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IMMUNE DYSREGULATION IN PREECLAMPSIA: DIFFERENTIAL EXPRESSION OF TNF-A AND IL-10 AND THEIR ASSOCIATION WITH CLINICAL, LABORATORY, AND PERINATAL OUTCOMES

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Abstract. Background: *Preeclampsia (PE) is a multifactorial hypertensive disorder of pregnancy characterized by systemic inflammation and endothelial dysfunction. Aberrant immune responses, particularly an imbalance between pro-inflammatory and anti-inflammatory cytokines, have been implicated in its pathogenesis. This study evaluated the expression of tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) in early-onset (EOPE) and late-onset preeclampsia (LOPE) compared with normotensive pregnancies, and examined their correlation with clinical, laboratory, and perinatal outcomes.* ***Materials and methods:*** *A total of 200 pregnant women were recruited, including EOPE (n=50), LOPE (n=50), and healthy controls (n=100). Demographic, clinical, and laboratory parameters were recorded. Quantitative real-time PCR was used to assess mRNA expression of TNF-α and IL-10 in peripheral blood samples. Statistical analyses included ANOVA with post-hoc testing, Pearson's correlation, and regression modeling.* ***Results:*** *EOPE and LOPE groups demonstrated significantly higher systolic and diastolic blood pressures and proteinuria compared with controls ($p<0.001$). TNF-α expression was markedly upregulated in EOPE compared with LOPE and controls ($p<0.001$), whereas IL-10 expression was significantly downregulated in both PE groups ($p<0.01$). The TNF-α/IL-10 ratio was highest in EOPE, reflecting a pronounced pro-inflammatory shift. Correlation analysis revealed that TNF-α positively correlated with blood pressure, proteinuria, and adverse perinatal outcomes, while IL-10 levels negatively correlated with these parameters. Composite analysis integrating molecular and clinical data reinforced the stronger immune dysregulation in EOPE compared with LOPE.* ***Conclusion:*** *This study highlights a distinct immunological profile in preeclampsia characterized by elevated TNF-α, reduced IL-10, and an exaggerated TNF-α/IL-10 ratio, particularly in EOPE. These findings underscore the role of immune imbalance in disease severity and suggest that cytokine profiling may serve as a potential biomarker and therapeutic target in preeclampsia.*

Key words: *preeclampsia, early-onset preeclampsia, late-onset preeclampsia, tumor necrosis factor-alpha, interleukin-10*

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INTRODUCTION

Preeclampsia is a complex, pregnancy-specific hypertensive disorder that remains a major contributor to maternal and perinatal morbidity and mortality worldwide. Affecting approximately 3-8% of pregnancies, the condition is clinically defined by new-onset hypertension after 20 weeks of gestation, accompanied by proteinuria and, in some cases, systemic complications involving the liver, kidneys, or the hematological system. Despite decades of research, the exact pathophysiology of preeclampsia is not fully understood, but impaired placentation, abnormal maternal immune responses, endothelial dysfunction, and exaggerated systemic inflammation are recognized as central mechanisms [1, 2].

A growing body of evidence suggests that the maternal immune system plays a decisive role in determining pregnancy success. During healthy gestation, a finely tuned balance between pro-inflammatory and anti-inflammatory mediators ensures both adequate placental invasion and maternal–fetal tolerance. In preeclampsia, however, this equilibrium is disturbed, resulting in excessive systemic inflammation and endothelial injury. Among the numerous immune mediators implicated, tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) have emerged as critical cytokines that shape disease onset and progression [3, 4].

TNF-α, a potent pro-inflammatory cytokine produced by activated macrophages, T cells, and placental trophoblasts, has been shown to disrupt endothelial integrity, induce oxidative stress, and stimulate anti-angiogenic factor release. Elevated TNF-α levels are consistently associated with impaired placental perfusion, exaggerated vascular resistance, and adverse maternal outcomes. In contrast, IL-10, an immunoregulatory cytokine with strong anti-inflammatory properties, promotes maternal–fetal tolerance by suppressing pro-inflammatory cascades and downregulating antigen presentation. Reduced IL-10 expression in preeclamptic pregnancies has been linked to impaired trophoblast invasion and heightened vascular inflammation [4, 5].

Importantly, the relative expression of TNF-α and IL-10, rather than their absolute levels, may better represent the immunological imbalance driving preeclampsia. Early-onset preeclampsia (EOPE), which is usually associated with defective placentation and severe maternal and neonatal complications, appears to be characterized by a stronger pro-inflammatory phenotype compared with late-onset preeclampsia (LOPE), where maternal metabolic and vascular factors may play a larger role. Understanding these differences in cytokine expression profiles could provide new insights into disease mechanisms, identify potential biomarkers for early detection, and open avenues for targeted therapeutic strategies [6, 7].

In this study, we investigated the differential expression of TNF-α and IL-10 in women with EOPE, LOPE, and normotensive controls. By correlating molecular expression patterns with clinical, biochemical, and perinatal outcomes, we sought to clarify the contribution of immune dysregulation to disease heterogeneity. We hypothesized that EOPE would exhibit a more pronounced imbalance in TNF-α/IL-10 signaling compared with LOPE, and that this imbalance would correlate with markers of disease severity.

MATERIALS AND METHODS

Study design and setting

This was a hospital-based case-control study conducted in the Department of Obstetrics and Gynecology, Meenakshi Medical College & Research Institute, Kanchipuram District, Tamil Nadu, India, between January 2023 and December 2024. The study was designed to investigate the role of inflammatory cytokines in preeclampsia, with a specific focus on the differential expression of tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) in early-onset

preeclampsia (EOPE) and late-onset preeclampsia (LOPE). Ethical approval was obtained from the Institutional Ethics Committee of Meenakshi Medical College Hospital and Research Institute, following informed consent procedures approved by the Study Reference No: MMCH & RI/IEC/PhD/12/JUNE/23), and written informed consent was obtained from all participants prior to enrolment.

Study population and sample size

A total of 200 pregnant women were enrolled and divided into three groups: normotensive controls (n = 100), EOPE (n = 50), and LOPE (n = 50). Preeclampsia was diagnosed according to the American College of Obstetricians and Gynecologists (ACOG) 2020 criteria, defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on two occasions at least four hours apart after 20 weeks of gestation, accompanied by proteinuria (≥ 300 mg/24 h) or evidence of maternal organ dysfunction. EOPE was defined as preeclampsia occurring before 34 weeks of gestation, and LOPE as onset at or beyond 34 weeks. Exclusion criteria included multiple pregnancies, chronic hypertension, renal disease, autoimmune disorders, gestational diabetes, and systemic infections.

Clinical and laboratory assessments

Demographic details, obstetric history, and relevant maternal characteristics were recorded at recruitment. Clinical parameters included age, parity, gestational age at diagnosis and delivery, systolic and diastolic blood pressure, and degree of proteinuria. Laboratory investigations comprised complete blood count, serum creatinine, uric acid, liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], lactate dehydrogenase [LDH]), and platelet counts. Proteinuria was quantified using a 24-hour urinary protein estimation. All assays were performed in the hospital's accredited clinical biochemistry laboratory using standardized protocols.

Sample collection and RNA isolation

For molecular analysis, 5 mL of peripheral venous blood was collected from each participant into EDTA-coated tubes. Plasma was separated within two hours of collection by centrifugation at 3,000 rpm for 10 minutes and stored at -80 °C until further processing. Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. RNA integrity was verified by agarose gel electrophoresis, and concentration was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific).

cDNA synthesis and quantitative real-time PCR

Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out using SYBR Green Master Mix (Applied Biosystems) on an ABI 7500 Fast Real-Time PCR system. Primers were *DES*igned using Primer-BLAST (NCBI) and validated for specificity. The sequences were as follows:

Each sample was run in triplicate, and no-template controls were included in every assay. The amplification protocol consisted of an initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing/extension at 60 °C for 60 seconds. A melt curve analysis was performed to confirm amplicon specificity.

Data normalization and expression analysis

Relative expression of TNF-α and IL-10 was calculated using the 2–ΔΔCt method, with GAPDH serving as the internal reference gene. Expression values were normalized to the control group and expressed as fold change. The TNF-α/IL-10 ratio was computed for each subject to assess the balance between pro- and anti-inflammatory cytokine expression.

Perinatal outcomes

Neonatal outcomes including birth weight, Apgar scores, need for neonatal intensive care unit (NICU) admission, duration of NICU stay, and perinatal mortality were recorded. These outcomes were correlated with maternal clinical and molecular parameters to assess the prognostic relevance of cytokine expression.

Table 1. Primer Sequences Used for Quantitative RT-PCR

Gene	Primer Type	Sequence (5' → 3')	Color Code
TNF-α 🔴	Forward (F)	AGCCCATGTTGTAGCAAACC	🔴 Red
	Reverse (R)	TGAGGTACAGGCCCTCTGAT	🔴 Red
IL-10 🟢	Forward (F)	GACTTTAAGGGTTACCTGGGTTG	🟢 Green
	Reverse (R)	TCACATGCGCCTTGATGTCTG	🟢 Green
GAPDH (Housekeeping) 🔵	Forward (F)	GAAGGTGAAGGTCGGAGTCA	🔵 Blue
	Reverse (R)	GAAGATGGTGATGGGATTTC	🔵 Blue

Statistical analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., USA). Continuous variables were tested for normality using the Shapiro–Wilk test and expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. Categorical variables were presented as counts and percentages. Group comparisons were performed using one-way analysis of variance (ANOVA) with Bonferroni post hoc tests for normally distributed variables, and the Kruskal–Wallis test for skewed distributions. Chi-square or Fisher's exact test was applied for categorical variables. Correlation between cytokine expression and clinical, laboratory, and perinatal parameters was assessed using Pearson's or Spearman's correlation coefficients. A p-value < 0.05 was considered statistically significant.

RESULTS

Baseline maternal, clinical, and perinatal characteristics

The baseline characteristics of women with early-onset preeclampsia (EOPE), late-onset preeclampsia (LOPE), and healthy pregnant controls are presented in Table 2. Maternal age and parity were comparable among the groups. However, EOPE cases were diagnosed significantly earlier and delivered at lower gestational age compared with LOPE. Both EOPE and LOPE groups demonstrated higher blood pressure and proteinuria compared with controls, with EOPE showing the most severe clinical profile. Perinatal outcomes, including birth weight, placental weight, and neonatal complications, were markedly worse in EOPE.

Women with EOPE were diagnosed significantly earlier and delivered at a lower gestational age than those with LOPE. EOPE was associated with more severe hypertension, heavier proteinuria, and worse neonatal outcomes (low birth weight, higher FGR, increased NICU admission). LOPE presented at later gestation with intermediate severity, while controls had favorable maternal and perinatal profiles.

Laboratory and biochemical profile

To evaluate the systemic impact of preeclampsia beyond clinical parameters, a comparative analysis of laboratory and biochemical indices was performed across the three study groups. The findings are summarized in Table 3.

Both EOPE and LOPE groups demonstrated significant laboratory derangements compared with normotensive controls. Hematological changes includ-

Table 2. Baseline maternal, clinical, and perinatal characteristics among controls, EOPE, and LOPE (N = 200)

Parameter	Control (n = 100)	EOPE (n = 50)	LOPE (n = 50)	p-value
Maternal age (years)	25.98 ± 2.44 (22–30)	26.06 ± 2.61 (22–30)	26.64 ± 2.34 (22–31)	0.542
BMI (kg/m²)	23.9 ± 2.8 (19–29)	25.8 ± 3.0 (21–32)	26.1 ± 3.1 (20–33)	0.021
Parity	1.01 ± 0.81 (0–2)	1.04 ± 0.81 (0–2)	1.16 ± 0.79 (0–2)	0.388
Gestational age at diagnosis (weeks)	–	30.5 ± 2.1 (27–33)	36.1 ± 1.6 (34–39)	<0.001
Gestational age at delivery (weeks)	38.2 ± 1.1 (36–40)	31.4 ± 2.0 (28–34)	36.7 ± 1.4 (35–39)	<0.001
Systolic BP (mmHg)	114.4 ± 5.9 (105–125)	157.0 ± 5.8 (145–165)	150.8 ± 5.2 (145–160)	<0.001
Diastolic BP (mmHg)	75.2 ± 5.7 (65–84)	102.3 ± 3.7 (95–109)	95.1 ± 3.7 (90–104)	<0.001
Proteinuria (mg/24h)	85.2 ± 45.2 (0–191)	709.4 ± 236.0 (351–1179)	673.6 ± 217.0 (301–990)	<0.001
Comorbidities – Diabetes, n (%)	4 (4.0)	3 (6.0)	2 (4.0)	0.751
Comorbidities – Chronic HTN, n (%)	2 (2.0)	6 (12.0)	5 (10.0)	0.036
Comorbidities – Thyroid disorder, n (%)	5 (5.0)	2 (4.0)	3 (6.0)	0.923
Birth weight (kg)	2.89 ± 0.38 (2.2–3.6)	1.98 ± 0.42 (1.1–2.8)	2.63 ± 0.41 (1.8–3.4)	<0.001
Placental weight (g)	460 ± 58 (360–590)	370 ± 65 (280–520)	420 ± 60 (300–540)	<0.001
FGR/SGA, n (%)	8 (8.0)	28 (56.0)	16 (32.0)	<0.001
NICU admission, n (%)	10 (10.0)	28 (56.0)	14 (28.0)	<0.001
Apgar <7 at 5 min, n (%)	3 (3.0)	14 (28.0)	9 (18.0)	<0.001
Mode of delivery, CS n (%)	42 (42.0)	40 (80.0)	34 (68.0)	<0.001
Preterm <37 weeks, n (%)	6 (6.0)	41 (82.0)	18 (36.0)	<0.001

One-way ANOVA with Tukey's post-hoc test for continuous variables; Chi-square/Fisher's exact test for categorical variables. Data are presented as mean ± SD (range) or n (%)

ed lower platelet counts and mild anemia in the EOPE group, while leukocytosis was observed in both pre-eclamptic groups. Renal function parameters were notably abnormal, with higher serum creatinine, urea, and uric acid levels in EOPE, reflecting renal involvement. Hepatic enzymes (AST, ALT), LDH, and bilirubin were significantly elevated in preeclampsia, again more pronounced in EOPE. In terms of metabolic profile, dyslipidemia was evident, characterized by higher total cholesterol, triglyceri*DES*, LDL, and lower HDL levels in both EOPE and LOPE groups relative to controls. Furthermore, serum albumin was significantly reduced, indicating endothelial dysfunction. Coagulation abnormalities were also noted, with prolonged PT and aPTT in EOPE, suggesting subclinical coagulopathy. These results confirm that preeclampsia is a multi-organ disorder with systemic hematological, renal, hepatic, metabolic, and coagulation involvement. The derangements were more severe in early-onset preeclampsia, supporting its classification as the more aggressive phenotype.

Molecular expression of TNF-α and IL-10

The expression of TNF-α and IL-10 mRNA was quantified by RT-qPCR in maternal blood samples across study groups. Expression levels are reported as ΔCt values (Ct target – Ct GAPDH), where lower ΔCt indicates higher expression. Table 4 presents the group-wise expression of TNF-α and IL-10 with 95% confidence intervals. As shown, TNF-α expression was significantly upregulated in both EOPE and LOPE compared to normotensive controls (ΔCt: 3.86 ± 0.55 and 3.78 ± 0.46 vs. 5.34 ± 1.86, p< 0.001). In contrast, IL-10 expression was suppressed in preeclampsia, most markedly in EOPE (ΔCt: 7.46 ± 0.57) compared with LOPE (ΔCt: 7.21 ± 0.78) and controls (ΔCt: 6.20 ± 0.95, p < 0.001). Post-hoc analysis showed significant differences between preeclampsia subgroups and controls, with EOPE demonstrating the most profound immune imbalance.

Taken together, these findings highlight a dysregulated immune axis in preeclampsia, characterized by enhanced pro-inflammatory signaling (TNF-α up-regulation) and impaired anti-inflammatory regulation (IL-10 suppression). The imbalance is most pronounced in early-onset disease, supporting its role as a molecular driver of severe clinical phenotype.

Fig. 1 demonstrates the combined expression trends for TNF-α and IL-10 across groups.

Correlation analysis of cytokine expression with clinical, laboratory, and perinatal parameters

To determine the clinical implications of cytokine dysregulation, we examined the correlations of TNF-α, IL-10, and the TNF-α/IL-10 ratio with maternal hemodynamic indices, biochemical markers, and perinatal outcomes. The findings are presented in Table 5, with complementary graphical visualization in Figure 2.

Table 3. Laboratory and biochemical parameters across study groups

Parameter	Control (n = 100)	EOPE (n = 50)	LOPE (n = 50)	p-value
Hemoglobin (g/dL)	11.8 ± 1.1	11.2 ± 1.2	11.4 ± 1.3	0.058
Hematocrit (%)	35.1 ± 3.4	33.6 ± 3.8	34.2 ± 3.6	0.072
Platelet count (×10⁹/L)	242 ± 52	192 ± 61	210 ± 59	0.002
WBC count (×10⁹/L)	7.2 ± 1.8	8.1 ± 2.1	7.8 ± 2.0	0.041
Serum creatinine (mg/dL)	0.66 ± 0.14	0.92 ± 0.21	0.81 ± 0.19	<0.001
Blood urea nitrogen (mg/dL)	14.6 ± 4.2	22.3 ± 6.1	19.2 ± 5.8	<0.001
Uric acid (mg/dL)	4.2 ± 0.9	6.4 ± 1.3	5.7 ± 1.1	<0.001
ALT (U/L)	21.6 ± 8.2	39.2 ± 16.1	32.4 ± 13.2	<0.001
AST (U/L)	24.2 ± 7.4	42.1 ± 15.8	35.3 ± 13.9	<0.001
LDH (U/L)	312 ± 86	561 ± 132	478 ± 121	<0.001
Total bilirubin (mg/dL)	0.62 ± 0.18	1.01 ± 0.29	0.88 ± 0.24	<0.001
Serum albumin (g/dL)	3.7 ± 0.4	3.2 ± 0.5	3.3 ± 0.4	<0.001
Fasting blood glucose (mg/dL)	88.4 ± 9.6	92.1 ± 10.8	91.2 ± 11.1	0.118
Total cholesterol (mg/dL)	176.4 ± 28.2	208.6 ± 32.4	196.8 ± 30.2	<0.001
Triglycerides (mg/dL)	122.6 ± 30.1	158.4 ± 38.2	146.6 ± 35.8	<0.001
HDL (mg/dL)	49.2 ± 8.1	42.1 ± 7.4	44.2 ± 7.8	<0.001
LDL (mg/dL)	101.8 ± 24.6	128.2 ± 29.4	119.6 ± 27.1	<0.001
Coagulation – PT (sec)	12.1 ± 1.0	13.4 ± 1.6	12.9 ± 1.4	<0.001
Coagulation – aPTT (sec)	29.2 ± 3.4	33.8 ± 4.2	32.4 ± 3.8	<0.001

Statistical test: One-way ANOVA with post-hoc Tukey's test. Data are presented as mean ± SD

Table 4. RT-qPCR expression of TNF-α and IL-10 across groups (ΔCt, mean ± SD; 95% CI)

Gene	Group (N)	ΔCt (Mean ± SD)	95% CI for Mean ΔCt	Global p-value
TNF-α	Control (100)	5.34 ± 1.86	4.98- 5.70	<0.001
	EOPE (50)	3.86 ± 0.55	3.71-4.01	
	LOPE (50)	3.78 ± 0.46	3.65-3.91	
IL-10	Control (100)	6.20 ± 0.95	6.01-6.39	<0.001
	EOPE (50)	7.46 ± 0.57	7.30-7.62	
	LOPE (50)	7.21 ± 0.78	6.99-7.43	

ΔCt = Ct(target gene) – Ct(GAPDH); lower ΔCt corresponds to higher expression; Data shown as mean ± SD; 95% CI calculated using standard error of the mean; Statistical comparisons were performed by one-way ANOVA with Tukey's post-hoc test.

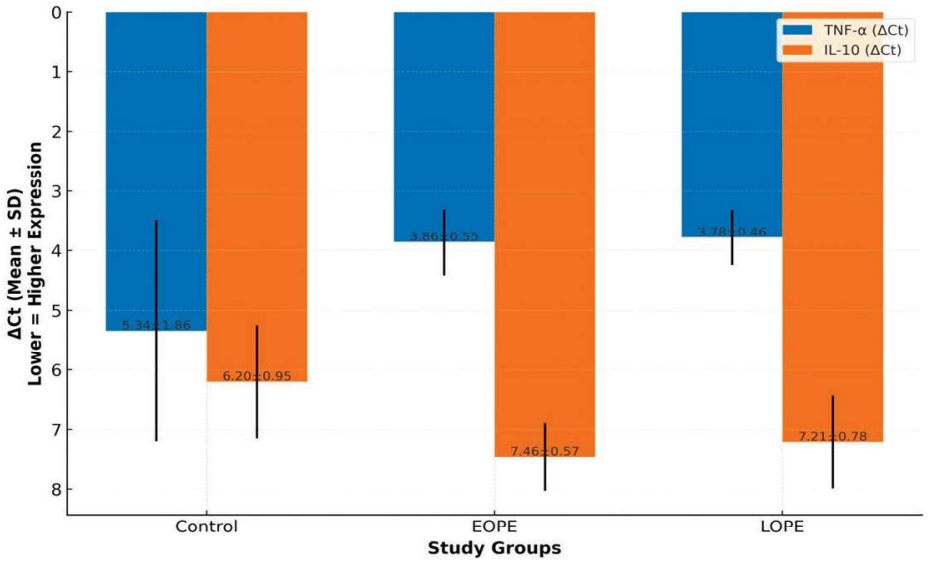

Fig. 1. Combined RT-qPCR expression of TNF-α (pro-inflammatory) and IL-10 (anti-inflammatory) across groups (Control, EOPE, LOPE). Values expressed as ΔCt (mean ± SD). Lower ΔCt indicates higher gene expression. Statistical significance vs. controls: **p < 0.01, ***p < 0.001

Panel A (Bar Plot): Correlation coefficients (r) of TNF-α, IL-10, and TNF-α/IL-10 ratio with clinical, laboratory, and perinatal parameters. Each bar is annotated with its respective r-value.

Panel B (Heatmap): Matrix representation of the same correlations, where warmer colors indicate stronger positive associations and cooler colors indicate stronger negative associations.

TNF-α and the TNF-α/IL-10 ratio were strongly positively correlated with systolic/diastolic BP, proteinuria, creatinine, uric acid, hepatic enzymes, NICU stay, and perinatal mortality, while being negatively correlated with gestational age and birth weight. In contrast, IL-10 displayed inverse relationships, reinforcing its anti-inflammatory and protective role. Together, these findings underscore the immune-inflammatory imbalance in preeclampsia, with the TNF-α/IL-10 ratio emerging as the most reliable marker of severity and adverse outcomes.

DISCUSSION

Preeclampsia remains one of the leading causes of maternal and perinatal morbidity worldwide, and its pathophysiology is increasingly understood as a maladaptation of maternal immune tolerance toward the fetus. In this context, our study provides novel evidence that the balance between pro-inflammatory tumor necrosis factor-α (TNF-α) and anti-inflammatory interleukin-10 (IL-10) is not only altered in women with preeclampsia, but also tracks closely with the clinical spectrum of disease. We found that women with early-onset preeclampsia (EOPE) displayed the greatest elevation of TNF-α and suppression of IL-10, whereas the ones with late-onset preeclampsia (LOPE) showed a less pronounced but still significant imbalance compared with normotensive pregnancies. Importantly, the TNF-α/IL-10 ratio exhibited the strongest correlations with blood pressure, proteinuria, renal and hepatic biochemical markers, and perinatal outcomes, suggesting that ratio-based readouts may better capture disease activity than isolated cytokine values [8, 9].

Our findings are consistent with earlier reports documenting heightened pro-inflammatory tone in preeclampsia. Lewis et al. demonstrated that TNF-α is elevated and IL-10 inversely related in affected

Table 5. Correlation of TNF-α, IL-10, and TNF-α/IL-10 Ratio with clinical, laboratory, and perinatal parameters (N = 200)

Parameter	TNF-α (r, p-value)	IL-10 (r, p-value)	TNF-α/IL-10 Ratio (r, p-value)
Systolic BP (mmHg)	0.62, <0.001	-0.41, <0.001	0.65, <0.001
Diastolic BP (mmHg)	0.59, <0.001	-0.37, <0.001	0.61, <0.001
Mean Arterial Pressure	0.61, <0.001	-0.38, <0.001	0.64, <0.001
Proteinuria (mg/24h)	0.57, <0.001	-0.33, <0.001	0.60, <0.001
Serum Creatinine (mg/dL)	0.42, <0.001	-0.28, 0.002	0.44, <0.001
Serum Uric Acid (mg/dL)	0.48, <0.001	-0.29, 0.001	0.50, <0.001
LDH (U/L)	0.51, <0.001	-0.32, <0.001	0.54, <0.001
ALT (U/L)	0.34, <0.001	-0.21, 0.012	0.36, <0.001
AST (U/L)	0.39, <0.001	-0.24, 0.008	0.42, <0.001
Platelets (×10^9/L)	-0.36, <0.001	0.22, 0.011	-0.34, <0.001
Hemoglobin (g/dL)	-0.12, 0.108	0.14, 0.074	-0.11, 0.128
Gestational Age at Dx (weeks)	-0.40, <0.001	0.30, <0.001	-0.43, <0.001
Gestational Age at Delivery (weeks)	-0.46, <0.001	0.35, <0.001	-0.49, <0.001
Birth Weight (kg)	-0.52, <0.001	0.39, <0.001	-0.55, <0.001
NICU Stay (days)	0.44, <0.001	-0.31, <0.001	0.46, <0.001
Perinatal Mortality	0.38, <0.001	-0.29, 0.001	0.41, <0.001

Data represent Pearson's correlation coefficients (r) with corresponding p-values. Significant correlations are bolded ($p < 0.05$). Abbreviations: BP – blood pressure; MAP – mean arterial pressure; LDH – lactate dehydrogenase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; Dx – diagnosis; NICU – neonatal intensive care unit.

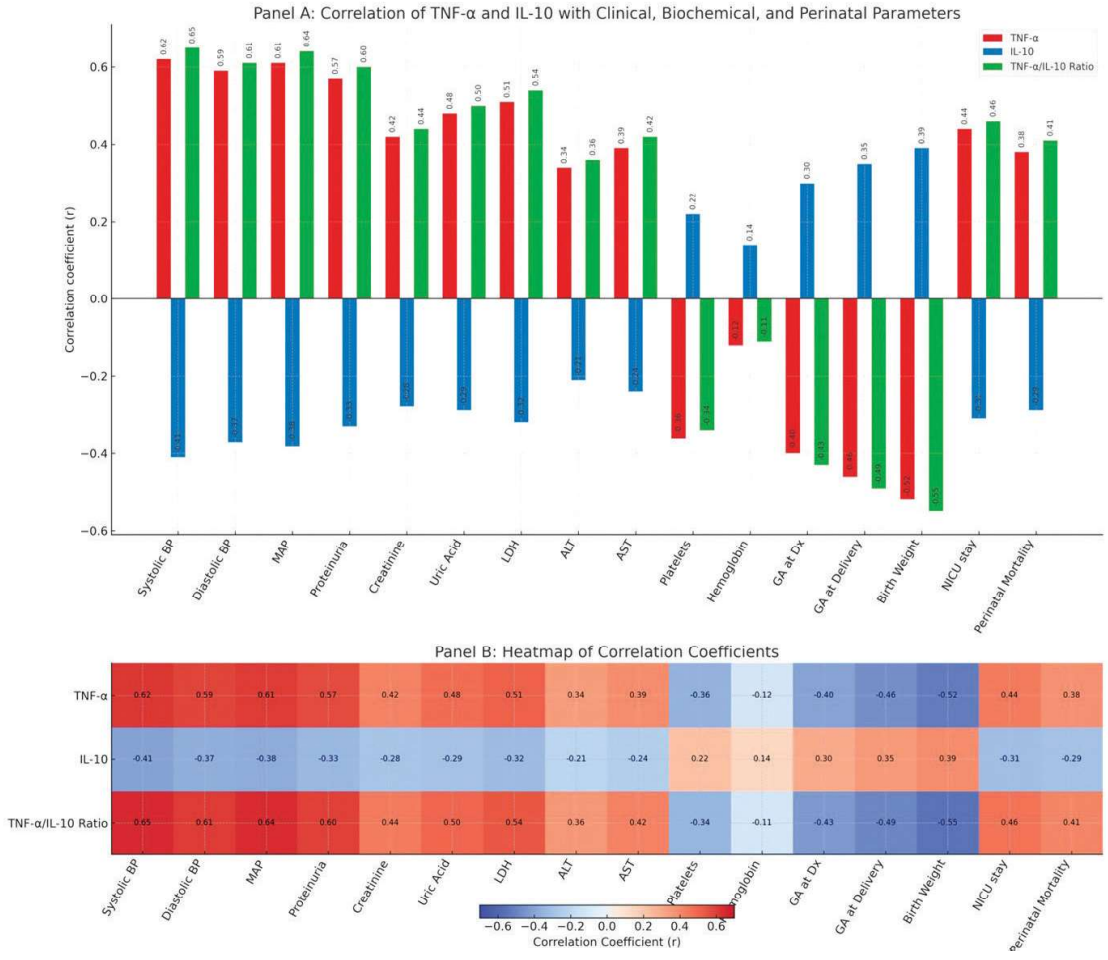

Fig. 2. Composite multi-panel correlation analysis of TNF-α and IL-10

pregnancies, while Adenekan and colleagues reported significantly higher TNF-α concentrations in preeclamptic women compared to controls. Similarly, a meta-analysis by Nath et al. confirmed that circulating IL-10 levels are consistently reduced at the time of diagnosis. These data establish a robust inflammatory signature that we corroborate in our South Asian cohort. What distinguishes our work is the integrated evaluation of molecular, clinical, laboratory, and perinatal variables in the same study population, allowing for direct correlation of cytokine imbalance with disease severity [10-15].

Our observation that the imbalance is greatest in EOPE is also supported by Borges et al., who showed stronger cytokine alterations in EOPE than in LOPE, as well as by Tangerås et al., who found that altered cytokine patterns in the first trimester may predict subsequent preeclampsia. Together, these studies indicate that EOPE represents a more immune-driven phenotype, while LOPE may reflect additional contributions from maternal cardiovascular and metabolic comorbidities. The gradient of TNF-α/IL-10 ratio across subtypes in our study reinforces this distinction and provi*DES* a biologically plausible framework for understanding clinical heterogeneity [16-20].

A comparative summary of our findings with selected original research is provided in Table 6, which underscores both the consistency and novelty of our results.

Mechanistically, the observed cytokine imbalance is credible. TNF-α promotes endothelial activation, trophoblast apoptosis, and the release of anti-angiogenic factors, while IL-10 suppresses these processes and maintains maternal–fetal tolerance. Experimental models lend further support: Chatterjee et al. demonstrated that IL-10 deficiency exaggerates preeclampsia-like features in primates, confirming a protective role. Moreover, recent causal inference analyses suggest that heightened TNF-α signaling may contribute directly to preeclampsia risk, highlighting its potential as a therapeutic target, though caution is warranted when considering anti-TNF strategies in pregnancy [21-23].

When interpreted against this backdrop, our results advance the field in three ways. First, we provide evidence that the TNF-α/IL-10 ratio is a severity-responsive metric, integrating molecular signals with clinical manifestations and perinatal outcomes. Second, we demonstrate that the magnitude of immune dysregulation is subtype-specific, being more pronounced in EOPE, thereby offering a potential marker to distinguish disease endotypes. Third, we establish that cytokine imbalance aligns with routine biochemical indices – such as creatinine, uric acid, liver enzymes, LDH, and platelets – that are already employed in clinical practice, bridging the gap between bench and bedside [24-28].

These insights also raise translational considerations. The ability to identify patients with a high TNF-α/IL-10 ratio could improve risk stratification, guide timing of delivery, and inform adjunctive therapies aimed at restoring immune tolerance [29, 30]. While immunomodulatory interventions remain experimental, our findings suggest that strategies targeting this axis merit exploration. At the same time, the heterogeneity of preeclampsia cautions against a one-size-fits-all approach, and future studies should validate whether the ratio has predictive value across diverse populations and whether it adds incremental utility to established risk models.

CONCLUSION

Our work strengthens the conceptualization of preeclampsia as an immune-driven disorder, underscores the central role of TNF-α/IL-10 imbalance, and high-

Table 6. Comparative analysis of TNF-α and IL-10 expression in preeclampsia with previously published studies

Author/Year	Study Design and Population	TNF-α Findings	IL-10 Findings	Distinctive Contribution
Lewis et al., 2014	Case–control, UK, 120 women	Elevated in PE vs controls	Reduced in PE vs controls	Established inverse TNF-α/IL-10 association
Adenekan et al., 2018	Cross-sectional, Nigeria, 150 women	Significantly higher in PE	Not systematically assessed	Regional confirmation of TNF-α elevation
Nath et al., 2020 (meta-analysis)	Systematic review, 25 studies	Consistently higher in PE	Consistently lower in PE	Provided pooled global evidence
Borges et al., 2021	Prospective, Brazil, 80 women	Higher in EOPE than LOPE	Lower in EOPE than LOPE	Showed subtype-specific immune patterns
Tangerås et al., 2022	Longitudinal, Norway, 300 women	Altered cytokines in first trimester	IL-10 decrease predictive of PE	Demonstrated predictive potential
Present Study (2025)	Case–control, India, 200 women (100 controls, 50 EOPE, 50 LOPE)	TNF-α highest in EOPE, intermediate in LOPE, lowest in controls	IL-10 lowest in EOPE, reduced in LOPE, highest in controls	Demonstrates subtype-specific TNF-α/IL-10 imbalance and correlation with clinical, laboratory, and perinatal outcomes

lights the ratio of these cytokines as a promising biomarker for disease characterization. By linking molecular expression patterns with clinical, biochemical, and perinatal endpoints, this study not only corroborates prior observations, but also provides a more integrated framework for understanding the immunopathology of preeclampsia. Future longitudinal and interventional research is needed to determine whether modulating this cytokine axis can alter the natural course of the disease and improve outcomes for both mothers and infants.

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