IMMUNE DYSREGULATION IN PREECLAMPSIA: DIFFERENTIAL EXPRESSION OF TUMOUR NECROSIS FACTOR-ALPHA AND INTERLEUKIN-10 AND THEIR ASSOCIATION WITH CLINICAL, LABORATORY, AND PERINATAL OUTCOMES

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ABSTRACT

Objective: 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 their pathogenesis. This study evaluated the expression of tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) in early-onset PE (EOPE) and late-onset PE (LOPE) compared with normotensive pregnancies, and examined their correlation with clinical, laboratory, and perinatal outcomes.

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 polymerase chain reaction was used to assess messenger RNA expression of TNF-α and IL-10 in peripheral blood samples. Statistical analyses included analysis of variance with *post hoc* testing, Pearson's correlation, and regression modeling.

Results: EOPE and LOPE groups demonstrated significantly higher systolic and diastolic blood pressures (BP) 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 BP, 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 PE 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 PE management.

Keywords: Preeclampsia, Early-onset preeclampsia; Late-onset preeclampsia, Tumor necrosis factor-alpha, Interleukin-10.

INTRODUCTION

Preeclampsia (PE) is a complex, pregnancy-specific hypertensive disorder and a leading cause of maternal and perinatal morbidity and mortality worldwide [1,2]. Affecting approximately 3–8% of pregnancies [3], it is clinically defined by new-onset hypertension (HTN) after 20 weeks of gestation, accompanied by proteinuria or, in some cases, maternal organ dysfunction involving the liver, kidneys, or hematological system [3,4]. Although extensive research over several decades has been conducted, the precise pathophysiology of PE remains incompletely elucidated. Nevertheless, defective placentation, dysregulated maternal immune responses, endothelial dysfunction, and heightened systemic inflammation are established as core pathogenic mechanisms [5].

Extensive evidence now implicates the maternal immune system as a pivotal factor in pregnancy viability [5]. During healthy gestation, a finely tuned balance between pro-inflammatory and anti-inflammatory mediators ensures both adequate placental invasion and maternal-fetal tolerance. In PE, this finely tuned equilibrium is disrupted, precipitating amplified systemic inflammation and endothelial dysfunction. Tumor necrosis factor- alpha (TNF-α) and interleukin-10 (IL-10) represent key cytokines among the spectrum of implicated immune mediators that govern disease pathogenesis and trajectory [5].

TNF-α, a potent proinflammatory cytokine secreted by activated macrophages, T cells, and placental trophoblasts, disrupts endothelial integrity, elicits oxidative stress, and promotes the release of anti-angiogenic factors. Elevated TNF-α levels correlate consistently with diminished placental perfusion, increased vascular resistance, and adverse maternal outcomes [6]. 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 [7].

Importantly, rather than their absolute levels, the relative expression of TNF-α and IL-10 may better reflect the immunological imbalance

underlying PE. Early-onset PE (EOPE), typically associated with defective placentation and severe maternal and neonatal complications, exhibits a stronger pro-inflammatory phenotype than late-onset PE (LOPE), in which maternal metabolic and vascular factors play a larger role. Understanding these differences in cytokine expression profiles could yield new insights into disease mechanisms, help identify biomarkers for early detection, and pave the way for targeted therapeutic strategies [7,8]. Furthermore, the intricate interplay between these cytokines influences trophoblast differentiation and invasion, crucial processes for successful implantation and placental development [9].

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

METHODS

Study design and setting
This was a hospital-based case–control study conducted in the Department of Obstetrics and Gynecology, Government medical college and hospital, Mahbubnagar, Telangana between January 2023 and December 2024. The study was designed to investigate the role of inflammatory cytokines in PE, with a specific focus on the differential expression of TNF-α and IL-10 in EOPE and LOPE. Ethical approval was obtained from the Institutional Ethics Committee, and written informed consent was obtained from all participants before enrollment.

Study population and sample size
A total of 200 pregnant women were recruited and categorized into two groups: Pregnant women with PE and healthy controls who were normotensive controls (n=100). The PE group was further divided into EOPE (n=50), LOPE (n=50). PE was diagnosed according to the American College of Obstetricians and Gynecologists 2020 criteria, defined as systolic blood pressure (BP) ≥140 mmHg and/or diastolic BP ≥90 mmHg on two occasions at least 4 h apart after 20 weeks of gestation, accompanied by proteinuria (≥300 mg/24 h) or evidence of maternal organ dysfunction [10,11]. EOPE was defined as PE occurring before 34 weeks of gestation, and LOPE as onset at or beyond 34 weeks. Exclusion criteria included multiple pregnancies, chronic HTN, renal disease, autoimmune disorders, gestational diabetes, and systemic infections [12,13].

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 BP, 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-h 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 ethylenediaminetetraacetic acid-coated tubes. Plasma was separated within 2 h of collection by centrifugation at 3000 rpm for 10 min and stored at −80°C until further processing. Total RNA was extracted from peripheral blood mononuclear cells 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).

Complementary DNA (cDNA) synthesis and quantitative real-time PCR (qRT-PCR)
cDNA was synthesized from 1 μg of total RNA using the high-capacity cDNA reverse transcription kit (Applied Biosystems, USA). qRT-PCR was carried out using SYBR green master mix (Applied Biosystems) on an Applied Biosystems (ABI) 7500 Fast RT-PCR system. Primers were designed using Primer-BLAST (NCBI) and validated for specificity. The forward and reverse primer sequences used for inflammatory markers were mentioned in table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as housekeeping gene (reference gene).

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 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 60 s. A melt curve analysis was performed to confirm amplicon specificity.

Data normalization and expression analysis
The relative levels of TNF-α and IL-10 messenger RNA (mRNA) expression were quantified using the $2^{-\Delta\Delta Ct}$ (double delta Ct analysis) method, with GAPDH as the endogenous reference gene [14]. These values were normalized to the control group and reported as fold changes relative to controls. The TNF-α/IL-10 ratio was determined for each participant to quantify the pro- versus anti-inflammatory cytokine balance.

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.

Statistical analysis
Data were analyzed using the Statistical Package for the Social Sciences version 26.0. Continuous variables were tested for normality using the Shapiro-–Wilk test and expressed as mean±standard deviation or median (interquartile range), as appropriate. Categorical variables were presented as counts and percentages. For group comparisons, one-way analysis of variance with Tukey's *post hoc* tests was used for normally distributed continuous variables, and the Kruskal–Wallis test was employed for non-normally distributed variables. Chi-square and Fisher's exact tests were applied for categorical variables. Correlations

Table 1: Primer sequences used for qRT-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

qRT-PCR: Quantitative real-time polymerase chain reaction, TNF-α: Tumour necrosis factor-alpha, IL-10: Interleukin-10

between cytokine expression and clinical, laboratory, and perinatal parameters were assessed using Pearson's or Spearman's coefficients, as appropriate. A $p<0.05$ was considered statistically significant.

RESULTS

Baseline maternal, clinical, and perinatal characteristics

The baseline characteristics of women with EOPE, 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 a lower gestational age compared with LOPE. Both EOPE and LOPE groups demonstrated higher BP 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 HTN, 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 PE 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 included lower platelet counts and mild anemia in the EOPE group, while leukocytosis was observed in both preeclamptic 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 PE, again more pronounced in EOPE. In terms

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/24 h)	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
Pre-term <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 (%). ANOVA: Analysis of variance, EOPE: Early-onset preeclampsia, LOPE: Late-onset preeclampsia, HTN: Hypertension, BP: Blood pressure, BMI: Body mass index, NICU: Neonatal intensive care unit

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 *po sthoc* Tukey's test. Data are presented as mean±SD. EOPE: Early-onset preeclampsia, LOPE: Late-onset preeclampsia, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, WBC: White blood cells, SD: Standard deviation, PT: Prothrombin time, aPTT: Activated partial thromboplastin time

of metabolic profile, dyslipidemia was evident, characterized by higher total cholesterol, triglycerides, Low-density lipoprotein, and lower high-density lipoprotein 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 prothrombin time and activated partial thromboplastin time in EOPE, suggesting subclinical coagulopathy. These results confirm that PE is a multi-organ disorder with systemic hematological, renal, hepatic, metabolic, and coagulation involvement. The derangements were more severe in EOPE, 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 groupwise 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 PE, 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 PE subgroups and controls, with EOPE demonstrating the most profound immune imbalance.

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. ANOVA: Analysis of variance, TNF-α: Tumour necrosis factor-alpha, IL-10: Interleukin-10, EOPE: Early-onset pre-eclampsia, LOPE: Late-onset pre-eclampsia, RT-qPCR: Reverse transcription-quantitative polymerase chain reaction, SD: Standard deviation, CI: Confidence interval

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 Fig. 1.

- 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 PE, with the TNF-α/IL-10 ratio emerging as the most reliable marker of severity and adverse outcomes.

DISCUSSION

PE continues to rank among the primary contributors to maternal and perinatal morbidity globally, with its underlying pathophysiology increasingly attributed to a disruption in maternal immune tolerance to the fetus. Within this framework, our investigation furnishes original data indicating that the equilibrium between the pro-inflammatory cytokine TNF-α and the anti-inflammatory cytokine IL-10 is disrupted in pre-eclamptic women and closely corresponds to the clinical severity spectrum of the condition. Specifically, patients with EOPE exhibited the most pronounced upregulation of TNF-α and downregulation of IL-10, while those with LOPE displayed a comparatively attenuated yet statistically significant disequilibrium relative to normotensive controls. Notably, the TNF-α/IL-10 ratio demonstrated the most robust associations with BP, proteinuria, renal and hepatic laboratory parameters, and perinatal outcomes, implying that this composite metric may more effectively reflect disease severity than individual cytokine measurements [7,15].

Our findings are consistent with earlier reports documenting heightened proinflammatory tone in PE. The majority of the studies demonstrated that TNF-α is elevated and IL-10 inversely related in affected pregnancies, while a few researches reported significantly

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/24 h)	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 (×10⁹/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). BP: Blood pressure; MAP: Mean arterial pressure, LDH: Lactate dehydrogenase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Dx: Diagnosis, NICU: Neonatal intensive care unit, TNF-α: Tumour necrosis factor-alpha
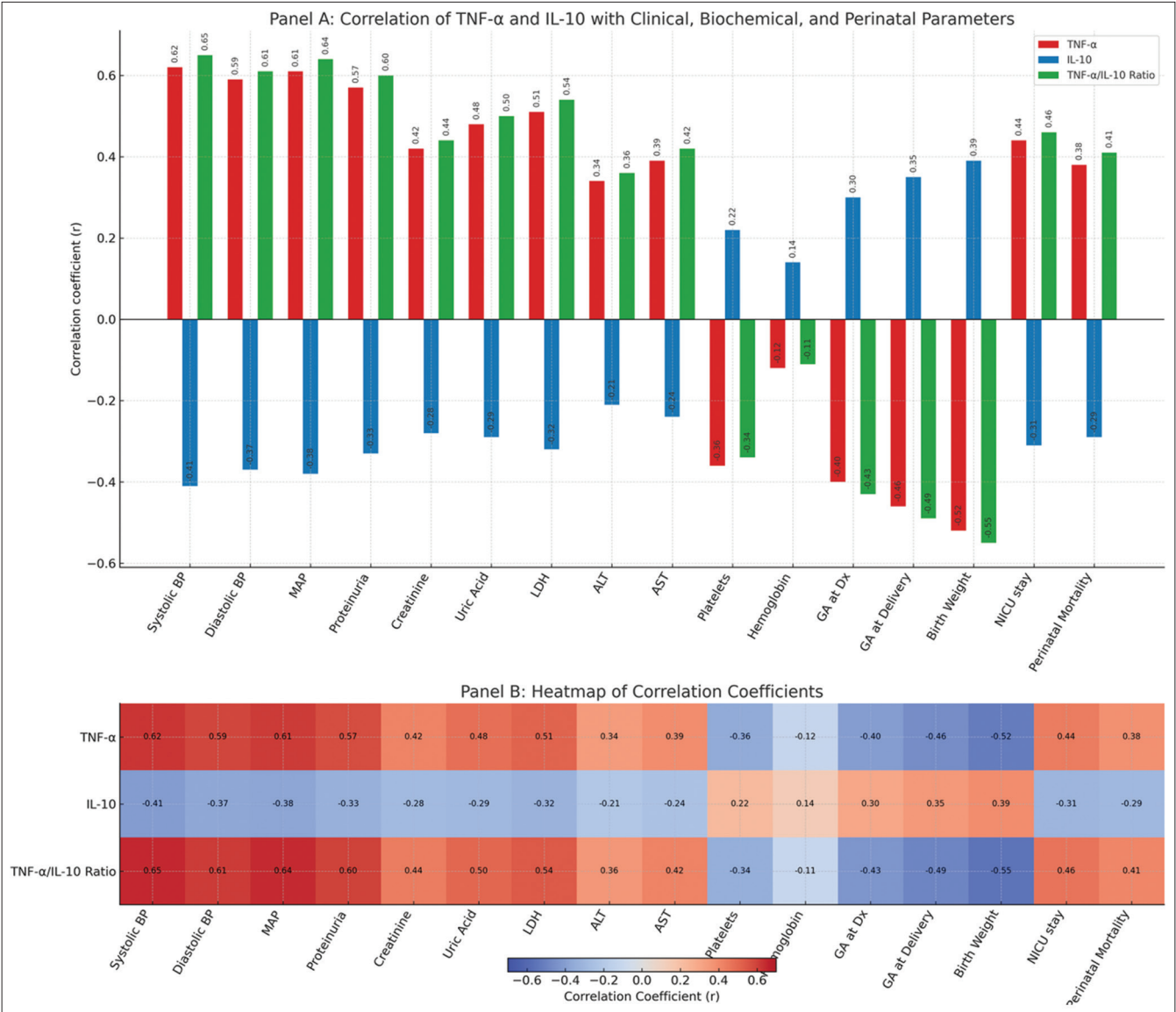
Fig. 1: Composite multi-panel correlation analysis of tumor necrosis factor-alpha and interleukin-10

higher TNF-α concentrations in pre-eclamptic women compared to controls [16,17]. Similarly, a study conducted by Szarka *et al.* confirmed that circulating IL-10 levels are consistently reduced at the time of diagnosis [18]. 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.

Our observation that the cytokine imbalance is most pronounced in EOPE aligns with Boij *et al.,* who reported more marked cytokine dysregulation in EOPE versus LOPE [19], and with Jarmund *et al.*, who linked first-trimester cytokine profiles to PE prediction [20]. In aggregate, these investigations support EOPE as chiefly immune-mediated, with LOPE additionally influenced by maternal cardiovascular and metabolic derangements. The stepwise increase in TNF-α/IL-10 ratio across subtypes in our cohort strengthens this sub-classification, yielding a pathophysiologic rationale for the disorder's clinical diversity.

Mechanistically, the observed cytokine imbalance aligns with established pathophysiology. 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. Cunningham *et al.* [21] and Azizieh *et al.* [22] demonstrated that IL-10 deficiency exacerbates preeclampsia-like features in primates, confirming its protective role which supports the current study. Moreover, recent causal inference analyses suggest that heightened TNF-α signaling may contribute directly to PE risk, highlighting its potential as a therapeutic target, though caution is warranted when considering anti-TNF strategies in pregnancy.

In light of these observations, our findings contribute to the field in three key ways. First, they provide evidence that the TNF-α/IL-10 ratio serves as a marker of disease severity by integrating molecular, clinical, and perinatal indicators [23,24]. Second, they show subtype-specific immune dysregulation that is most pronounced in EOPE, potentially defining distinct disease endotypes [25,26]. Third, they demonstrate strong alignment between cytokine imbalance and established biochemical markers such as creatinine, uric acid, hepatic enzymes, LDH, and platelet counts that were routinely used in clinical practice, thereby bridging the gap from laboratory research to patient care [27,28].

These insights also hold important translational implications. Identifying patients with elevated TNF-α/IL-10 ratios could

enhance risk stratification, optimize delivery timing, and guide adjunctive therapies to restore immune tolerance [29]. Although immunomodulatory interventions remain experimental, our findings suggest that strategies targeting this cytokine axis warrant further investigation [30]. However, given PE's heterogeneity, a one-size-fits-all approach should be avoided; future studies must validate the ratio's predictive value across diverse populations and its incremental utility beyond established risk models [31]. Furthermore, the elevated TNF-α levels, particularly in pre-term PE with severe clinical manifestations, and the reduced IL-10 levels observed in our study align with previous research indicating a sustained proinflammatory state in PE [32].

CONCLUSION

Our investigations and comparisons in this study bolster the paradigm of PE as an immune-mediated pathology, accentuate the pivotal disequilibrium in TNF-α and IL-10, and position their ratio as a prospective biomarker for delineating disease severity. By correlating molecular profiles with clinical, biochemical, and perinatal metrics, the present study substantiates prior findings while providing a cohesive framework for understanding the immunopathogenesis of PE. Prospective longitudinal and interventional studies are essential to determine whether modulating this cytokine axis can improve disease trajectory and optimize maternal and neonatal outcomes.

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