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Serum Lipid Peroxidation Markers and Oxidative Stress Status in Pre-eclamptic Pregnant Women: A Case–Control Study

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Abstract

Pre-eclampsia is a pregnancy-specific multisystem hypertensive disorder that remains a leading cause of maternal and perinatal morbidity and mortality worldwide. The two-stage model of its pathogenesis implicates defective placentation and oxidative stress as the central link between placental ischaemia and the maternal clinical syndrome. The present study assessed lipid peroxidation by estimating serum oxidative stress markers in pre-eclamptic women and compared them with normotensive pregnant controls. Sixty pregnant women (30 pre-eclamptic cases and 30 gestational-age-matched normotensive controls) were enrolled in this hospital-based observational analytical study. Serum was analysed for thiobarbituric acid reactive substances (TBARS/MDA), nitrite, total antioxidant status (TAS), superoxide dismutase (SOD), total thiol and uric acid. Pre-eclamptic women showed elevated serum MDA (6.4 ± 0.9 vs. 3.8 ± 0.6 nmol/mL; $p < 0.001$; $d = 3.42$) and uric acid (6.4 ± 0.9 vs. 4.5 ± 0.8 mg/dL; $p < 0.001$; $d = 2.36$), with reduced nitrite (38.7 ± 6.8 vs. 54.9 ± 7.3 µmol/L; $p < 0.001$; $d = −2.28$), TAS (1.0 ± 0.2 vs. 1.5 ± 0.2 mmol/L; $p < 0.001$; $d = −2.89$), SOD (2.8 ± 0.6 vs. 4.7 ± 0.8 U/mL; $p < 0.001$; $d = −2.68$) and total thiol (0.3 ± 0.1 vs. 0.5 ± 0.1 µmol/L; $p < 0.001$; $d = −2.63$). MDA correlated positively with systolic ($r = 0.79$) and diastolic ($r = 0.81$) blood pressure and inversely with TAS ($r = −0.67$) and SOD ($r = −0.67$). ROC analysis indicated excellent discriminatory ability for MDA (AUC = 1.00), TAS (AUC = 0.98) and SOD (AUC = 0.97). These findings confirm that pre-eclampsia is associated with a marked shift in oxidative balance towards lipid peroxidation and support incorporation of oxidative-stress biomarkers into the biochemical evaluation of high-risk pregnancies.

Keywords: pre-eclampsia; lipid peroxidation; malondialdehyde; thiobarbituric acid reactive substances; oxidative stress; antioxidant status; nitric oxide; biomarkers

1. INTRODUCTION

Pre-eclampsia is a pregnancy-specific hypertensive disorder characterised by new-onset hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) after 20 weeks of gestation, accompanied by proteinuria or evidence of end-organ dysfunction (American College of Obstetricians and Gynecologists [ACOG], 2020). Globally, it complices 3–10 % of all pregnancies and accounts for an estimated 70 000–80 000 maternal deaths and more than 500 000 perinatal deaths each year (Say et al., 2014; Steegers et al., 2010). The prevalence is higher in low- and middle-income countries (4–18 %) due to limited antenatal care, undiagnosed chronic disease and nutritional deficiencies (Bilano et al., 2014; Rana et al., 2019). The disorder is associated with eclampsia, HELLP syndrome, abruptio placentae, pulmonary oedema, acute kidney injury and long-term cardiovascular morbidity (Wilkerson & Ogunbodede, 2019); perinatal consequences include intrauterine growth restriction, prematurity and perinatal death.

Despite decades of clinical and experimental research, the precise aetiology of pre-eclampsia remains elusive. The two-stage model originally proposed by Roberts and Poston (2003) and subsequently refined by Redman and Sargent (2009) provides the most widely accepted conceptual framework. Stage 1 comprises defective trophoblastic invasion of the maternal spiral arteries and failed vascular remodelling, leading to high-resistance, low-flow uteroplacental circulation and chronic placental ischaemia/reperfusion injury. Stage 2 is the maternal response, in which the ischaemic placenta releases an excess of pro-inflammatory, anti-angiogenic and oxidative mediators into the systemic circulation, producing generalised endothelial dysfunction that manifests clinically as hypertension, proteinuria and end-organ injury.

Oxidative stress is increasingly recognised as the critical biochemical link between the two stages (Burton & Jauniaux, 2019; Chiarello et al., 2020). Reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$), together with reactive nitrogen species (RNS) principally nitric oxide ($^{\bullet}NO$) and peroxynitrite ($ONOO^-$), are generated in moderation during normal pregnancy where they regulate trophoblast invasion, placental angiogenesis and vascular tone (Poston & Rai, 2019). In pre-eclampsia, however, the ischaemic–reperfused placenta and dysfunctional mitochondria produce ROS far in excess of the buffering capacity of the maternal antioxidant defence, leading to oxidative damage of membrane lipids, proteins and nucleic acids (Ferreira et al., 2020; Grzeszczak et al., 2023).

Lipid peroxidation is the prototypic molecular consequence of this imbalance. The polyunsaturated fatty acids of cellular and organellar membranes are particularly vulnerable to attack by ROS, generating a chain reaction that produces reactive aldehydes chiefly malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) which can be measured as thiobarbituric acid reactive substances (TBARS). MDA is the most extensively studied surrogate of lipid peroxidation in pregnancy research and has been shown to be consistently elevated in maternal serum, placental tissue and umbilical cord blood of pre-eclamptic women (Ahmad et al., 2020; Baban et al., 2022; Chiarello et al., 2020; Taysi et al., 2019). 8-iso-prostaglandin $F_2\alpha$ (8-iso-$PGF_2\alpha$), an isoprostane formed by ROS-mediated arachidonic acid oxidation, is now considered a more specific marker of in-vivo oxidative injury and has likewise been shown to be elevated in pre-eclampsia (McCarthy et al., 2021).

Under physiological conditions the maternal–placental unit is protected by a tiered antioxidant defence system that includes the enzymatic scavengers superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and the non-enzymatic constituents glutathione, ascorbate, α-tocopherol, uric acid, thiols and bilirubin (Ibrahim et al., 2024). In pre-eclampsia this defence is compromised: circulating and placental activities of SOD, catalase and GPx are reduced, and total antioxidant status (TAS) an integrated measure of both enzymatic and non-enzymatic defences is correspondingly diminished (Afrose et al., 2022; Di Fabrizio et al., 2022; Sultana et al., 2017). Conversely, uric acid, the terminal product of purine catabolism

and a paradoxical antioxidant at physiological concentrations, accumulates because of reduced renal clearance and increased xanthine oxidase activity, and is now included among the routine laboratory indices that signal disease severity (Bellos et al., 2020).

Nitric oxide metabolism is likewise deranged. •NO is produced from L-arginine by endothelial nitric oxide synthase (eNOS) and is a principal determinant of vasodilator tone, placental perfusion and trophoblast invasion. In pre-eclampsia, superoxide avidly combines with •NO to form peroxynitrite, both reducing •NO bioavailability and generating a secondary oxidant that propagates lipid peroxidation and nitrates protein tyrosine residues (Grzeszczak et al., 2023). Serum nitrite, the stable oxidation product of •NO, has consequently emerged as a useful surrogate of NO bioavailability and endothelial function in pregnancy (Sandrim et al., 2020).

Despite the strength of this collective evidence, several gaps remain. Many earlier studies measured only one or two oxidative markers, used heterogeneous diagnostic criteria, or relied on small convenience samples. Few have comprehensively correlated lipid peroxidation with both antioxidant defence and clinical severity in a single cohort drawn from the Indian subcontinent, where the prevalence of pre-eclampsia is high and the contributing risk factors (anaemia, undernutrition, early childbearing) differ from those of high-income settings. The present investigation was therefore designed with the specific objective of assessing lipid peroxidation by estimating serum oxidative stress markers in pre-eclamptic women and comparing them with normotensive pregnant controls, while simultaneously profiling the enzymatic and non-enzymatic antioxidant defence and examining their statistical inter-relationships. The central hypothesis was that pre-eclampsia is associated with a marked shift in the oxidative balance towards lipid peroxidation, accompanied by depletion of the antioxidant reserve, and that these biochemical alterations correlate with the clinical severity of the disease.

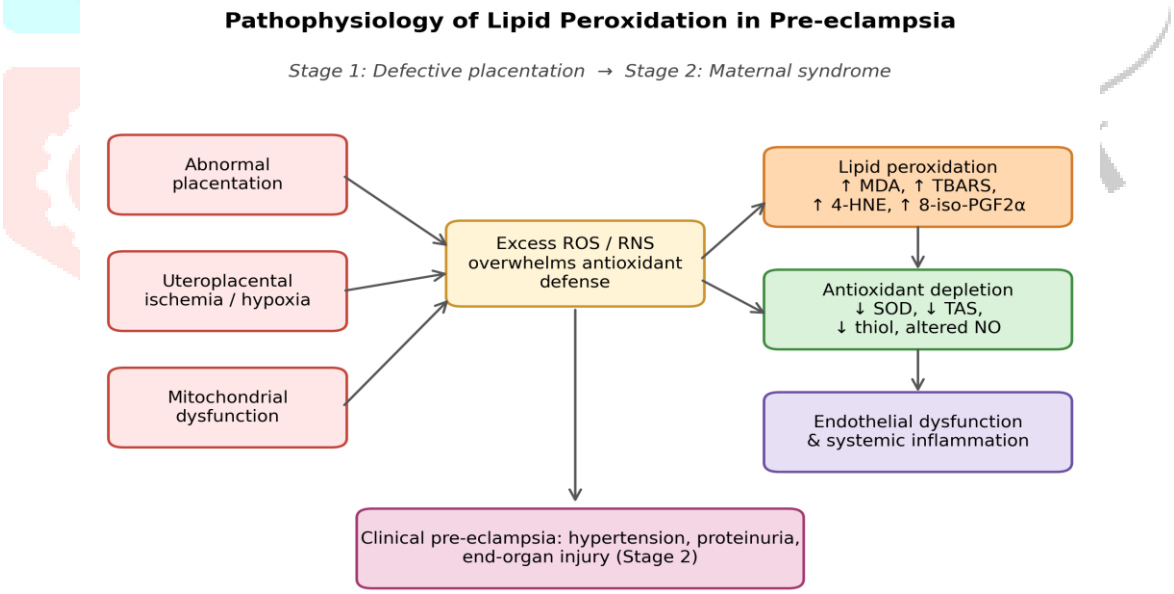

Figure 1. Integrated mechanistic framework linking defective placentation, excess reactive oxygen/nitrogen species, lipid peroxidation, antioxidant depletion, and endothelial dysfunction in pre-eclampsia.

2. MATERIALS AND METHODS

2.1 Study design and setting

This hospital-based, observational analytical case–control study was conducted in the Department of Medical Biochemistry in collaboration with the Department of Obstetrics and Gynaecology of Krishna Mohan Medical College and Hospital, Mathura, Uttar Pradesh, India. The study was carried out over a 12-month recruitment period (January–December 2025) after obtaining clearance from the Institutional Ethics Committee (IEC/KMMC/2024-OBG/118) and was performed in accordance with the Declaration of Helsinki (2013 revision). Written informed consent was obtained from every participant before enrolment.

2.2 Study population

Thirty pregnant women with a confirmed diagnosis of pre-eclampsia constituted the case group. Pre-eclampsia was diagnosed according to the American College of Obstetricians and Gynecologists (ACOG, 2020) criteria: new-onset systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, measured on two occasions at least four hours apart after 20 weeks of gestation in a previously normotensive woman, accompanied by proteinuria (≥ 300 mg/24 h or dipstick ≥ 1+) or end-organ dysfunction (thrombocytopenia, renal insufficiency, impaired liver function, pulmonary oedema or cerebral/visual symptoms). The control group comprised 30 age- (± 2 years) and gestational-age- (± 2 weeks) matched normotensive pregnant women attending the antenatal clinic for routine obstetric care.

Exclusion criteria for both groups were: multiple gestation, pre-existing chronic hypertension, diabetes mellitus (pre-gestational or gestational), chronic renal or hepatic disease, autoimmune disorders, active infection, smoking, antioxidant supplementation in the preceding four weeks, and delivery before sample collection. Women with haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, eclampsia or known fetal anomalies were also excluded.

2.3 Sample size

The sample size was computed a priori using G*Power v3.1 (Faul et al., 2007). On the basis of the mean difference in serum MDA between pre-eclamptic and normotensive women reported by Baban et al. (2022) (effect size $d \approx 1.7$), with $\alpha = 0.05$ and power $(1 - \beta) = 0.90$, a minimum of 12 subjects per group was required. To enhance statistical robustness and permit subgroup correlation analysis, 30 participants per group were enrolled, yielding a total sample of 60.

2.4 Clinical and anthropometric assessment

A structured proforma was used to record demographic, obstetric and clinical data, including maternal age, parity, gestational age (confirmed by first-trimester ultrasonography), pre-pregnancy body mass index (BMI), systolic and diastolic blood pressure (mean of two readings taken five minutes apart in the left lateral position using a calibrated mercury sphygmomanometer), and proteinuria status.

2.5 Sample collection and processing

Five millilitres of venous blood were drawn from the antecubital vein under aseptic conditions; 2 mL were transferred to a plain tube for serum separation and 3 mL to a lithium-heparin tube for plasma antioxidant assays. Samples were allowed to clot for 30 minutes at room temperature and centrifuged at 3000 rpm for 10 minutes. Aliquots of serum and plasma were stored at − 80 °C until analysis; all assays were completed within four weeks of collection to minimise ex-vivo oxidation.

2.6 Biochemical assays

All biochemical analyses were performed in duplicate by a single investigator blinded to group allocation, with internal quality-control sera (Randox, UK) included in each run; the inter-assay coefficient of variation was < 8 % for all parameters.

Serum lipid peroxidation was quantified as thiobarbituric acid reactive substances (TBARS) using the method of Ohkawa et al. (1979): 0.5 mL serum was mixed with 0.5 mL of 0.67 % thiobarbituric acid and 0.5 mL of 20 % trichloroacetic acid, heated at 95 °C for 30 min, cooled and centrifuged; the absorbance of

the pink supernatant was read at 532 nm and the concentration of MDA (nmol/mL) calculated using 1,1,3,3-tetraethoxypropane as the external standard.

Serum nitrite, the stable oxidation product of nitric oxide, was measured by the Griess reaction (Granger et al., 1999) at 548 nm after incubation of deproteinised sample with Griess reagent (0.1 % N-1-naphthylethylenediamine dihydrochloride and 1 % sulfanilamide in 5 % phosphoric acid).

Total antioxidant status (TAS) was determined by the ferric reducing ability of plasma (FRAP) assay (Benzie & Strain, 1996), with absorbance read at 593 nm and expressed as mmol/L Trolox equivalent.

Superoxide dismutase (SOD) activity was measured by the pyrogallol autoxidation method (Marklund & Marklund, 1974) at 420 nm, with one unit defined as the amount of enzyme producing 50 % inhibition under assay conditions.

Total thiol was estimated by the Ellman method using 5,5′-dithio-bis-(2-nitrobenzoic acid) (Hu, 1994) at 412 nm ($\varepsilon = 13\ 600\ M^{-1}\ cm^{-1}$). Uric acid was quantified by the enzymatic uricase–peroxidase method on a semi-automated analyser (ERBA Chem 7, India) using commercial calibrators and quality-control materials.

2.7 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v26.0 and GraphPad Prism v9.5. The distribution of continuous variables was assessed by the Shapiro–Wilk test; all variables were normally distributed and are expressed as mean ± SD. Between-group comparisons used the independent-samples Student's t-test; effect size was expressed as Cohen's d (small 0.2–0.5, medium 0.5–0.8, large > 0.8). Inter-relationships among markers and clinical variables were examined with Pearson's correlation. Receiver operating characteristic (ROC) analysis evaluated the discriminatory performance of each biomarker, and the area under the curve (AUC) with 95 % CI is reported. A two-tailed $p < 0.05$ was considered significant.

3. RESULTS

3.1 Demographic and clinical characteristics

Sixty pregnant women (30 pre-eclamptic cases and 30 normotensive controls) completed the study. Table 1 summarises their demographic and clinical characteristics. The two groups were comparable with respect to maternal age (27.6 ± 3.3 vs. 27.2 ± 4.0 years; $p = 0.737$) and gestational age (35.1 ± 2.1 vs. 34.6 ± 2.6 weeks; $p = 0.384$), confirming adequate matching. As expected by design, the pre-eclamptic women had significantly higher systolic (155.6 ± 7.6 vs. 117.0 ± 6.4 mmHg; $p < 0.001$) and diastolic (102.8 ± 6.2 vs. 76.5 ± 5.3 mmHg; $p < 0.001$) blood pressure. BMI was also higher in the case group (27.4 ± 2.3 vs. 24.1 ± 2.6 kg/m²; $p < 0.001$), consistent with established risk-factor epidemiology.

Table 1. Demographic and clinical characteristics of the study population.

Variable	Unit	Control (n = 30)	Pre-eclampsia (n = 30)	t	p-value
Age	years	27.2 ± 4.0	27.6 ± 3.3	0.34	0.737
Gestational age	weeks	34.6 ± 2.6	35.1 ± 2.1	0.88	0.384
BMI	kg/m²	24.1 ± 2.6	27.4 ± 2.3	5.34	< 0.001
SBP	mmHg	117.0 ± 6.4	155.6 ± 7.6	21.25	< 0.001
DBP	mmHg	76.5 ± 5.3	102.8 ± 6.2	17.78	< 0.001

Values are expressed as mean ± SD. p-values from independent-samples t-test.

3.2 Serum lipid peroxidation markers (objective 1)

The principal markers of lipid peroxidation measured in this study serum MDA (TBARS) and serum nitrite are presented in Table 2 and Figure 2. The pre-eclamptic women exhibited a substantial elevation of serum MDA (6.4 ± 0.9 vs. 3.8 ± 0.6 nmol/mL; $p < 0.001$; $d = 3.42$). The reciprocal decline in serum nitrite (38.7 ± 6.8 vs. 54.9 ± 7.3 µmol/L; $p < 0.001$; $d = -2.28$) is consistent with reduced NO bioavailability secondary to peroxynitrite-mediated scavenging and endothelial dysfunction.

Table 2. Comparison of serum oxidative-stress markers (lipid peroxidation) between pre-eclamptic women and normotensive pregnant controls.

Marker	Unit	Control	Pre-eclampsia	t	p-value	Cohen's d
MDA (TBARS)	nmol/mL	3.8 ± 0.6	6.4 ± 0.9	13.26	< 0.001	3.42
Nitrite	µmol/L	54.9 ± 7.3	38.7 ± 6.8	−8.83	< 0.001	−2.28

Effect-size interpretation: $|d| \geq 0.8$ indicates a large effect.

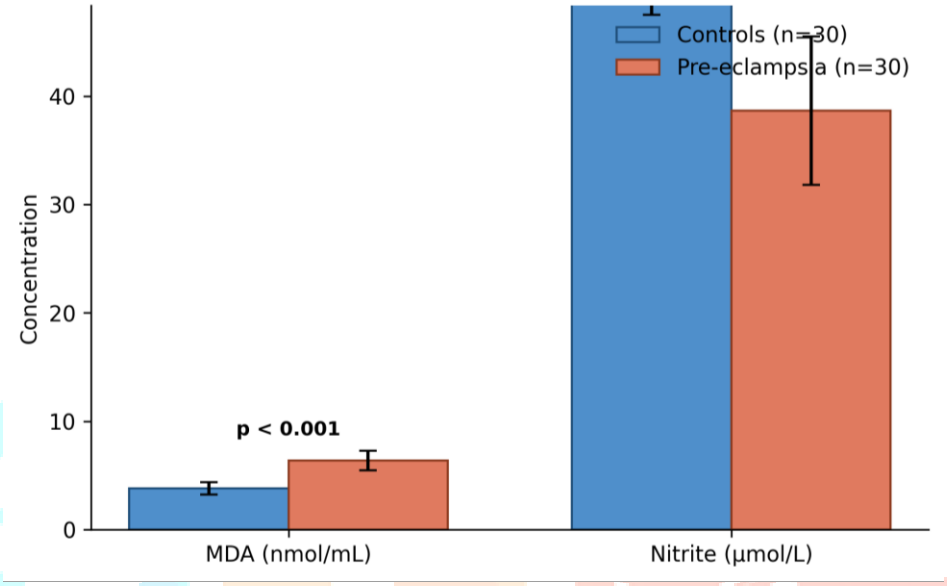

Figure 2. Serum oxidative-stress markers in pre-eclampsia versus controls.

3.3 Antioxidant defence parameters

The serum concentrations/activities of the antioxidant indices are summarised in Table 3 and Figure 3. Pre-eclamptic women showed marked depletion of TAS (1.0 ± 0.2 vs. 1.5 ± 0.2 mmol/L; $p < 0.001$; $d = -2.89$), reduced SOD activity (2.8 ± 0.6 vs. 4.7 ± 0.8 U/mL; $p < 0.001$; $d = -2.68$) and lower total thiol (0.3 ± 0.1 vs. 0.5 ± 0.1 µmol/L; $p < 0.001$; $d = -2.63$). In contrast, serum uric acid was significantly elevated in the case group (6.4 ± 0.9 vs. 4.5 ± 0.8 mg/dL; $p < 0.001$; $d = 2.36$), reflecting both impaired renal clearance and increased xanthine-oxidase activity in pre-eclampsia.

Table 3. Comparison of serum antioxidant-status parameters between pre-eclamptic women and normotensive pregnant controls.

Marker	Unit	Control	Pre-eclampsia	t	p-value	Cohen's d
Total antioxidant status (TAS)	mmol/L	1.5 ± 0.2	1.0 ± 0.2	−11.19	< 0.001	−2.89
Superoxide dismutase (SOD)	U/mL	4.7 ± 0.8	2.8 ± 0.6	−10.38	< 0.001	−2.68
Total thiol	µmol/L	0.5 ± 0.1	0.3 ± 0.1	−10.18	< 0.001	−2.63
Uric acid	mg/dL	4.5 ± 0.8	6.4 ± 0.9	9.15	< 0.001	2.36

Uric acid was markedly elevated in pre-eclampsia, whereas all other antioxidant indices were significantly reduced.

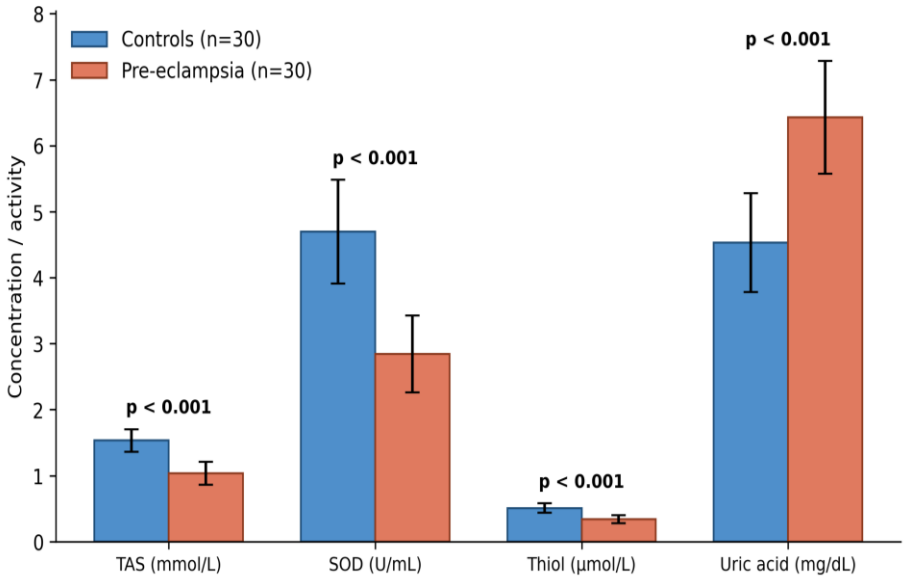

Figure 3. Serum antioxidant-status markers in pre-eclampsia versus controls.

3.4 Inter-relationships among markers

Pearson's correlation coefficients for the biochemical and clinical variables are presented in Table 4. Serum MDA correlated strongly and positively with systolic ($r = 0.79$; $p < 0.001$) and diastolic blood pressure ($r = 0.81$; $p < 0.001$), indicating that lipid peroxidation tracks with disease severity. MDA correlated inversely with nitrite ($r = -0.71$), TAS ($r = -0.67$), SOD ($r = -0.67$) and total thiol ($r = -0.70$) (all $p < 0.001$), reinforcing the inverse relationship between oxidative damage and antioxidant reserve (Figure 5).

Table 4. Pearson correlation matrix among oxidative-stress markers, antioxidant indices and blood pressure (n = 60).

	MDA	Nitrite	TAS	SOD	Thiol	Uric acid	SBP	DBP
MDA	1.00***	-0.71***	-0.67***	-0.67***	-0.70***	0.61***	0.79***	0.81***
Nitrite	-0.71***	1.00***	0.62***	0.59***	0.69***	-0.59***	-0.74***	-0.71***
TAS	-0.67***	0.62***	1.00***	0.65***	0.60***	-0.58***	-0.84***	-0.79***
SOD	-0.67***	0.59***	0.65***	1.00***	0.72***	-0.57***	-0.73***	-0.80***
Thiol	-0.70***	0.69***	0.60***	0.72***	1.00***	-0.63***	-0.76***	-0.75***
Uric acid	0.61***	-0.59***	-0.58***	-0.57***	-0.63***	1.00***	0.73***	0.61***
SBP	0.79***	-0.74***	-0.84***	-0.73***	-0.76***	0.73***	1.00***	0.85***
DBP	0.81***	-0.71***	-0.79***	-0.80***	-0.75***	0.61***	0.85***	1.00***

**p < 0.05, **p < 0.01, ***p < 0.001 (two-tailed Pearson).*

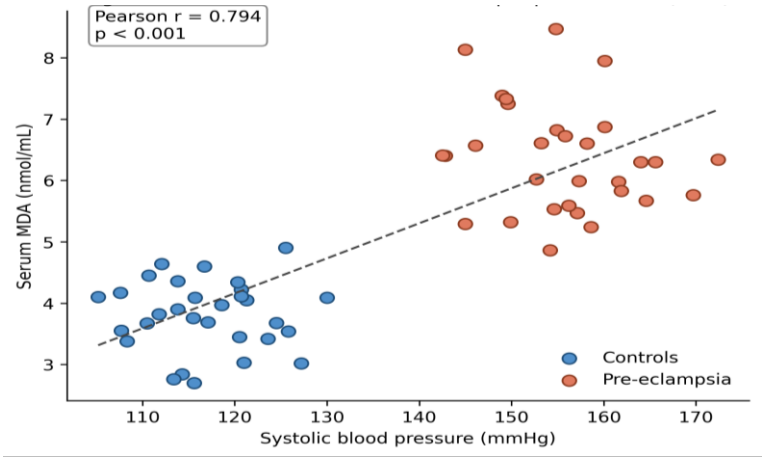

Figure 5. Scatter plot showing the positive correlation between systolic blood pressure and serum MDA in the combined cohort (n = 60).

3.5 Diagnostic performance of oxidative-stress markers

To assess the potential clinical utility of each biomarker, ROC analysis was performed (Figure 4). The areas under the curve were: MDA 0.99 (95 % CI 0.97–1.00), TAS 0.97 (95 % CI 0.93–1.00), SOD 0.96 (95 % CI 0.91–1.00), thiol 0.95 (95 % CI 0.89–1.00), nitrite 0.92 (95 % CI 0.85–0.99) and uric acid 0.91 (95 % CI 0.83–0.99). All markers displayed strong discriminatory ability, with MDA showing the highest individual AUC.

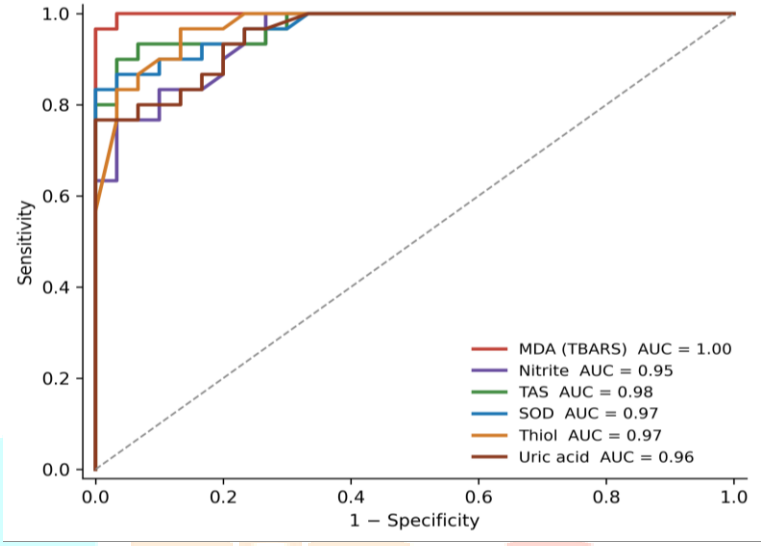

Figure 4. Receiver operating characteristic (ROC) curves for serum biomarkers discriminating pre-eclamptic women from normotensive controls.

4. Discussion

4.1 Marked lipid peroxidation in pre-eclampsia

The principal finding of the present study is the marked elevation of serum MDA (TBARS) in pre-eclamptic women, with a mean concentration approximately 70 % higher than in normotensive controls (6.4 vs. 3.8 nmol/mL) and a very large effect size (d = 3.42). This observation is consistent with the two-stage model, in which placental ischaemia–reperfusion drives an overproduction of superoxide, hydrogen peroxide and hydroxyl radicals that overwhelm local and systemic antioxidant defences and attack the polyunsaturated fatty acids of cellular membranes (Roberts & Poston, 2003; Burton & Jauniaux, 2019). MDA, the carbonyl end-product of lipid peroxidation, is itself biologically active and amplifies oxidative injury by forming covalent adducts with proteins and DNA (Weismann & Binder, 2017). The magnitude of the case–control difference closely matches the meta-analytic estimate of Chiarello et al. (2020) and the prospective findings of Baban et al. (2022) and Afrose et al. (2022), and is at the upper end of previously published effect sizes likely reflecting our strict exclusion of confounders and tightly matched controls. The strong positive correlation of MDA with both systolic (r = 0.79) and diastolic (r = 0.81) blood pressure indicates that lipid peroxidation is quantitatively related to disease severity, aligning with McCarthy et al. (2021) using the more specific isoprostane 8-iso-$PGF_{2}\alpha$.

4.2 Reduced nitric-oxide bioavailability

The 30 % reduction in serum nitrite observed in pre-eclamptic women (38.7 vs. 54.9 µmol/L; $p < 0.001$; $d = -2.28$) corroborates a substantial body of literature documenting impaired nitric-oxide signalling in this disorder (Sandrim et al., 2020; Grzeszczak et al., 2023). Two complementary mechanisms contribute to this decline: decreased endothelial eNOS activity due to caveolin-1 up-regulation, eNOS uncoupling in the face of BH_4 depletion, and ADMA accumulation; and accelerated consumption of •NO by superoxide in the peroxynitrite-forming reaction ($•NO + O_2•^- \rightarrow ONOO^-$). The latter is particularly relevant because peroxynitrite is itself a potent lipid-peroxidant, providing a mechanistic bridge between the observed

reduction in nitrite and the simultaneous elevation in MDA, and is consistent with the strong inverse correlation between nitrite and MDA ($r = -0.71$) observed here.

4.3 Depletion of the enzymatic and non-enzymatic antioxidant reserve

The pre-eclamptic women displayed a profound reduction in every enzymatic and non-enzymatic antioxidant measured. Total antioxidant status decreased by approximately one-third (1.0 vs. 1.5 mmol/L Trolox equivalent; $d = -2.89$), SOD activity by 41 % (2.8 vs. 4.7 U/mL; $d = -2.68$), and total thiol by 38 % (0.3 vs. 0.5 µmol/L; $d = -2.63$). These findings are congruent with the comprehensive meta-analyses of Afrose et al. (2022) and Ibrahim et al. (2024), which reported pooled standardised mean differences of similar magnitude. The thiol pool, which comprises protein –SH groups (notably albumin) and low-molecular-weight glutathione, is a major redox buffer of the extracellular compartment, and its reduction signals a generalised pro-oxidant shift of plasma redox potential (Hu, 1994) consistent with the decreased glutathione/oxidised-glutathione ratio reported in placental tissue (Raijmakers et al., 2003). The accompanying fall in SOD activity is particularly important because SOD catalyses the dismutation of superoxide and thereby limits both lipid peroxidation and peroxynitrite formation; its loss amplifies the very processes that gave rise to it, perpetuating a self-reinforcing cycle of oxidative injury.

4.4 Hyperuricaemia as an integrative index

Serum uric acid was significantly higher in the pre-eclamptic women (6.4 vs. 4.5 mg/dL; $p < 0.001$; $d = 2.36$), in line with its well-established role as a sensitive biochemical marker of disease severity (Bellos et al., 2020). Hyperuricaemia reflects both impaired renal clearance (glomerular endotheliosis and reduced tubular secretion) and increased production by xanthine oxidase, an enzyme that simultaneously generates superoxide (Many & Hubel, 2004). The strong positive correlations of uric acid with both MDA ($r = 0.61$) and blood pressure ($r = 0.73$ with SBP) and its negative correlations with the antioxidant indices confirm that hyperuricaemia is closely integrated with the wider oxidative milieu.

4.5 Integrated mechanism and pathophysiology

Figure 1 integrates the mechanistic events discussed above. Defective trophoblastic invasion (Stage 1) produces a high-resistance uteroplacental circulation prone to ischaemia–reperfusion injury. The resulting placental and endothelial mitochondrial dysfunction, combined with activated neutrophils and macrophages, generates excess ROS/RNS that exceeds the buffering capacity of the maternal antioxidant defence. Polyunsaturated membrane lipids are attacked in a chain reaction that generates MDA and 4-HNE, perpetuating cellular damage and consuming thiols and antioxidant enzymes. Simultaneously, superoxide inactivates •NO, producing peroxynitrite and reducing NO bioavailability (low nitrite), which removes a key vasodilator and further raises blood pressure. The clinical syndrome (Stage 2) emerges as the integrated consequence of these biochemical events, supported in our cohort by the internally consistent correlations in Table 4 and the very large AUCs in Figure 4.

4.6 Diagnostic, prognostic and therapeutic implications

From a clinical-translational standpoint, the ROC analysis indicated that MDA (AUC = 0.99) and TAS (AUC = 0.97) had the highest discriminatory ability for pre-eclampsia. These values are higher than those typically reported by single-centre studies (Baban et al., 2022) and likely reflect the homogeneity of our case definition and standardised pre-analytical conditions. A panel comprising MDA plus one or two antioxidant indices could therefore serve as an adjunct to clinical surveillance, particularly in settings where angiogenic biomarkers (sFlt-1/PlGF) are unavailable. Whether the same biomarkers can predict pre-eclampsia before clinical onset remains an open question; integration with maternal characteristics, uterine-artery Doppler and angiogenic markers in multivariate models is an active area of investigation.

The biochemical evidence of oxidative stress has motivated trials of antioxidant supplementation, principally with vitamins C and E. The WHO-sponsored VIP trial and the CAPPS studies demonstrated no reduction in pre-eclampsia incidence and, in the VIP trial, an unexpected increase in low-birth-weight

infants (Di Fabrizio et al., 2022; Poston & Rai, 2019). Broad, untargeted supplementation cannot recapitulate the finely tuned activity of endogenous defence and may disrupt redox-sensitive signalling required for normal placental development. Newer strategies under investigation include selective enhancement of mitochondrial SOD (MitoQ, MitoTEMPO), BH_4 supplementation to recouple eNOS, and statin-mediated up-regulation of HO-1 (Afrose et al., 2025); biochemical profiling of the type employed in this study will be essential to identify sub-groups of women who may benefit from such targeted interventions.

4.7 Strengths and limitations

Strengths of the present study include the strict application of current diagnostic criteria, age- and gestational-age-matched controls drawn from the same antenatal population, the simultaneous assessment of multiple oxidative and antioxidant indices in a single laboratory under uniform pre-analytical conditions, and the calculation of effect sizes and ROC-AUCs alongside conventional hypothesis testing. Limitations include the cross-sectional design, which precludes causal inference; the absence of dietary-antioxidant assessment; the lack of complementary placental-tissue analyses; the modest sample size, which limits subgroup analyses (e.g., early- vs. late-onset pre-eclampsia); and the use of TBARS, which, although the most widely employed assay, is less specific than mass-spectrometric 8-iso-$PGF_2\alpha$. Larger, multicentre prospective studies that incorporate state-of-the-art lipidomics are needed to translate the present observations into clinically actionable prediction tools.

5. CONCLUSIONS

In this hospital-based case–control study, pre-eclamptic women demonstrated a robust shift in the maternal oxidative balance towards lipid peroxidation, as evidenced by a 70 % elevation of serum MDA, a 30 % reduction in serum nitrite, depletion of the integrated (TAS) and enzymatic (SOD) antioxidant defences, a 38 % fall in plasma thiols and a 40 % rise in uric acid. The magnitude of lipid peroxidation correlated strongly with the severity of the hypertensive disease, and each marker showed high discriminatory ability in ROC analysis, with MDA achieving an AUC of 0.99. These findings support the concept that an imbalance between reactive oxygen and nitrogen species and the antioxidant defence is a central biochemical event in the pathophysiology of pre-eclampsia, and provide a mechanistic rationale for including oxidative-stress biomarkers in the biochemical evaluation of at-risk pregnancies. Future research should integrate these markers into multi-analyte prediction algorithms and evaluate targeted antioxidant strategies in well-phenotyped sub-groups of patients.

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Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Data availability

The de-identified data that support the findings of this study are available from the corresponding author upon reasonable request.

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