubaneswar after getting ethical clearance from the Institute (Ref no: IEC/AIIMS BBSR/ PG THESIS/ 2022-23/50). We included 29 women diagnosed

with preeclampsia and 36 healthy pregnant women as case (Group-A) and control (Group-B) groups from the Department of Obstetrics and Gynaecology. Participants provided informed consent to join this study, during which we adhered to the ACOG guidelines for diagnosing PE. Healthy pregnant women were included if they were over 20 weeks of gestation. The gestational age of each participant was noted at the time of blood and urine collection.

The ACOG (American College of Obstetricians and Gynecologists) guidelines for diagnosing preeclampsia include pregnant women with two separate blood pressure readings at ≥ 140/90 mmHg with proteinuria of either 300 mg within 24 hours or protein/creatinine ratio of 0.3 mg/dL or more, or a dipstick test showing +2 for protein. This is done only if other quantitative methods unavailable [1].

In Group A, all participants had early-onset preeclampsia that is PE occurring before 34 weeks gestation.

Women diagnosed with Hemolysis, Elevated Liver Enzymes, and Low Platelet Count (HELLP) syndrome, eclampsia, diabetes mellitus, other inflammatory diseases, autoimmune disorders or prior kidney disease were excluded from the study.

Method: Five millilitres of blood was collected from each participant; 2 ml in Ethylenediaminetetraacetic acid (EDTA) containing vacutainers and the other 3 ml in plain vacutainers. The EDTA sample was used immediately for analysis of Tregs by flow cytometry (Beckman Coulter Navios and Dx flex cytometers) while serum was isolated from plain vacutainers via centrifugation and stored at -20 C for estimation of sFlt-1 and IL-10 levels by Enzyme Linked Immunosorbent Assay (ELISA) (ELK biotechnology human sFlt-1 ELISA Kit and EliKine™ Human IL-10 ELISA Kit). Urine was collected from the enrolled participants and stored at -80°C for the assessment of KIM-1 levels using ELISA (ELK Biotechnology Human Urinary KIM-1 ELISA Kit). All three ELISA kits employed a sandwich ELISA technique. Complete blood counts, liver function tests (TBIL, DBIL, AST, ALT, ALP, TP, albumin, globulin, A:G) and kidney function tests (Blood urea, serum creatinine, uric acid, Sodium, Potassium, Chloride) were performed for all study participants.

Flow cytometric analysis

After collecting blood samples in EDTA vials, samples were processed following Beckman Coulter's DURAClone IM Treg tubes instruction manual for estimation of Tregs. The processed samples were analyzed using a Beckman Coulter Navios flow cytometer and Beckman Coulter Dx Flex flow cytometer. The computer system connected to the flow cytometer archives the data for each individual cell, which are subsequently analyzed using the built-in software, CXP. The DURAClone IM Treg panel facilitated the detection and characterization of FoxP3+ T regulatory cells through a rapid permeabilization protocol. These tubes incorporate eight markers in various fluorochrome combinations that support the reliable identification of cell populations, including CD3, CD4, CD25, FoxP3, CD39, CD45, CD45RA, and Helios.

Tregs were defined as CD4+CD25+FOXP3+ using flow cytometry. Gating strategies were standardized and applied equally across groups. Only co-expressing cells were analyzed. Markers such as Helios and CD45RA were collected but not statistically explored due to sample size limitations.

Statistical Analysis

Data were anonymously collected and electronically stored for analysis. Data analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) v26. Data were checked for normal distribution, and all data were reported accordingly. Continuous data are expressed as median (IQR), and discrete data as counts (percentage). Comparisons between groups were performed using the Mann Whitney U test. Spearman's correlation coefficient was used to determine the correlation between the studied parameters. Receiver operating characteristic (ROC) analysis was utilized to assess the diagnostic capability of biomarkers at the time of sampling. The area under the curve (AUC) and its 95% confidence intervals were determined and the optimal threshold for each biomarker was established through Youden's J index, calculated as (sensitivity + specificity – 1). At this determined threshold, sensitivity and specificity were recorded. ROC analyses were conducted to differentiate between preeclampsia and control groups, but they do not offer predictions before the disease develops. A p-value of less than 0.05 was considered statistically significant.

Results

Of all the 65 participants included (29 in Group-A and 36 in Group-B) we could not collect urine samples from three normal pregnant women because of patient unavailability. Owing to technical issues, we were unable to perform flowcytometry analysis for the two samples in the preeclampsia group. Hence, the flow cytometric analysis included 27 women with PE and 36 normal pregnant women, and urinary KIM-1 was estimated in 29 cases and 33 control women. Among anthropometric measurements as shown in Table 1, height, weight, pulse and systolic and diastolic blood pressure (BP) were significantly higher in patients than in controls ($p<0.05$). No significant difference was found between the cases and controls with respect to the various parameters of the complete blood picture (shown in Table 2). Among all Liver function tests (LFT's) (Table 3), Aspartate Transaminase (AST) was significantly higher in case group than in the control group while total protein and albumin levels were significantly higher in the control group. Among the Renal Function Tests (RFT's) (shown in Table 3), creatinine levels were significantly higher in the case group than in the control group while serum sodium levels were significantly higher in the control group than in the case group. Binary logistic regression was performed to account for the baseline anthropometric characteristics of the individuals, and none of the parameters were found to be significant in the model.

Among the immunological parameters (shown in Table 4), the percentages (%) of CD4+CD25+ and CD 4+FOX P3+ Tregs (in % of lymphocytes) were significantly higher in the control group than in the case group. The serum sFlt-1 and urinary KIM-1 levels were significantly higher in the case group than in the control group. The serum IL-10 levels were significantly higher in the control group than cases group. According to the correlation statistics (shown in Table 5), within the overall population, a significant negative correlation was found between the following parameters: a) CD4+ FOXP3+ and serum sFLT-1 b) CD4+ FOX P3+ & Urinary KIM-1, and c) serum sFlt-1 and serum IL-10. Within the case group, a significant negative correlation existed between CD4+ FOXP3+ and urinary KIM-1, whereas in the control group no significant correlation was found between any of the parameters. The correlation heatmap of Regulatory T Cells and serum and urine parameters in the overall population is shown in Figure 1. According to the Receiver Operating Characteristic (ROC) curve as shown in Figure 2, serum sFlt-1 served as the best marker with a higher sensitivity and specificity of approximately 93% and 92% respectively (Area Under Curve (AUC) =0.955), followed by urinary KIM-1 with a sensitivity and specificity of 76% and 58 % respectively (AUC=0.671). The AUC curve analysis data with cut-off values for different parameters are shown in Table 6.

The flowcytometry flow page of T-regs of preeclampsia women (Group A) is shown in Figures 3A, 3B, 3C and normal pregnant women (Group B) are shown in 4A, 4B and 4C respectively.

Table 1: Demographic anthropometric characteristics of cases and controls.

Parameters		Cases [n=29] Median (IQR)	Controls [n=36] Median (IQR)	p-value MW
Age (in years)		28 (25-30)	28 (25.5-29.5)	0.942
Primi Gravida#	No	13 (44.83)	14 (38.89)	0.800χ
	Yes	16 (55.17)	22 (61.11)	
Gestational age <37 weeks#	No	9 (31.03)	8 (22.22)	0.571χ
	Yes	20 (68.97)	28 (77.78)	
Height (in cms)		153 (150-156)	150.5 (149-154)	0.048*
Weight (in kgs)		66 (62-68.5)	61 (56.65-66)	0.021*
BMI (kg/m2)		27.63 (26.77-28.19)	27.02 (25.85-27.63)	0.066
Weight Gained During Pregnancy (kgs)		7.5 (6.5-9)	7.95 (7-9)	0.735
Pulse (in bpm)		96 (88-100)	88 (81.5-93)	0.003*
Systolic Blood Pressure (in mm of Hg)		144 (139-150)	110 (105.5-115.5)	0.001*
Diastolic Blood Pressure (in mm of Hg)		92 (90-96)	71 (67-76)	0.001*

#Count (%). MW: p-value based on Mann Whitney U test. χ : Chi-Square test. *Significant difference-value <0.05.

Table 2: Showing Complete blood picture of cases and controls.

Parameter	Cases (n=29)	Controls (n=36)	p- valueMW
	Median(IQR)	Median (IQR)	
Hb (in g/dl)	11.1 (10.5-11.6)	11.35 (10.35-12.2)	0.44
RBC count(in millions/ cu mm)	4.15 (3.8-4.58)	4.365 (3.875-4.695)	0.44
PCV (in %)	34.7 (32.5-38.6)	36 (32.4-38.9)	0.319
MCV (in fl)	82.7 (76.5-86.7)	82.45 (73.15-90.05)	0.995
MCH(in pg)	26.7 (23.9-29.4)	25.75 (22.2-28.15)	0.295
MCHC(in g/dl)	31.6 (29.9-32.7)	30.25 (29.15-31.85)	0.122
RDW CV (in %)	14.8 (13.5-16.8)	15.6 (14-16.5)	0.191
WBC count (10^3/cu mm)	10.74 (8.81-12.61)	10.68 (9.125-12.19)	0.522
Neutrophils (in %)	74.9 (70.5-78.8)	77.45 (73.85-83.05)	0.251
Lymphocytes (in %)	18 (15.3-20.25)	16.75 (13.7-19.5)	0.402
Eosinophils (in %)	1.2 (0.6-1.8)	1.15 (0.6-1.95)	0.716
Monocytes (in %)	2.8 (2.4-3.1)	3 (2.5-3.5)	0.253

Basophils (in %)	0.3 (0.1-0.4)	0.3 (0.2-0.45)	0.459
Platelet count(in 10^3/cu mm)	238 (185-274)	232.5 (184-269.5)	0.874

Table 3: shows liver function tests and renal function tests among the cases and controls.

Parameter	Cases (n=29)	Controls (n=36)	p- valueMW
	Median(IQR)	Median (IQR)	
Total Bilirubin (T.Bil) (in mg/dl)	0.6 (0.4-0.92)	0.72 (0.455-0.895)	0.761
Direct Bilirubin (D.Bil) (in mg/dl)	0.1 (0.1-0.15)	0.1 (0.08-0.13)	0.771
Indirect bilirubin (IBil) (in mg/dl)	0.43 (0.3-0.72)	0.61 (0.375-0.8)	0.209
Aspartate Transaminase (AST) (in U/L)	30 (21.1-43)	20.25 (16-30.55)	0.017*
Alanine Transaminase (ALT) (in U/L)	28 (15-39)	18.95 (10.5-35)	0.409
Alkaline Phosphatase (ALP) (in U/L)	165 (127-196)	145 (109.5-173)	0.162
Total Protein (TP) (in g/dl)	6.2 (5.8-6.3)	6.51 (6.15-6.75)	0.001*
Albumin (in g/dl)	3.12 (3-3.3)	3.45 (3.2-3.6)	0.001*
Globulin (in g/dl)	3 (2.8-3.1)	3 (2.8-3.3)	0.31
Albumin to Globulin Ratio (A:G)	1.06 (1.03-1.09)	1.11 (1.03-1.27)	0.109
Urea (in mg/dl)	19 (17-24)	18.2 (15.3-23.5)	0.667
Creatinine (in mg/dl)	0.83 (0.79-0.91)	0.76 (0.665-0.835)	0.006*
Uric acid (in mg/dl)	4.8 (3.92-6.2)	4.4 (3.9-5.65)	0.366
Sodium (in mEq/L)	132 (129-134)	134.5 (131.5-138.5)	0.005*
Potassium (in mEq/L)	3.87 (3.51-4.4)	3.98 (3.675-4.29)	0.501
Chloride (in mmol/L)	101 (100-104)	102 (101-104)	0.328

MW: p-value based on Mann Whitney U test. *: Significant difference-value <0.05.

Table 4: Immunological parameters and serum sFlt-1, IL-10 and urinary KIM-1 among the two study groups.

Parameter	Cases (n=29)	Controls (n=36)	p- valueMW
	Median(IQR)	Median (IQR)	
CD4+ CD 25+ (in % of lymphocytes)	3.5 (2.15-4.67)	5.83 (4.48-6.64)	0.001 *
CD4+ FOX P3+ (in % of lymphocytes)	1.92 (1.3-2.8)	4.35 (3.455-5.08)	0.001 *
Serum sFlt-1 (in ng/ml)	7.84 (5.6-9.96)	2.031 (1.41-3.345)	0.001 *
Serum IL-10 (in pg/ml)	1.69 (1.51-15.4)	11.185 (1.845-27.9)	0.021 *
Urinary KIM-1 (in pg/ml)	560.47 (481.07-723.37)	445.42 (387.7-536.7)	0.008 *

MW: p-value based on Mann Whitney U test. *: Significant difference-value <0.05.

Table 5: Correlation between Regulatory T-Cells and Serum & Urine parameters related to Preeclampsia.

Compared Parameter Pair	Overall Population		Cases		Controls	
	Coeff.	**p-value**	**Coeff.**	**p-value**	**Coeff.**	**p-value**
CD4+ FOX P3+ & Serum sFlt-1	**-0.664**	**0 .001***	-0.026	0.882	-0.181	0.346
CD4+ FOX P3+ & Serum IL-10	0.164	0.193	-0.25	0.142	0.023	0.904
CD4+ FOX P3+ & Urinary KIM-1	**-0.351**	**0.005***	**-0.357**	**0.041***	0.074	0.701
Serum sFlt-1 & Serum IL-10	**-0.268**	**0.031***	-0.071	0.683	0.025	0.898
Serum sFlt-1 & Urinary KIM-1	0.25	0.05	0.023	0.06	-0.037	0.847
Serum IL-10 & Urinary KIM-1	-0.097	0.451	0.009	0.9	0.066	0.734

Significant correlation is known to exist when p value <0.05, denoted by *.

*p value <0.05, **- p value <0.01, ***- p value 0.001

Figure 1: Correlation heatmap of Regulatory T Cells and Serum and Urine Parameters in overall population.

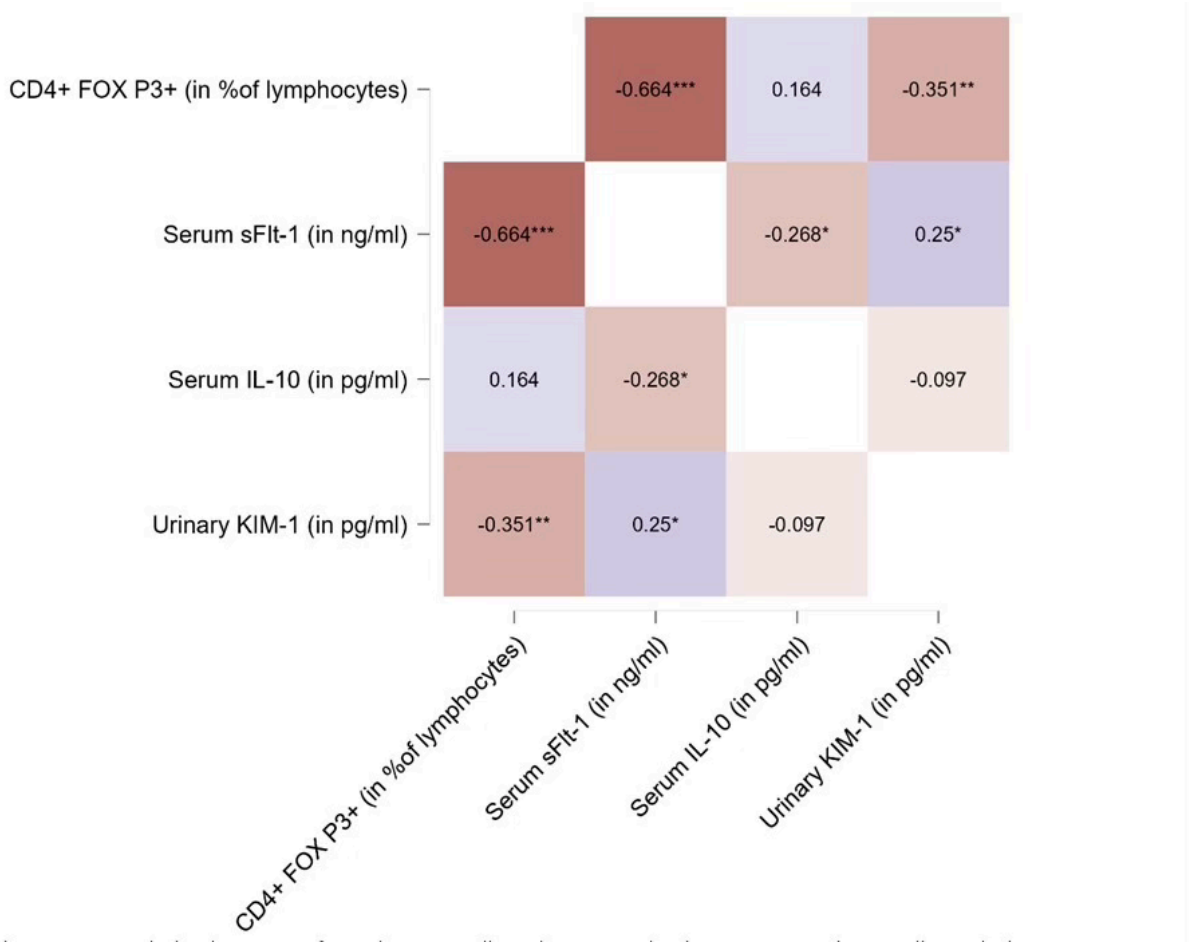


Figure 2: ROC Curve Analysis for prediction of preeclampsia.

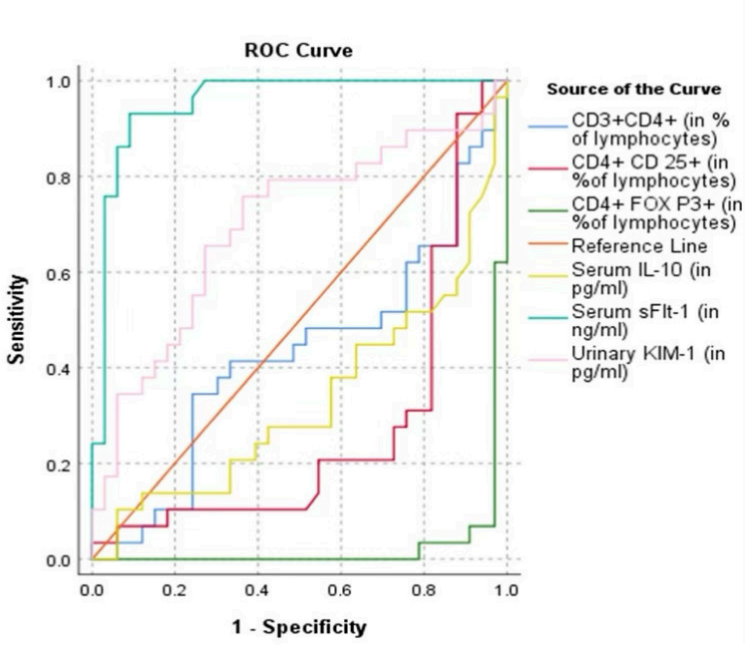
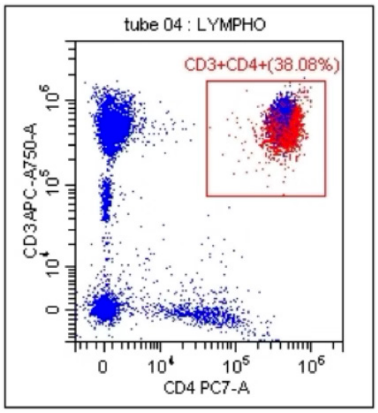


Table 6: Area Under Curve analysis for Prediction of Preeclampsia with Cut-Off estimation by Youden's Index.

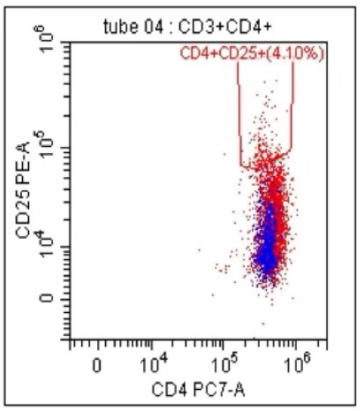
Parameters	AUC	p-value	Cutoff	Sensitivity	Specificity
CD3+CD4+ (in %of lymphocytes)	0.581	0.344	> 39.35	34%	75%
CD4+ CD 25+ (in %of lymphocytes)	0.733	0.001	< 0.60	100%	6%
CD4+ FOX P3+ (in % of lymphocytes)	0.973	0.001	> 6.60	0%	100%
Serum sFlt-1 (in ng/ml)	0.955	0.001	> 3.96	93%	92%
Serum IL-10 (in pg/ml)	0.667	0.021	< 0.96	97%	6%
Urinary KIM-1 (in pg/ml)	0.671	0.005	< 481.07	76%	58%

Figure 3: Flowcytometry flow page of T-regs of preeclampsia women (Group A).

3A: CD3+ CD4+



3B: CD4+CD25+



3C: CD4+FOXP3+

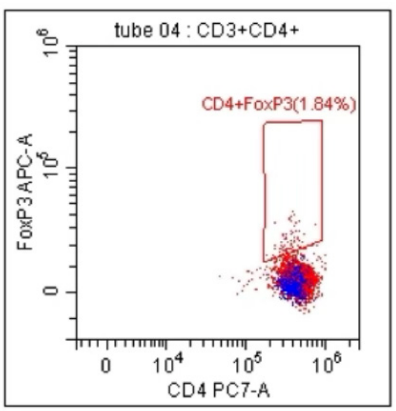


Figure 4: Flowcytometry flow page of T-regs of normal pregnant women (Group B).

4A: CD3+CD4+ **4B:** CD4+CD25+ **4C:** CD4+FOXP3+

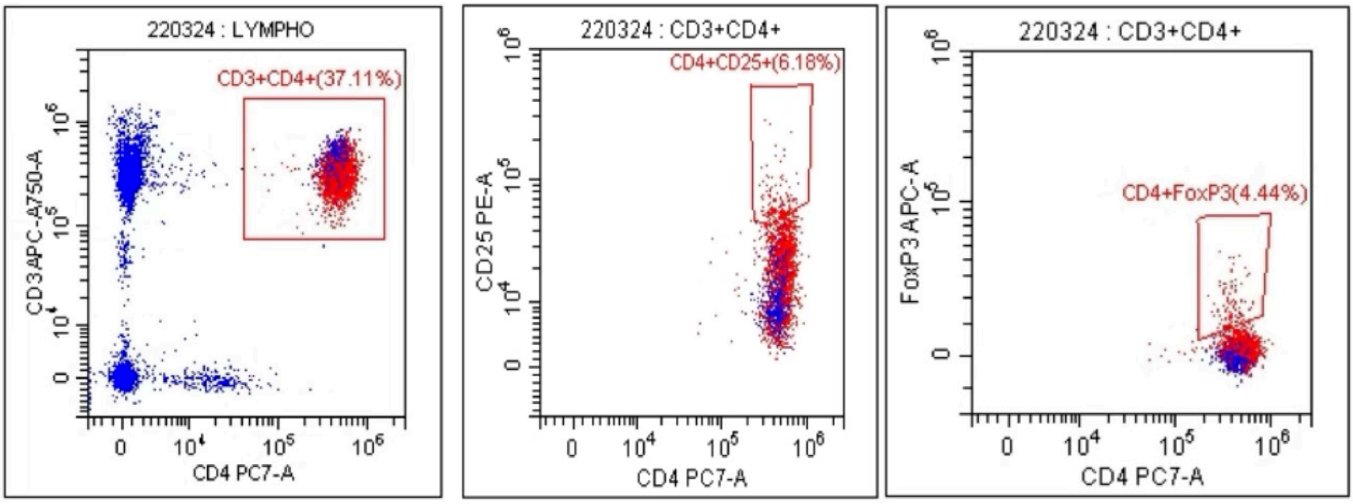


Table 7: Correlation of Novel Biomarkers with routine anthropometric, clinical and biochemical parameters.

Parameter	$CD3^+CD4^+$	$CD4^+CD25^+$	$CD4^+FOXP3^+$	Serum sFlt-1	Serum IL-10	Urinary KIM-1
Age (yrs)	−0.172 (0.171)	0.035 (0.784)	0.049 (0.697)	0.102 (0.419)	−0.116 (0.358)	0.081 (0.534)
Height (cm)	0.070 (0.582)	−0.108 (0.392)	−0.092 (0.466)	0.110 (0.382)	−0.032 (0.801)	0.034 (0.794)
Weight (kg)	0.151 (0.228)	−0.137 (0.277)	−0.057 (0.654)	0.117 (0.353)	−0.089 (0.482)	0.065 (0.616)
Pulse (bpm)	0.167 (0.183)	−0.192 (0.125)	−0.271 (0.029)	0.358 (0.003)	−0.096 (0.446)	0.310 (0.014)
SBP (mm Hg)	−0.007 (0.953)	−0.254 (0.041)	−0.653 (<0.001)	0.658 (<0.001)	−0.311 (0.012)	0.284 (0.025)
DBP (mm Hg)	−0.105 (0.406)	−0.313 (0.011)	−0.687 (<0.001)	0.704 (<0.001)	−0.161 (0.201)	0.186 (0.147)
AST (U/L)	0.025 (0.842)	−0.227 (0.069)	−0.278 (0.025)	0.077 (0.544)	−0.192 (0.125)	0.104 (0.421)
ALT (U/L)	0.143 (0.256)	−0.177 (0.158)	−0.148 (0.238)	−0.057 (0.653)	−0.107 (0.398)	0.219 (0.087)
ALP (U/L)	0.016 (0.901)	−0.371 (0.002)	−0.106 (0.399)	0.085 (0.503)	−0.262 (0.035)	0.173 (0.179)
Total Protein (g/dl)	0.161 (0.201)	0.272 (0.028)	0.324 (0.008)	−0.453 (<0.001)	0.055 (0.666)	0.208 (0.105)
Albumin (g/dl)	0.069 (0.585)	0.304 (0.014)	0.381 (0.002)	−0.413 (0.001)	0.106 (0.402)	0.106 (0.411)
Globulin (g/dl)	0.155 (0.217)	0.029 (0.818)	0.076 (0.546)	−0.197 (0.116)	−0.005 (0.968)	0.237 (0.063)
A/G Ratio	−0.040 (0.755)	0.183 (0.144)	0.195 (0.120)	−0.103 (0.414)	0.064 (0.610)	−0.070 (0.588)
Urea (mg/dl)	0.090 (0.475)	−0.083 (0.510)	−0.035 (0.781)	−0.032 (0.797)	−0.030 (0.813)	−0.005 (0.972)
Creatinine (mg/dl)	−0.145 (0.249)	−0.094 (0.456)	−0.289 (0.020)	0.232 (0.063)	−0.181 (0.149)	0.226 (0.077)
Uric acid (mg/dl)	0.073 (0.562)	−0.059 (0.638)	0.012 (0.924)	0.048 (0.701)	−0.037 (0.767)	0.243 (0.057)
Sodium (mEq/L)	−0.012 (0.925)	0.170 (0.176)	0.311 (0.012)	−0.148 (0.240)	0.077 (0.542)	−0.008 (0.949)
Potassium (mEq/L)	0.064 (0.614)	0.050 (0.691)	0.012 (0.927)	0.005 (0.967)	0.136 (0.280)	0.040 (0.755)
Chloride (mmol/L)	−0.110 (0.382)	0.045 (0.721)	0.116 (0.357)	0.088 (0.485)	0.169 (0.178)	0.028 (0.826)
Urine protein	−0.140 (0.265)	−0.402 (0.001)	−0.815 (<0.001)	0.783 (<0.001)	−0.288 (0.020)	0.340 (0.007)

Spearman's rank correlation analysis was performed to explore the relationship between immunological markers ($CD3^+CD4^+$, $CD4^+CD25^+$, $CD4^+FOXP3^+$, sFlt-1, IL-10, and urinary KIM-1) and clinical-biochemical variables. A strong negative correlation was observed between $CD4^+FOXP3^+$ T-regulatory cell frequency and both systolic ($\rho = -0.653$, $p < 0.001$) and diastolic blood pressure ($\rho = -0.687$, $p < 0.001$), as well as urinary protein levels ($\rho = -0.815$, $p < 0.001$). Conversely, serum sFlt-1 showed a strong positive correlation with systolic ($\rho = 0.658$, $p < 0.001$) and diastolic pressures ($\rho = 0.704$, $p < 0.001$), and with urine protein excretion ($\rho = 0.783$, $p < 0.001$). $CD4^+CD25^+$ cells demonstrated modest inverse associations with blood pressure parameters ($\rho \approx -0.25$ to -0.31, $p < 0.05$) and alkaline phosphatase ($\rho = -0.371$, $p = 0.002$), but positive correlations with total protein and albumin levels. In contrast, serum IL-10 correlated negatively with systolic pressure ($\rho = -0.311$, $p = 0.012$) and ALP ($\rho = -0.262$, $p = 0.035$). Urinary KIM-1, a marker of tubular injury, showed a significant positive correlation with systolic pressure ($\rho = 0.284$, $p = 0.025$) and urinary protein ($\rho = 0.340$, $p = 0.007$). Collectively, these findings indicate that rising anti-angiogenic activity and renal injury markers are associated with higher blood pressure and proteinuria, whereas reduced T-regulatory cell populations reflect immune dysregulation contributing to the pathophysiology of preeclampsia.

Discussion

In this study, the relationship between regulatory T cells (Tregs), serum IL-10, sFlt-1, and urinary KIM-1 with glomerular endothelial dysfunction in preeclampsia was explored. The results suggest possible biomarker and immunological connections to endothelial damage. However, the cross-sectional nature of the study restricts the ability to draw causal conclusions and to validate the underlying mechanisms functionally. As our findings are observational, mechanistic studies (e.g., animal models, prospective cohorts) are warranted.

Although height and weight were significantly higher in the case group than controls, BMI and weight gain showed no differences between groups, nullifying these differences. This helps exclude anthropometric measurements interference in concluding that observed changes in T-regulatory cells, IL-10, sFlt-1 and KIM-1 are primarily due to preeclampsia. The findings of the current study gain credibility when these possible confounding variables are eliminated. The case group showed significantly higher pulse rate and blood pressure than controls, confirming the diagnostic criteria of PE.

Our findings revealed a marked decrease in the presence of CD4+CD 25+ Treg cells, CD4+FOXP3+ Treg cells and lower levels of serum IL-10 in PE women. This observation aligns with previous research that associates reduced Treg function with compromised maternal–fetal immune tolerance and increased inflammation [15] [16].

Extensive clinical and experimental evidence indicates a reduction in CD4+CD25+FOXP3+ Tregs and diminished IL-10 levels in PE, which compromises maternal–fetal tolerance [11,15]. Mechanistically, the lack of Treg/IL-10 intensifies pro-inflammatory Th1/Th17 responses, hinders placental angiogenesis, and triggers endothelial activation, leading to glomerular damage [17,18]. Hu et al. also discovered a decrease in fetal thymic Tregs in PE, which can be reversed with maternal acetate supplementation [19].

New approaches are being investigated to enhance Treg function, such as adoptive Treg transfer, IL-10 analogues, and Short Chain Fatty Acid (SCFA) supplementation, or to create Treg-based biomarkers for assessing PE risk [20]. Gaining insight into the disruptions of the Treg-IL-10 axis is essential for precise immunomodulation in PE.

Consistent with the findings of Daneva et al [21] and Nath et al [11], our study identified a marked reduction in serum IL-10 levels in PE cases when compared to healthy pregnant controls, which tilts the cytokine balance towards pro-inflammatory mediators [11,22]. The lack of IL-10 facilitates endothelial activation, leading to the increased expression of adhesion molecules like Vascular cell adhesion molecule-1 (VCAM-1) and the attraction of leukocytes to the glomerular endothelium [23]. A decrease in IL-10 also diminishes antioxidant capacity, thereby elevating oxidative stress and causing direct harm to the endothelium [24].

Insufficient IL-10 may allow for unchecked complement activation, resulting in endothelial damage through membrane attack complex pathways [25]. Furthermore, the absence of IL-10 intensifies angiotensin II–driven vasoconstriction, further compromising renal blood flow [25]. Together, these processes-cytokine imbalance, complement activation, oxidative stress, and Renin–Angiotensin–Aldosterone System (RAAS) sensitization-hinder endothelial repair mechanisms, leading to glomerular damage in PE.

As reported by Maynard et al [26], De vivo et al [27] and Moghaddas et al [28], our findings also indicate a significant rise in sFlt-1 levels in PE women when compared to normotensive women. This protein binds to VEGF/PlGF, causing a disruption in glomerular endothelial fenestrations and podocyte function, which leads to proteinuria [6,26]. The reduction of VEGF due to sFlt-1 also exacerbates oxidative stress and triggers complement activation, further impairing endothelial repair and lowering Glomerular filtration rate (GFR) [24,29]. These interconnected mechanisms are central to the renal dysfunction associated with sFlt-1 in PE.

Another novel marker that we have studied is KIM-1 protein in urine. In our study we found that urinary KIM-1 levels were significantly higher in the preeclampsia group than in the normal pregnant women.

KIM-1, a proximal tubular injury marker, is elevated in PE and correlates with glomerular endothelial damage, reflecting hypertension- and oxidative stress–mediated tubular injury [30,31]. Elevated KIM-1 augments vascular permeability, and its degree of upregulation parallels glomerular injury severity

[31]. Exploring the relationship between KIM-1 and injury to glomerular endothelial cells could deepen our understanding of preeclampsia's pathophysiology. This insight might pave the way for the development of specific therapeutic interventions and enhanced diagnostic techniques, ultimately leading to better health outcomes for both mothers and their infants.

The Receiver Operating Characteristic (ROC) curve analysis revealed that serum sFlt-1 is the most effective marker for diagnosing preeclampsia, with impressive sensitivity (93%) and specificity (92%). This finding highlights the potential of sFlt-1 as a diagnostic biomarker for preeclampsia. However, our data were not adjusted for gestational age, and all preeclampsia cases were early-onset, occurring before 34 weeks. As a result, although sFlt-1 levels were elevated in these cases, the small sample size and lack of gestational age normalization limit the predictive conclusions we can draw. Previous literature emphasizes the variability of sFlt-1 levels with gestational age and disease severity, which should be considered in future prospective studies assessing its predictive utility (Matin et al., 2020 [32]; Vogtmann et al., 2021[6]]. Urinary KIM-1 also showed promise as a marker, albeit with lower sensitivity and specificity. It is important to emphasize that these ROC analyses illustrate diagnostic differentiation at the point of sample collection, rather than predicting when the disease will begin.

The study revealed negative correlations between CD4+FOXP3+ Tregs and both serum sFlt-1 and urinary KIM-1, suggesting their interplay in preeclampsia pathogenesis. One hypothesis explaining this negative correlation was that increased sFlt-1 levels during PE are associated with oxidative stress and inflammation, which reduces Treg cell numbers and thereby impair their suppressive function [33]. The negative correlation between Tregs and urinary KIM-1 can be explained by inflammatory state of PE where KIM-1 acts as protective factor by suppressing inflammation during acute kidney injury [34]. The negative correlation between serum sFlt-1 and IL-10 indicates an imbalance between pro- and anti-inflammatory factors. A rodent study by Lai et al found that hypoxic conditions as observed in preeclampsia result in increased sFlt-1 expression and this expression increases further especially in IL-10 deficient IL-10-/- mice suggesting a possible negative correlation between serum sFlt-1 and IL-10, as noted in our study [35].

By simultaneously assessing Tregs, IL-10, sFlt-1, and urinary KIM-1, this study offers new insights into the interrelated roles of immunological dysregulation, angiogenic imbalance, and renal damage in preeclampsia. Being one of the first Indian studies to examine this combination in a clinical context, the integrated analysis supports the use of these indicators for early diagnosis and monitoring and points to a possible immune-vascular-renal axis in disease etiology.

However, this study has certain limitations, including its cross-sectional design, which precludes the establishment of causal relationships. The relatively small sample size may limit the generalizability of the findings. Future longitudinal studies with larger cohorts could provide more robust evidence and potentially elucidate the temporal relationships between these markers and the development of preeclampsia. Another key limitation is the variability of immune and angiogenic mediators across pregnancy and the lack of precise gestational-age matching, which may have influenced biomarker levels.

To conclude, this study reveals that preeclampsia is associated with decreased levels of Tregs and IL-10, alongside increased concentrations of sFlt-1 and urinary KIM-1. These consistent relationships suggest their potential as important biomarkers for clinical diagnosis and risk assessment. Future prospective research is essential to confirm these findings and to explore their role in informing management strategies. These findings may contribute to the development of improved diagnostic tools and targeted therapeutic strategies (immune modulators) for managing preeclampsia, potentially leading to better outcomes for both mothers and babies affected by this condition.

Novelty of the study

This research differs from earlier studies that examined angiogenic, immune, or renal markers separately by combining all three pathways within a single cohort. It reveals that a decrease in Tregs and IL-10 is associated with an increase in sFlt-1 and urinary KIM-1. This finding establishes an immune–angiogenic–renal axis in preeclampsia and introduces new evidence from an Indian population, emphasizing urinary KIM-1 as a potential non-invasive indicator of renal involvement. It also supports the clinical value of using multi-marker panels for diagnosis and risk assessment.

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The study did not receive any external funding.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical statement

After receiving approval from the Institutional Ethics Committee (Ref no: IEC/AIIMS BBSR/PG THESIS/2022-23/50), a cross-sectional study was executed at the All India Institute of Medical Sciences (AIIMS), Bhubaneswar. The research included 29 women diagnosed with preeclampsia (Group A) and 36 healthy pregnant women (Group B), all sourced from the Department of Obstetrics and Gynaecology. Written informed consent was obtained from each participant before collecting blood and urine samples for

research purposes. The study was conducted in alignment with the ethical standards of the Declaration of Helsinki.

Data Availability Statement

The data presented in this study is available on request from the corresponding author. The data is not publicly available due to privacy and intellectual property reasons.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used AI tools in order to reformulate some sentences. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Credit authorship contribution statement

- Kasala Farzia: Conceptualization, Data curation, Investigation, Writing – original draft.
- Prakruti Dash: Supervision, Methodology, Writing – review & editing, Project administration.
- Gautom Kumar Saharia: Resources, Data collection, Validation.
- Saubhagya Kumar Jena: Patient recruitment, Clinical support, Validation.
- Saurav Nayak: Formal analysis, Visualization, Writing – review & editing.

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