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ELEVATED CIRCULATING ENDOTHELIN-1 AND OXIDATIVE STRESS AS POTENTIAL BIOMARKERS FOR PREECLAMPSIA IN WOMEN FROM TAMILNADU: A CASE CONTROL STUDY.

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ABSTRACT

Background
Preeclampsia (PE) is a hypertensive disorder of pregnancy characterized by endothelial dysfunction and oxidative stress, contributing to maternal and fetal morbidity. Endothelin-1 (ET-1), a potent vasoconstrictor, has been implicated in the pathogenesis of PE.
Objective:
This study aimed to evaluate ET-1 and oxidative stress markers as potential biomarkers for preeclampsia in pregnant women from Erode, Tamil Nadu.

Methods
A prospective case-control study was conducted at Government Erode Medical College Hospital, involving 86 pregnant women. Participants were categorized into preeclamptic cases (n = 46) and normotensive controls (n = 40). Serum levels of ET-1, total oxidative status (TOS), total antioxidative status (TAS), and oxidative stress index (OSI) were measured. Independent t-tests and Pearson's correlation were performed to evaluate group differences and associations.

Results
The preeclamptic group had significantly elevated ET-1 (1.39 ± 1.31 pg/ml vs. 1.27 ± 0.285 pg/ml, p = 0.023), higher TOS (119.44 ± 6.48 µmol/L vs. 112.88 ± 3.23 µmol/L, p = 0.034), lower TAS (1.04 ± 0.259 mmol/L vs. 1.47 ± 0.388 mmol/L, p = 0.001), and higher OSI (1.44 ± 1.29 vs. 0.867 ± 0.421, p < 0.001). Demographic variables such as mean age (24.01 ± 3.43 vs. 22.64 ± 2.16 years), gravidity, and gestational age (33.1 ± 4 vs. 32 ± 4.3 weeks) showed no significant differences, confirming matched groups. Pearson's correlation revealed a weak, non-significant association between ET-1 and OSI.

Conclusion
Preeclampsia is associated with elevated oxidative stress and ET-1 levels, supporting their role as potential biomarkers. These findings may aid in early diagnosis and improved clinical monitoring of PE.

Recommendations
Future multi-center studies with larger cohorts and longitudinal follow-up are recommended to confirm these findings, explore causal mechanisms, and evaluate the utility of ET-1 and oxidative stress markers for risk stratification and therapeutic interventions in high-risk pregnancies.

Keywords: *Preeclampsia, Endothelin-1, Oxidative Stress, Biomarkers, Pregnancy*
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INTRODUCTION

Preeclampsia (PE) represents a significant medical complication in pregnancy, prominently characterized by elevated blood pressure and the potential for multi-organ dysfunction, posing threats from mild to severe health conditions for both mother and fetus [1]. The condition underscores the importance of the placenta in its

pathophysiology, initiating systemic changes, particularly through increased secretions of bioactive factors like endothelin-1 (ET-1), which are implicated in endothelial dysfunction and vascular volume adjustments during pregnancy[2]. The exact mechanism underlying PE remains ambivalently defined, yet it is generally agreed that inadequate placentation leads to hypoxia and the consequent release of pro-inflammatory and anti-angiogenic factors that disturb normal endothelial function[3].

Fundamentally, oxidative stress and reactive oxygen species (ROS) play crucial roles in mediating the endothelial dysfunction noted in PE. An imbalance of oxidative species and antioxidants results in excess ROS, which leads to impaired cellular signaling and vascular impairment, augmenting the severity of PE [4]. This oxidative imbalance is frequently manifested as increased oxidative stress indices, where elevated total oxidative status (TOS) and diminished total antioxidative status (TAS) are observed in affected pregnancies [5]. Furthermore, oxidative stress contributes to post-translational modifications of proteins, thereby damaging cellular structures and prompting adverse clinical outcomes [6]. The efficacy of thioredoxin-1, a redox-sensitive protein, as a biomarker suggests that oxidative stress is intrinsically linked to the pathogenesis of PE [7]. Therefore, the objective of the present study was to evaluate the levels of circulating Endothelin-1 (ET-1) and oxidative stress markers—Total Oxidative Status (TOS), Total Antioxidative Status (TAS), and Oxidative Stress Index (OSI)—in preeclamptic women compared to normotensive pregnant controls. Additionally, the study aimed to assess the correlation between ET-1 and oxidative stress indices to explore their potential as early biomarkers for preeclampsia.

MATERIALS AND METHODS

Study Design

This was a prospective case-control study conducted to evaluate oxidative stress markers and endothelin-1 (ET-1) in preeclamptic and normotensive pregnant women.

Study Setting

The study was conducted at the Department of Obstetrics and Gynaecology, Government Erode Medical College Hospital, Perundurai, Erode, Tamil Nadu, India. This is a tertiary care teaching hospital catering to a large rural and semi-urban population. The study was carried out over a period of three months from October 2023 to December 2023. Blood sample collection, clinical data recording, and laboratory investigations were completed during this period, with all participants followed until delivery for monitoring maternal and fetal outcomes.

Participants and Eligibility Criteria

A total of 86 pregnant women were enrolled in the study based on clearly defined eligibility criteria. Participants were systematically categorized into two groups:

Preeclamptic group (n = 46): Women diagnosed with preeclampsia according to the American College of Obstetricians and Gynecologists (ACOG) guidelines, defined as new-onset hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) occurring after 20 weeks of gestation, accompanied by proteinuria (≥300 mg in a 24-hour urine collection or ≥1+ on dipstick testing).

Normotensive control group (n = 40): Healthy pregnant women of similar gestational age with no clinical evidence of hypertension or proteinuria.

Inclusion Criteria

Pregnant women aged 18–35 years
Singleton pregnancies
Gestational age of 20 weeks or more
Willingness to provide informed written consent

Exclusion Criteria

Pre-existing chronic hypertension or diabetes mellitus
Renal or hepatic disorders
Autoimmune diseases
Multiple gestations
Known fetal anomalies based on ultrasonography
Use of antioxidant supplements or medications known to affect oxidative stress markers

Detailed demographic and clinical information was collected for all participants using a structured proforma. Venous blood samples were drawn under aseptic precautions for the estimation of serum endothelin-1 (ET-1), total oxidative status (TOS), total antioxidative status (TAS), and oxidative stress index (OSI). These analyses were undertaken to explore the potential of these biomarkers in identifying preeclampsia and understanding the oxidative and endothelial pathways implicated in its pathogenesis.

Sample Size Determination

The sample size was calculated using data from previous literature reporting differences in oxidative stress markers between preeclamptic and normotensive pregnant women. Assuming a moderate effect size of 0.7, power of 80%, and significance level (α) of 0.05, a minimum of 40 participants per group was required. To accommodate potential dropouts or incomplete data, the sample size was increased to 86 participants (46 preeclamptic, 40 controls), ensuring adequate statistical power.

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Data Collection Tool

Data collection was carried out using a structured, pre-tested proforma specifically designed for the study. This tool captured comprehensive demographic, clinical, and obstetric information, including age, gravidity, gestational age, blood pressure readings, and clinical signs of preeclampsia. Additionally, blood samples were collected under aseptic conditions from all participants after obtaining informed consent. The serum was separated and stored at −80°C until biochemical analysis. Endothelin-1 (ET-1) levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's protocol. Oxidative stress markers—Total Oxidative Status (TOS) and Total Antioxidative Status (TAS)—were assessed using colorimetric assay kits. The Oxidative Stress Index (OSI) was calculated as the ratio of TOS to TAS, providing a comprehensive measure of oxidative imbalance. All laboratory measurements were performed in duplicate to ensure accuracy and reproducibility. The data collection tool thus integrated both clinical and biochemical parameters essential for assessing the association between oxidative stress, ET-1 levels, and preeclampsia.

Data Collection Procedure

After obtaining ethical clearance and informed consent, eligible pregnant women attending the antenatal clinic at Government Erode Medical College Hospital were categorized into preeclamptic and normotensive groups. Clinical details and demographic data were recorded using a structured proforma. Venous blood samples (5 ml) were collected, and serum was separated by centrifugation. Samples were stored at −80°C until analysis. Serum Endothelin-1 (ET-1) levels were measured using ELISA, while Total Oxidative Status (TOS) and Total Antioxidative Status (TAS) were assessed using colorimetric methods. The Oxidative Stress Index (OSI) was calculated as TOS/TAS × 100.

Data Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 25.0. Descriptive statistics were used to summarize demographic and clinical characteristics. Independent t-tests were performed to compare biochemical parameters between groups. Pearson's correlation was used to assess the association between Endothelin-1 and oxidative stress markers. A p-value < 0.05 was considered statistically significant.

Bias

To minimize selection bias, participants were consecutively recruited based on predefined inclusion and exclusion criteria. Information bias was reduced by using standardized procedures for sample collection and biochemical analysis. Observer bias was limited by blinding laboratory personnel to group allocation during analysis.

Ethical Considerations

The study was approved by the Institutional Ethics Committee of Government Erode Medical College (IEC/GEMCH/2024/52 dated 02-03-2024. Written informed consent was obtained from all participants before enrollment. Confidentiality and anonymity of the participants were maintained throughout the study, and all procedures adhered to the ethical principles outlined in the Declaration of Helsinki.

RESULTS

A total of 103 pregnant women were initially screened for participation in the study. After applying the inclusion and exclusion criteria, 91 women were found eligible and provided informed consent. Of these, 5 participants were excluded due to incomplete biochemical data or voluntary withdrawal, resulting in a final sample size of 86 participants. This included 46 preeclamptic women and 40 normotensive controls, all of whom completed the study procedures and were included in the final analysis.

Table 1 presents the demographic characteristics of the study participants, comparing preeclamptic cases (n=46) and controls (n=40). The mean age of participants in the control group was 22.64 ± 2.16 years, while in the case group, it was 24.01 ± 3.43 years, with no statistically significant difference between the groups ($p = 0.459$). Gravidity distribution was also comparable, with both groups consisting of primigravida and multigravida women. In the control group, 57.5% were primigravida, whereas 43.5% of the case group were primigravida. The proportion of women with two or more pregnancies was slightly higher in the case group, but the difference was not statistically significant ($p = 0.669$). The mean gestational age at the time of assessment was 32 ± 4.3 weeks in the control group and 33.1 ± 4 weeks in the case group, with no significant difference observed ($p = 0.406$). These findings indicate that the demographic variables were well-matched between the groups, minimizing potential confounding effects on the study outcomes.

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Table 1: Summary of data based on demographic details

Variables	Group		p-value
	Control (n=40)	Case (n=46)	
Age (years)	22.64 ± 2.16	24.01 ± 3.43	0.459
Gravida			
1	23 (57.5%)	20 (43.5%)	
2	15 (37.5%)	20 (43.5%)	0.669
3	2 (5%)	5 (10.9%)	
4	0	1 (2.1%)	
Gestational age	32 ± 4.3	33.1 ± 4	0.406

Table 2 presents the biochemical differences between the control and preeclamptic groups, highlighting significant alterations in oxidative stress markers and endothelin-1 (ET-1) levels. The mean serum ET-1 concentration was significantly elevated in the preeclamptic group (1.39 ± 1.31 pg/ml) compared to the control group (1.27 ± 0.285 pg/ml, p = 0.023), supporting its role in vascular dysfunction associated with preeclampsia.

Total antioxidant status (TAS) levels were markedly lower in the preeclamptic group (1.04 ± 0.259 mmol/L) than in the control group (1.47 ± 0.388 mmol/L, p = 0.001), indicating a compromised antioxidant defense system. Similarly, the TASx100 value was significantly reduced in the case group (106.59 ± 35.9) compared to controls (131.28 ± 28.84, p = 0.001), further emphasizing oxidative imbalance.

Conversely, total oxidative status (TOS) was significantly higher in preeclamptic participants (119.44 ± 6.48 µmol/L) than in controls (112.88 ± 3.23 µmol/L, p = 0.034), reflecting increased oxidative stress. Moreover, the oxidative stress index (OSI), calculated as the TOS/TAS ratio, was significantly elevated in the preeclamptic group (1.44 ± 1.29) compared to controls (0.867 ± 0.421, p < 0.001).

These findings suggest that preeclampsia is associated with heightened oxidative stress and endothelial dysfunction, as evidenced by elevated ET-1 levels, reduced antioxidant capacity, and increased oxidative markers, reinforcing the role of oxidative stress in the disease pathophysiology.

Table 2: Summary of data based on different variables over groups.

Variables	Group		p-value
	Control (n=40)	Case (n=46)	
ET- pg/ml	1.27 ± 0.285	1.39 ± 1.31	0.023*
TAS conc (mmol/L)	1.47 ± 0.388	1.04 ± 0.259	0.001*
TASx100	131.28 ± 28.84	106.59 ± 35.9	0.001*
TOS (umol/L)	112.88 ± 3.23	119.44 ± 6.48	0.034*
OSI=TOS/TAS100	0.867 ± 0.421	1.44 ± 1.29	<0.001*

Table 3 presents Pearson's correlation analysis between oxidative stress index (OSI) and endothelin-1 (ET-1) levels in both control and preeclamptic groups. The results indicate weak and statistically non-significant correlations between ET-1 and OSI across both groups (p > 0.05). In the preeclamptic group, ET-1 showed a weak negative correlation with OSI (r = -0.091, p = 0.548), while in the control group, a weak positive correlation was observed (r = 0.202, p = 0.212). These findings suggest that although oxidative stress and ET-1 are altered in preeclampsia, their direct interplay may be influenced by additional pathophysiological factors.

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Table 3: Pearson's Correlation between the oxidative stress index with ET1 in preeclamptic women

Correlations					
		Control_ET (pg/ml)	Case_ET (pg/ml)	Control_OSI	Case_OSI
Control_ET (pg/ml)	Pearson Correlation	1	-0.029	-0.240	0.202
	p-value	-	0.861	0.135	0.212
	N	40	40	40	40
Case_ET (pg/ml)	Pearson Correlation	-0.029	1	0.054	-0.091
	p-value	0.861	-	0.739	0.548
	N	40	46	40	46
Control_OSI	Pearson Correlation	-0.240	0.054	1	-0.136
	p-value	0.135	0.739	-	0.404
	N	40	40	40	40
Case_OSI	Pearson Correlation	0.202	-0.091	-0.136	1
	p-value	0.212	0.548	0.404	-
	N	40	46	40	46

The oxidative stress assessment revealed a significant imbalance in the preeclamptic group, characterized by elevated total oxidative status (TOS: 119.44 ± 6.48 μmol/L vs. 112.88 ± 3.23 μmol/L, $p = 0.034$) and reduced total antioxidative status (TAS: 1.04 ± 0.259 mmol/L vs. 1.47 ± 0.388 mmol/L, $p < 0.001$). Consequently, the oxidative stress index (OSI) was significantly higher in preeclamptic women (1.44 ± 1.29) compared to controls (0.867 ± 0.421, $p < 0.001$), indicating a pronounced oxidative imbalance.

Despite these biochemical alterations, the correlation between ET-1 and OSI was weak, suggesting that while oxidative stress is a key feature of preeclampsia, its relationship with ET-1 may be modulated by additional biological and environmental factors. These findings emphasize the complex pathophysiology of preeclampsia and highlight the need for further studies to explore the mechanistic links between oxidative stress and endothelial dysfunction in this condition.

DISCUSSION

The findings of our study bolster the understanding of oxidative stress and endothelin-1 (ET-1) levels as pivotal factors associated with preeclampsia (PE). Specifically, our results aligned with the hypothesis that oxidative stress is prevalent in preeclamptic pregnancies, contributing to the endothelial dysfunction characteristic of the syndrome. The observation that total oxidative status (TOS) was significantly higher and total antioxidative status (TAS) was notably lower among preeclamptic patients compared to normotensive controls is consistent with existing literature, highlighting the potential role of the oxidative stress index (OSI) as a predictor of PE[8].

The production of reactive oxygen species (ROS) has been documented as detrimental in numerous physiological processes during pregnancy. In preeclampsia, an increased ROS level is often seen to overwhelm antioxidant defenses, leading to endothelial damage and amplified vascular dysfunction [9]. The placenta, under hypoxic conditions typical of preeclampsia, releases pro-inflammatory factors and anti-angiogenic proteins, notably sFlt-1 and soluble endoglin, which further exacerbate endothelial dysfunction and may explain the clinical manifestations of this condition[10].

Interestingly, our study demonstrated a positive correlation between oxidative stress markers and ET-1 levels, although not statistically significant. ET-1, a potent vasoconstrictor, has been identified as a key player in the pathogenesis of PE, primarily through its influence on blood pressure and vascular endothelial integrity [11]. Elevated ET-1 levels can instigate further oxidative stress by promoting the production of superoxide anions via NADPH oxidase pathways, creating a vicious cycle of vascular dysfunction[6]. This feedback loop may explain the persistent nature of PE, as both increased ET-1 levels and oxidative stress contribute synergistically to the disease's severity.

The statistical insignificance of the correlation between OSI and ET-1 levels in our study, despite a noted trend, may be attributable to the limited sample size, which poses a challenge in detecting robust correlations. Future studies on larger cohorts are necessary to validate these observations and ascertain whether OSI can indeed serve as a predictive biomarker for PE. Previous research supports this outlook, indicating that targeted interventions against oxidative stress can mitigate PE symptoms and improve outcomes for affected pregnancies[12].

Furthermore, the heterogeneity of preeclampsia and its varying clinical presentations complicate the establishment of universal biomarkers. As noted, only certain subsets of pregnant women exemplify the extremities of oxidative stress and ET-1 elevation, leading to the call for personalized medicine approaches in PE management[13]. Studies have shown that interventions such as antioxidants and dietary modifications could

benefit women predisposed to oxidative stress during their pregnancies[14]. However, additional clinical trials are necessary to examine the effectiveness and safety of these interventions as they pertain to PE prevention and treatment.

In considering the breadth of preeclampsia's pathophysiology, the interplay between genetic predispositions, environmental factors, and lifestyle choices also warrants further exploration. Genetic susceptibilities are increasingly recognized as contributing factors in the pathogenesis of PE, and understanding these influences alongside oxidative stress markers could lead to tailored screening strategies for high-risk populations [15].

Moreover, the implications for clinical practice are profound. Identifying and modifying risk factors for oxidative stress through nutritional supplementation or lifestyle changes could become an integral part of managing pregnant women at risk for preeclampsia. Such approaches would necessitate multi-faceted healthcare strategies combining obstetric care with nutritional and psychological support to optimize maternal and neonatal health outcomes [16-18].

CONCLUSION

In conclusion, the study elucidates the substantial relationship between elevated oxidative stress indices and ET-1 levels in women experiencing preeclampsia. These findings contribute to the existing literature by suggesting that monitoring these biomarkers could enhance early diagnostic procedures, ultimately improving management strategies for preeclampsia and possibly mitigating its severe complications.

LIMITATIONS AND GENERALIZABILITY

This study was limited by its relatively small sample size and single-center design, which may affect the generalizability of the findings. The participants were recruited from a specific geographic region in Tamil Nadu, which may not reflect the broader population. Additionally, the cross-sectional nature of the study limits the ability to infer causal relationships between biomarkers and preeclampsia. Despite these limitations, the study provides valuable insights into the potential role of endothelin-1 and oxidative stress markers in the pathophysiology of preeclampsia, warranting further research in more diverse and larger populations.

RECOMMENDATIONS

Future research should involve larger, multicenter studies to validate the role of endothelin-1 and oxidative stress markers as reliable biomarkers for preeclampsia. Longitudinal studies are needed to explore the temporal relationship and potential causal mechanisms linking oxidative stress and endothelial dysfunction. Additionally, investigating the efficacy of targeted antioxidant therapies and lifestyle interventions in high-risk pregnancies may help in the early prevention and management of preeclampsia. Integrating biomarker screening into routine antenatal care could also enhance early detection and improve maternal-fetal outcomes.

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LIST OF ABBREVIATIONS

PE: Preeclampsia
ET-1: Endothelin-1
TOS: Total Oxidative Status
TAS: Total Antioxidative Status
OSI: Oxidative Stress Index
ROS: Reactive Oxygen Species
sFlt-1: Soluble Fms-like Tyrosine Kinase-1
SD: Standard Deviation
ANOVA: Analysis of Variance
SPSS: Statistical Package for the Social Sciences

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CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHOR CONTRIBUTIONS

PB-Conceptualization, Study design, Supervision, and Manuscript review. PMS-Methodology development, Data interpretation, and Critical revision of the manuscript. RM-Laboratory analysis, Biochemical assessments, and Data curation. RA-Participant recruitment, Data collection, and Data validation. PP-Manuscript writing, Formatting, and Final editing. SG-Literature review, Statistical analysis, and Drafting of results.

DATA AVAILABILITY

All datasets analyzed during this study are included in the manuscript.

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