

Association between Maternal Serum Vitamin D, Oxidative Stress, Angiogenic, and Inflammatory Biomarkers and the Risk of Preeclampsia in Pregnant Women

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ABSTRACT

Preeclampsia is a hypertensive pregnancy-associated multisystem disorder that is characterized by massive maternal and fetal morbidity. There is an emerging evidence that vitamin D deficiency, oxidative stress, inflammation, and angiogenic imbalance play a role in its pathophysiology. A case control study involving 60 pregnant women in Iraq was done; the subjects were recruited from Al-Basrah teaching hospital for gynaecology and maternity with 30 having preeclampsia and 30 healthy normotensive controls. Women with preeclampsia had markedly lower vitamin D levels than controls (14.06 ± 4.50 vs. 31.54 ± 7.20 ng/mL, $p < 0.001$). They had also elevated oxidative stress as indicated by elevated MDA (5.88 ± 0.93 vs. 2.76 ± 0.82), and decreased antioxidant defenses as measured by less SOD and TAC (all $p < 0.001$). There was a significant increase in inflammatory markers, especially CRP and IL-6 and angiogenic imbalance was observed in the form of increased sFlt-1 and decreased PlGF (all $p < 0.001$). Moreover, the preeclampsia group had lower nitric oxide and higher uric acid (both $p < 0.001$). Preeclampsia was associated with a distinct biochemical pattern involving vitamin D deficiency, oxidative stress, inflammation, angiogenic imbalance, and endothelial dysfunction, supporting the value of combined biomarker assessment in disease evaluation.

Keywords: Preeclampsia; Vitamin D; Oxidative stress; Inflammatory biomarkers; Angiogenic factors; sFlt-1; PlGF; Pregnancy.

INTRODUCTION

Preeclampsia is a complicated and poorly understood pathophysiology. A multifactorial hypothesis involving aberrant placentation, defective trophoblast invasion, placental hypoperfusion, endothelial dysfunction, immunological dysregulation, oxidative stress, and angiogenic imbalance interacting in a self-amplifying way has been proposed^{1,2}. It also remains a major cause of maternal and fetal morbidity worldwide³⁻⁵. This framework is supported by current research⁶⁻⁸. It is believed that placental stress causes the release of pro-inflammatory mediators, antiangiogenic factors, and reactive oxygen species into the mother's bloodstream, which leads to systemic endothelial damage and clinical illness⁹⁻¹¹. As a result, rather than being a single-pathway obstetric issue, preeclampsia is becoming recognized as a condition of interconnected biological pathways^{8,10,12}.

Among the possible routes associated with preeclampsia, vitamin D has drawn increased interest due to its immunomodulatory, anti-inflammatory, and vasculoprotective qualities^{13,14}. Preeclampsia and other adverse pregnancy outcomes have been associated with low maternal vitamin D levels¹⁵⁻¹⁷. Endothelium homeostasis, placental development, and trophoblast invasion are all related to vitamin D. Recent systematic reviews and meta-analyses indicate that lower maternal vitamin D concentrations during early and mid-pregnancy are

associated with a higher risk of later preeclampsia, albeit the strength of the association differs among populations and study designs^{13,16,18}.

There is growing evidence that the disease's molecular expression is mostly dependent on oxidative stress and inflammation^{8,19,20}. While total antioxidant capacity and superoxide dismutase are utilized to understand antioxidant defense, malondialdehyde is a sign of lipid peroxidation^{21,22}. Increased maternal immunological activation is indicated by the presence of inflammatory markers such as C-reactive protein, interleukin-6, and tumor necrosis factor-alpha^{23,24}. Angiogenic dysregulation is also a central component of the disease process²⁵⁻²⁷.

Gap in knowledge and novelty

There have been a number of studies examining individual biomarkers in preeclampsia, but the majority have only included studies on one biological pathway (e.g. vitamin D deficiency, oxidative stress, inflammation, and angiogenic imbalance). To date, the complex interactions between these pathways are not well-defined in a single clinical population^{13,20,25}. The present study was initiated to fill this void and to assess simultaneously the vitamin D status, oxidative stress, antioxidant defense, inflammatory activation, angiogenic regulation, endothelial function and metabolic disturbance, assessed by the use of multiple biomarkers which represent various interconnected biological

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domains. The study was not designed to consider these markers alone, but rather to investigate the interplay and interaction between these pathways in the biochemical milieu of preeclampsia^{14,21,24}.

This novelty of the work goes beyond merely quantifying multiple biomarkers. The scientific value is its ability to offer a comprehensive mechanistic link between vitamin D deficiency and oxidative stress, inflammatory activation, angiogenic dysregulation and endothelial dysfunction. These pathways are studied in the same cohort, allowing for a more comprehensive picture of the biological network involved in preeclampsia and identifying interactions that would not necessarily be obvious if the biomarkers were studied individually.

Problem statement

In spite of significant improvements in obstetric care, the detection of preeclampsia on the basis of clinical manifestations remains one of the key issues in practice, with many symptoms being revealed only when the pathological process is already developed. This constrains early biochemical risk stratification and the possibility of appreciating the mechanistic heterogeneity of the disorder at the maternal serum level. Although angiogenic biomarker testing has entered clinical guidance in selected settings, it does not reflect oxidative stress, inflammatory burden, antioxidant depletion, or micronutrient-related perturbations that may also contribute to the development and severity of the disease^{1,2,27}. Hence, research is required to assess the effectiveness of a larger set of serum biomarkers to more effectively differentiate preeclamptic and normotensive pregnancies and to elucidate the interconnections between these interrelated pathways^{8,13,25}.

Aim and hypothesis

The primary aim of this study was exploratory and mechanistic. Specifically, the study sought to investigate the association between maternal serum vitamin D levels and a multidimensional panel of oxidative stress, inflammatory, angiogenic, endothelial, and metabolic biomarkers in women with preeclampsia compared with normotensive pregnant controls.

This study was not conducted as a diagnostic validation study or as a predictive modeling study. It had rather, try to identify biological relationships between these pathways, and test if vitamin D deficiency relates to a wider biochemical dysfunction of preeclampsia.

We hypothesized that women who developed preeclampsia would have lower levels of vitamin D levels and greater concentrations of oxidative stress, the inflammatory markers, angiogenic molecules, deficient endothelial function and elevated serum uric acid. Moreover, we hypothesized that vitamin D levels would be related to these pathway biomarkers, which also supports the idea of a cross-interacting pathophysiological network involved in human disorders.

Conceptual Framework

The underlying concept of the present study is that suboptimal levels of vitamin D can be a catalyst to a chain of multiple and cross-connected biological dysfunctions associated with preeclampsia. Lower levels of vitamin D have been linked to diminished antioxidant activities and higher levels of oxidative stress that can activate inflammatory signaling pathways and endothelial damage. Hypoxia and endothelial dysfunction may then induce an excess of production of anti-angiogenic factors like sFlt-1, or a decrease of production of pro-angiogenic factors as PlGF. These alterations may collectively contribute to the maternal vascular dysfunction characteristic of preeclampsia. By simultaneously

assessing biomarkers from each of these biological domains, the present study aimed to evaluate this integrated mechanistic model within a single clinical cohort.

METHODOLOGY

Study design and setting: This hospital-based case-control study was carried out between January 2025 and January 2026 at Al-Basrah teaching hospital for gynaecology and maternity in Basrah, Iraq. Maternal serum vitamin D, oxidative stress, inflammatory, angiogenic, and specific biochemical indicators were compared between preeclamptic women and healthy, normotensive pregnant controls. Because it is suitable for assessing relationships between several biochemical exposures and a reasonably well-defined clinical outcome within a practical time and financial constraints, the case-control method was chosen.

In order to reduce the selection bias, cases and controls were selected within the same hospital setting and at the same study period. Cases were recruited in sequence as they satisfied the diagnostic criteria of preeclampsia and controls were recruited among pregnant women who attended regular antenatal follow-up and were assured to be normotensive and free of any clinical manifestations of hypertensive pregnancy diseases. The sampling methodology was aimed at enhancing comparability of groups in terms of referral patterns, geographic catchment and accessibility to obstetric care.

Study population: The study included a sample of 60 pregnant women in two groups. 20 puerpera presented with moderate preeclampsia and another 20 normotensive puerpera of singletons formed the control group. Thirty puerpera who presented with moderate preeclampsia comprised the case group and thirty healthy normotensive puerpera with singleton pregnancy made up the control group. The participants had all been recruited from the time of the onset of clinical signs and symptoms of preeclampsia, at week 20 of gestation. All women were clinically evaluated on the day of enrollment, and blood samples were taken for biochemical evaluation.

As to eliminating excess variables from the study, due to extreme maternal age only women between 18–40 years of age have been chosen. Because the authors recognized that multifetal pregnancies might introduce some confounding factors due to their strong association with abnormalities of placentation, angiogenic malady, and high-risk for hypertensive problems, they limited their study to singleton pregnancies.

Eligibility criteria: Participants were recruited based on eligibility criteria. Inclusion criteria were: pregnant women aged between 18 and 40 years old, gestational age of 20 weeks and above and single pregnancy. In case group, the subjects were required to meet the predetermined diagnostic criteria of preeclampsia. In the case of the control group, participants were required to be normotensive, without proteinuria, and not suspected of hypertensive pregnancy disorders.

Women were not eligible in case they had history of chronic hypertension, diabetes mellitus, renal disease, autoimmune disorders or they were currently taking vitamin D supplements in pregnancy. This was done to exclude the effects that each of these conditions can affect vitamin D metabolism, oxidative stress balance, inflammatory activity, endothelial activity or the angiogenic signaling, misleading the observed relationship between the biomarker panel and preeclampsia. Women who had incomplete clinical records, hemolysis of serum sample or insufficient sample volume to be analyzed in the laboratory were also excluded in the final analysis.

Table 1. Eligibility criteria and study groups

Component	Cases	Controls
Study design	Case-control	Case-control
Number of participants	30	30
Pregnancy status	Pregnant women ≥ 20 weeks	Pregnant women ≥ 20 weeks
Diagnostic status	Preeclampsia	Normotensive pregnancy
Age range	18–40 years	18–40 years
Pregnancy type	Singleton	Singleton
Key exclusions	"Chronic hypertension, diabetes mellitus, renal disease, autoimmune disorders, vitamin D supplementation"	Same exclusions applied

Diagnostic Criteria for Preeclampsia: The diagnosis of preeclampsia was established according to the recommendations of the American College of Obstetricians and Gynecologists (ACOG Practice Bulletin No. 222) and the International Society for the Study of Hypertension in Pregnancy (ISSHP). Preeclampsia was defined as new-onset hypertension occurring after 20 weeks of gestation, characterized by a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg on at least two measurements obtained at least four hours apart in a previously normotensive woman.

Proteinuria was defined as the presence of ≥ 300 mg of protein in a 24-hour urine collection, a urine protein-to-creatinine ratio ≥ 0.3 mg/mg, or a urine dipstick reading of at least 1+ when quantitative measurements were not available. Clinical diagnoses and laboratory findings were verified through review of hospital medical records and obstetric assessments performed by specialist physicians.

Both severe and non-severe preeclampsia cases were eligible for inclusion. Severe disease was defined according to established clinical criteria, including severe hypertension (systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg), significant end-organ involvement, or severe laboratory abnormalities. However, because of the limited sample size, subgroup analyses according to disease severity were not performed.

For the purposes of this exploratory study, early-onset (< 34 weeks of gestation) and late-onset (≥ 34 weeks of gestation) preeclampsia were not analyzed separately. All eligible cases were included in a single preeclampsia group to maximize statistical power for biomarker comparisons (Table 1).

Sample Size: The sample size was determined during the study planning phase using maternal serum 25-hydroxyvitamin D concentration as the primary biomarker of interest. Previous studies evaluating vitamin D levels in women with and without preeclampsia reported substantial between-group differences, with expected mean differences ranging from approximately 10 to 15 ng/mL and standard deviations between 10 and 12 ng/mL.

Sample size estimation was carried out with the aid of the G*Power Software Version 3.1.9.7 (Heinrich Heine University, Düsseldorf, Germany). Sample sizes were estimated to be around 58 participants to achieve 80% confidence, moderate-to-large effect size (Cohen's $d \approx 0.75$) and a conservative two-sided alpha level of 0.05 for independent group comparisons. A final sample of 60 patients with preeclampsia and 60 normotensive controls was ultimately recruited, in order to have balanced groups and allow recruitment.

The focus of the study was to assess differences in levels of the biomarkers between the groups, and to examine exploratory relationships among the biomarker variables. It was not specifically designed to accommodate large scale multivariable regression

modeling, or sub-group analyses of amount of disease severity, onset phenotype. Therefore, results of the secondary analyses must be interpreted with proper caution.

Data collection and study variables: All participants filled out a standard data collection instrument. Throughout the recruitment process, maternal age, gestational age, gravidity, parity, body mass index, systolic blood pressure, and diastolic blood pressure were collected, clinical diagnosis, and relevant medical and obstetric history were collected. Information regarding the severity of a clinical disease and the presence of proteinuria was also incorporated into the patient's clinical record, if available. BP was obtained from standardized obstetric records and was re-checked by the attending obstetric team and re-matched with standardized patient records for proteinuria detection and classification of the disease.

Blood sample collection and processing: Aseptic venous blood collection of 5-mL was performed on each participant by trained personnel. The samples were left to clot, and after that centrifuged to get serum. The serum was separated and aliquoted in labeled microtubes and stored at -20 C until biochemical analysis. To preserve analytical consistency, repeated freeze-thaw cycles were avoided as much as possible. All the specimens were coded before subjecting them to laboratory analysis to minimize the effect of an observer and laboratory measurements of both groups were done using the same operating procedure.

Biomarker analyses should, where possible, be done in batches to minimize inter-assay variability. Each assay kit should also report the manufacturer, catalog number, lot number, analytical sensitivity, detection range and intra-assay/inter-assay coefficients of variation, as such information is frequently demanded by reviewers when laboratory-based clinical studies are conducted.

Biochemical analysis: Established laboratory techniques were used to assess serum biomarkers. Enzyme-linked immunosorbent assay (ELISA) was used to measure serum levels of 25-hydroxyvitamin D, IL-6, TNF- α , sFlt-1, and PlGF. The thiobarbituric acid reactive substances method was used to measure MDA, the spectrophotometric assay for SOD, the colorimetric assay for TAC, the immunoturbidimetric method for CRP, the Griess reaction for nitric oxide, and the enzymatic colorimetric method for blood uric acid. Every procedure was performed in accordance with established laboratory operating procedures and the manufacturers' instructions.

Serum 25-hydroxyvitamin D, IL-6, TNF- α , sFlt-1, and PlGF concentrations were measured using commercially available ELISA kits (Elabscience, USA). The kits included 25-OH Vitamin D (Cat. No. E-EL-0015), IL-6 (Cat. No. E-EL-H6156), TNF- α (Cat. No. E-EL-H0109), sFlt-1 (Cat. No. E-EL-H2320), and PlGF (Cat. No. E-EL-H5458). All assays were performed according to the manufacturers' instructions. Calibration curves were generated using

supplied standards, and samples were analyzed in duplicate. Laboratory personnel were blinded to participants' clinical status during biomarker measurements (Table 2).

Statistical Analysis: Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of histograms and Q–Q plots. Normally distributed variables were expressed as mean $\pm$ standard deviation (SD), whereas non-normally distributed variables were presented as median and interquartile range (IQR).

For comparisons between the preeclampsia and control groups, independent-samples t-tests were used for normally distributed variables, while the Mann–Whitney U test was used for variables that did not meet normality assumptions. Categorical variables, when applicable, were compared using the chi-square test or Fisher's exact test. Correlations between serum vitamin D concentrations and other biomarkers were evaluated using Spearman's rank correlation coefficient because several variables demonstrated non-normal distributions.

A two-sided p-value <0.05 was considered statistically significant. Given the exploratory nature of the study and the relatively limited sample size, adjustment for multiple comparisons was not performed. Results should therefore be interpreted as exploratory and hypothesis-generating. Data completeness was verified before statistical analysis. No missing biomarker measurements were identified among the enrolled participants; therefore, no imputation procedures were required.

Table 2. Biomarker panel and analytical methods

Biomarker category	Biomarker	Analytical method
Vitamin D status	25-OH vitamin D	ELISA
Oxidative stress	MDA	TBARS method
Antioxidant defense	SOD	Spectrophotometric assay
Antioxidant defense	TAC	Colorimetric assay
Inflammation	CRP	Immunoturbidimetric method
Inflammation	IL-6	ELISA
Inflammation	TNF- α	ELISA
Angiogenic markers	sFlt-1	ELISA
Angiogenic markers	PlGF	ELISA
Endothelial/oxidative pathway	Nitric oxide	Griess reaction
Additional biochemical marker	Uric acid	Enzymatic colorimetric method

Table 3. Baseline demographic characteristics of the study population

Variable	Preeclampsia (n = 30)	Control (n = 30)	p-value
Age (years)	27.00 (24.00–30.75)	27.00 (23.00–30.00)	0.756
Gestational age (weeks)	27.50 (25.00–32.00)	27.50 (22.25–30.00)	0.509
BMI (kg/m ²)	28.67 $\pm$ 4.31	28.35 $\pm$ 3.72	0.760

Presented as median (interquartile range). Other values are mean $\pm$ standard deviation.

Table 4. Comparison of serum 25-hydroxyvitamin D concentrations between women with preeclampsia and controls

Variable	Preeclampsia group	Control group	p-value
Serum 25-hydroxyvitamin D (ng/mL)	14.06 $\pm$ 4.50	31.54 $\pm$ 7.20	<0.001

Table 5. Comparison of oxidative stress and antioxidant markers between women with preeclampsia and controls

Variable	Preeclampsia group	Control group	p-value
"Malondialdehyde (MDA)"	5.88 $\pm$ 0.93	2.76 $\pm$ 0.82	<0.001
Superoxide dismutase (SOD)	80.13 $\pm$ 9.92	118.82 $\pm$ 17.23	<0.001
Total antioxidant capacity (TAC)"	0.80 $\pm$ 0.18	1.48 $\pm$ 0.28	<0.001

RESULTS

Baseline characteristics of the study population

The investigation comprised 60 pregnant women in total, 30 of whom had preeclampsia and 30 of whom were normotensive pregnant controls. Table 3 displays the dataset's baseline demographic characteristics. Maternal age, gestational age, and body mass index did not differ statistically significantly between the two groups. Both groups' median age was 27.00 years, and their median gestational age was 27.50 weeks. Additionally, the preeclampsia and control groups' mean BMIs were similar.

Comparison of vitamin D levels between groups

See Table 4 and Figure 1.

Comparison of oxidative stress biomarkers

See Table 5 and Figure 2

Comparison of inflammatory biomarkers

See Table 6 and Figure 3

Additional biochemical markers

The preeclampsia group's nitric oxide levels were substantially lower than those of the controls. Preeclamptic women had a mean nitric oxide of 20.54 ± 4.60 , while controls had a mean of 34.77 ± 7.66 ($p < 0.001$). The preeclampsia group had considerably higher serum uric acid levels (7.28 ± 0.78) compared to the control group (3.90 ± 0.79 , $p < 0.001$) Table 7.

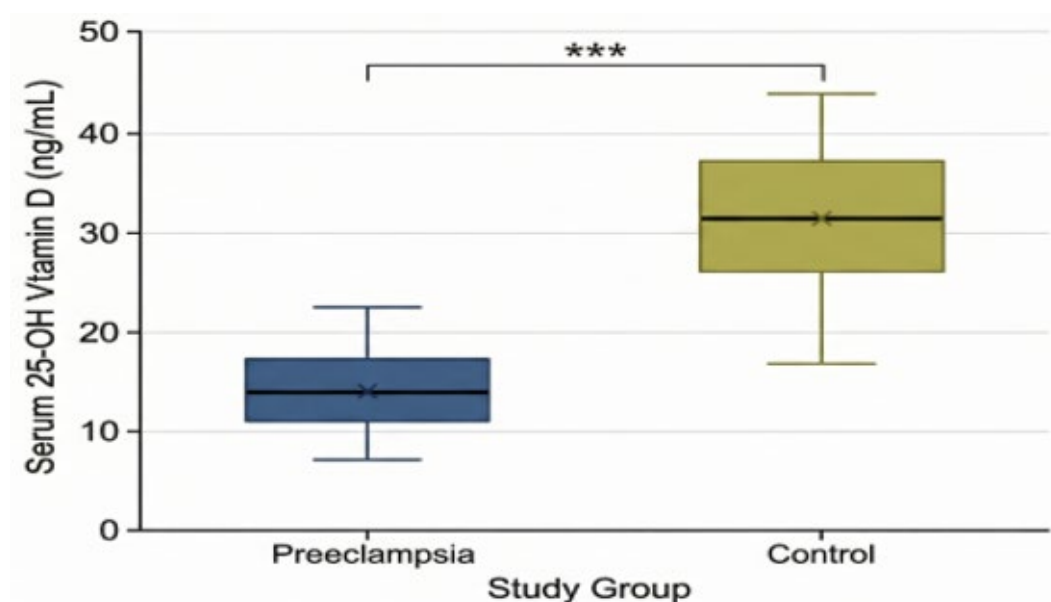


Figure 1. Boxplot comparison of maternal serum 25-OH vitamin D levels between study groups

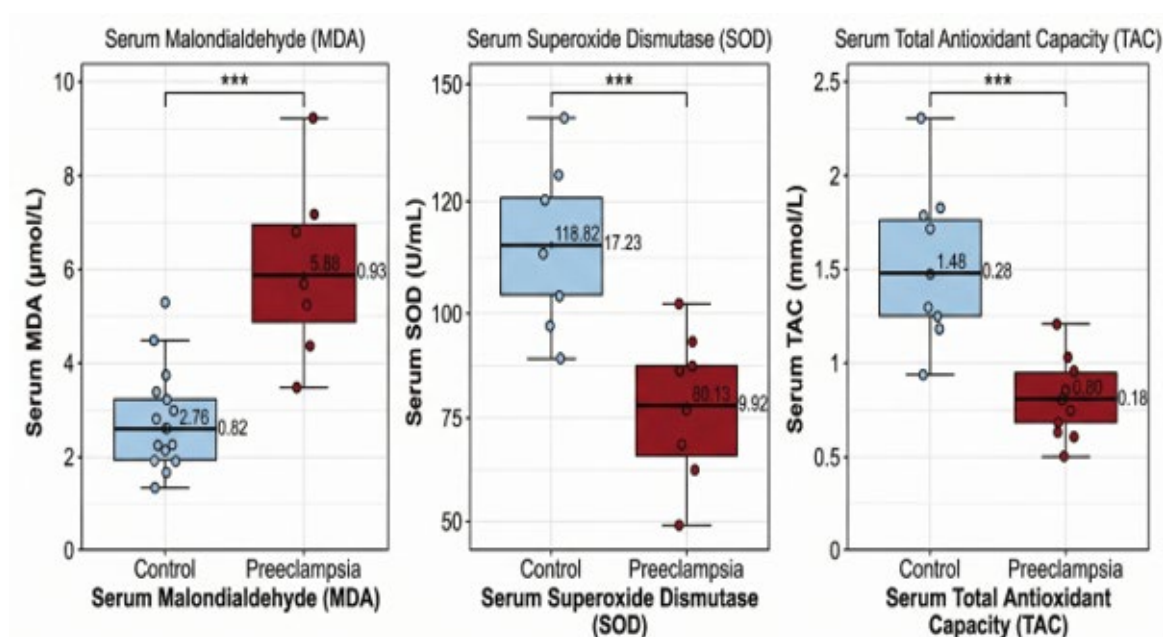


Figure 2. Oxidative stress and antioxidant biomarkers in women with preeclampsia and controls

Table 6. Comparison of inflammatory markers between women with preeclampsia and controls

Variable	Preeclampsia group	Control group	p-value
"C-reactive protein (CRP)	9.72 ± 3.07	3.45 ± 1.46	< 0.001
Interleukin-6 (IL-6)	13.12 ± 3.79	4.96 ± 1.95	< 0.001
Tumor necrosis factor-alpha (TNF-α), median (IQR)"	15.97 (10.73–17.58)	7.58 (6.09–8.53)	< 0.001

Table 7. Comparison of angiogenic biomarkers between women with preeclampsia and normotensive controls

Biomarker	Preeclampsia (n = 30)	Control (n = 30)	p-value
sFlt-1	7048.04 ± 1037.59	3058.06 ± 895.73	<0.001
PlGF	95.70 ± 34.76	244.10 ± 62.06	<0.001
sFlt-1/PlGF ratio†	75.37 (61.13–97.86)	11.65 (9.41–15.12)	<0.001
Nitric oxide	20.54 ± 4.60	34.77 ± 7.66	<0.001
Uric acid	7.28 ± 0.78	3.90 ± 0.79	<0.001

Presented as median (interquartile range). Other values are mean ± standard deviation.

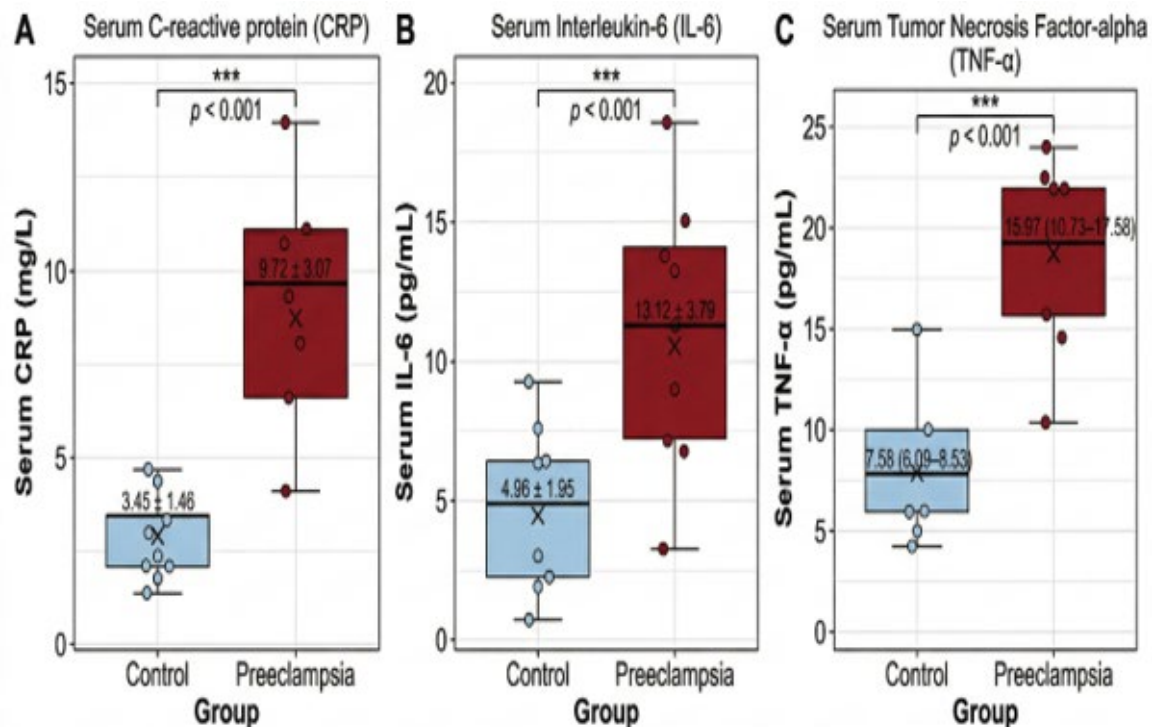


Figure 3. Inflammatory biomarker profile in preeclampsia and normotensive pregnancy

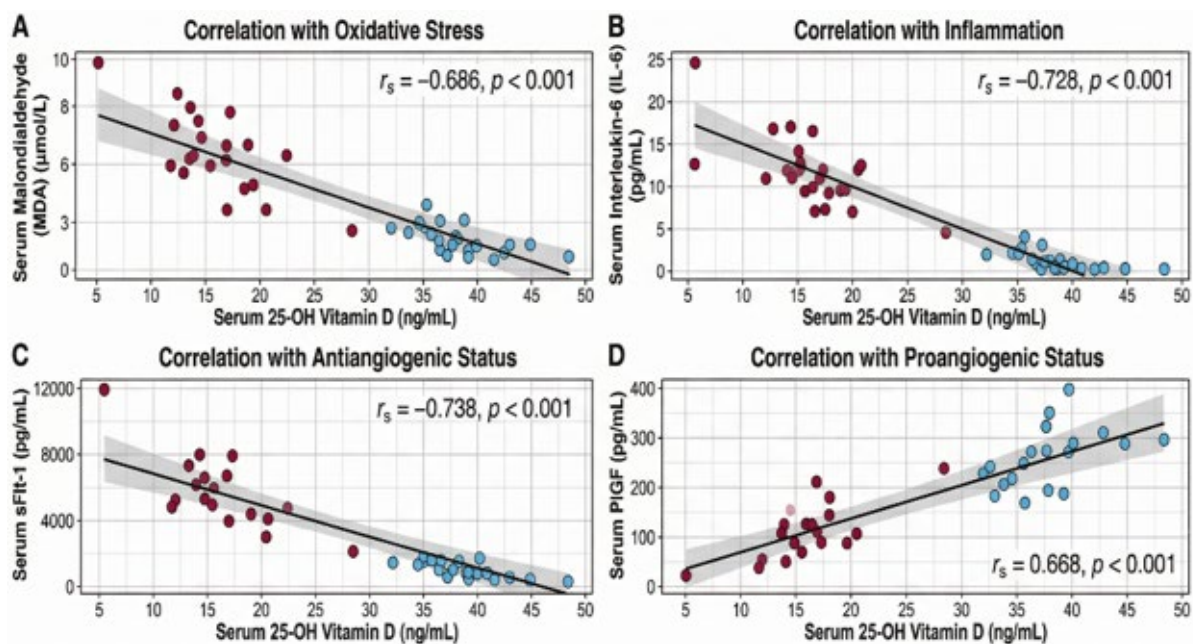


Figure 4. Correlation between maternal serum vitamin D and selected biomarkers

Table 8. Correlation of serum vitamin D with other biochemical biomarkers in the pooled sample (n = 60)

Biomarker	Spearman's rho	p-value
MDA	-0.686	<0.001
SOD	0.690	<0.001
TAC	0.676	<0.001
CRP	-0.717	<0.001
IL-6	-0.728	<0.001
TNF-α	-0.600	<0.001
sFlt-1	-0.738	<0.001
PlGF	0.668	<0.001
Nitric oxide	0.688	<0.001
Uric acid	-0.754	<0.001

Correlation analysis

Correlation analysis was performed between serum vitamin D and the remaining biochemical markers across the pooled study sample. Serum vitamin D showed significant **negative correlations** with MDA, CRP, IL-6, TNF- α , sFlt-1, and uric acid, and significant **positive correlations** with SOD, TAC, PIGF, and nitric oxide. The strongest inverse correlation was observed between vitamin D and uric acid ($\rho = -0.754$, $p < 0.001$), while the strongest positive correlation was observed between vitamin D and SOD ($\rho = 0.690$, $p < 0.001$) (Table 8) (Figure 4).

DISCUSSION

Interpretation of the results

The current evidence confirms that preeclampsia is a biologically unified disease that is associated with the dysregulation of micronutrients, oxidative damage, inflammatory activity, angiogenic imbalance, and endothelial dysfunction^{6,8,12}. Serum levels of vitamin D, SOD, TAC, PIGF, and nitric oxide among women with preeclampsia were significantly lower in our cohort, along with increased MDA, CRP, IL-6, TNF- α , sFlt-1/PIGF ratio, and uric acid. The trend and consistency of these modifications imply that preeclampsia in this group was marked by a transition to a pro-oxidant, pro-inflammatory, and antiangiogenic state. This general pattern is biologically plausible, since abnormal placentation and placental hypoxia are believed to induce oxidative stress, antiangiogenic signaling, and systemic endothelial injury, which further feed back on each other during disease progression⁸⁻¹⁰.

This interpretation is confirmed by the correlation analysis. Vitamin D levels were found to be inversely associated with MDA, CRP, IL-6, TNF- α , sFlt-1 and uric acid and positively to SOD, TAC, PIGF and nitric oxide. Such relationships suggest that alternative markers of a broader biochemical profile of inadequate antioxidant defense, increased level of inflammatory mediators, impaired endothelial vasodilatory function, and disturbed placental angiogenesis may be related to poor vitamin D status during a preeclamptic disorder^{13,22,23}. The relationships between these biological pathways were confirmed by further correlation analyses. There were prominent relationships found between vitamin D levels and oxidative stress, inflammatory, angiogenic regulation, endothelial function and metabolic disturbances markers. Based on these results, the authors conclude that vitamin D levels could be associated with overall biochemical profile of preeclampsia. But due to the observational case-control study design, causal relationships cannot be inferred.

All of these together indicate that there is no single opt bet to this disorder. Rather, there is evidence suggesting an interaction of oxidative stress, inflammatory factors, angiogenic mechanisms and endothelial dysfunction in the clinical picture of Preeclampsia, and that Vitamin D deficiency might provide a clue or factor in the unfavorable biochemical scenario^{8,22,25}.

A key finding of this present study is the discovery that these biological abnormalities are not independent. Although most previous studies have focused on angiogenic biomarkers there is now evidence to suggest an angiogenic imbalance in a greater biochemical network of vitamin D deficiency, oxidative stress, inflammatory activation, and endothelial dysfunction. The observed associations between vitamin D and various biomarkers in each of the biological domains fit the notion that these pathways could be mechanistically linked and are not separate events. Therefore, the use of an integrated biomarker approach is more representative of the pathophysiology of preeclampsia than studies investigating only angiogenic markers.

Comparison with the previous studies

Our findings agree with recent publications showing decreased vitamin D levels in mothers who develop preeclampsia. Recent systematic reviews and meta-analyses found that decreased vitamin D status assessed before late pregnancy was related to increased risk of preeclampsia, although the strength of association varied according to sampling time and study design¹³⁻¹⁵. This is in line with the considerable decrease in serum vitamin D observed in our preeclampsia group. Our findings on oxidative stress are also in agreement with previous reports. The pattern of increased MDA and decreased antioxidant defenses, such as lower SOD and TAC, is consistent with evidence from systematic reviews and recent clinical studies showing excessive lipid peroxidation and reduced antioxidant activity in women with preeclampsia^{20,21}.

The angiogenic and inflammatory results were also consistent with previous research. Recent reviews and clinical studies have documented higher circulating inflammatory markers in preeclampsia, especially IL-6 and CRP, together with broader immune dysregulation and inflammatory activation²²⁻²⁴. Similarly, the increased levels of sFlt-1 and decreased levels of PIGF and the markedly elevated sFlt-1/PIGF ratio in our study are in line with current knowledge that antiangiogenic imbalance is a fundamental characteristic of preeclampsia²⁵⁻²⁷. Additional validation studies have shown that the sFlt-1/PIGF ratio may perform better than either marker alone for short-term risk assessment and adverse outcome prediction^{28,29}. Our findings of lower nitric oxide and higher uric acid are also consistent with prior studies linking endothelial dysfunction and uric acid-related oxidative pathways to preeclampsia³⁰⁻³².

Clinical implications

From a clinical perspective, the present findings suggest a multi domain biomarker panel might provide more information regarding the risk of preeclampsia than individual tests. The association of vitamin D and oxidative stress, inflammatory and angiogenic markers, nitric oxide and uric acid are all disrupted, indicating the possibility of using joint biochemical profiling to further stratify risk and identify women who have an increased need for monitoring. The marked difference in the angiogenic markers sFlt-1 and PIGF as well as the sFlt-1/PIGF ratio are particularly in support of the increasing importance of angiogenic biomarkers in obstetric risk assessment²⁵⁻²⁷. These markers are available, however, they should not be used as a substitute for the use of clinical judgement. These biomarkers for angiogenesis are now validated and recommended to be used to complement the clinical indicators for diagnosis and management in selected women with hypertensive disorders, rather than replace them^{1,28,29}. In future, more research will provide better understanding of how to use integrated biomarker panels to optimize personalized monitoring algorithms, and uncover potentially modifiable biological targets^{16,24,31}.

A number of other factors might also affect vitamin D levels in mothers and/or inflammatory markers and the likelihood of preeclampsia. These include physical activity patterns (pregnancy and outside activity, in particular), socioeconomic status, smoking status, dietary intake of vitamin D and calcium, and seasonality (amount of sunshine, day light hours, etc.). In addition, parity and gravidity also may be important factors of maternal metabolic and inflammatory response variation. There was not enough measurement of these variables in the present study and the possibility of residual confounding exists. The associations observed therefore should be considered to suggest biological relationships but not necessarily cause/effect.

Strengths, limitations and future work

There are a number of strengths with this study. It reviewed a panel

of biological pathways in a clinical relevant obstetric patient group and applied a case-control design featuring a contemporaneous normotensive control group. This included all four markers of vitamin D, oxidative stress, inflammatory markers, angiogenic markers, nitric oxide and uric acid markers, giving a broader spectrum of indices related to preeclampsia than previous studies that focused mainly on single pathways. But the main thing about the study are also some limitations. This study was small, all participants were drawn from one centre, measurements of the biomarkers were at one time point, and the study design is case-control and does not permit causality inferences. Residual confounding is also possible, particularly due to diet, seasonal variation in sunlight exposure, timing of samples in maternal pregnancy and any unaccounted for clinical variables. The potential for using integrated panels of biomarkers to enhance prediction over traditional clinical parameters should be tested, and larger, multicenter, prospective cohorts with separation of early and late onset phenotypes and repeated sampling over gestation should be used in future studies^{8,15,24}. We further suggest that future studies should analyze the biomarker profiles separately as the early-onset and late-onset cases of preeclampsia as well as depending on the severity of the disease might have different characteristics or outcome, as these may possess a different biology.

CONCLUSION

In conclusion, this paper illustrates that preeclampsia is correlated with a unique biochemical profile of decreased levels of maternal serum vitamin D, disturbed antioxidant defense, higher levels of oxidative stress, increased inflammatory activity, angiogenic disproportion, lower levels of nitric oxide and high levels of uric acid. These data confirm the idea that preeclampsia is a multidimensional disease that is characterized by the interrelation of pathways of placental malfunction, endothelial damage, and maternal global response. The very strong correlations between vitamin D and other oxidative, inflammatory and angiogenic indicators also indicate that vitamin D status may be highly related to the pathophysiology of the disease as a whole. Combined biomarker strategy can enhance the knowledge of the disease pathology and can help in the earlier detection of risk, yet until the day when it can be used in practice on a large scale, bigger prospective multicentre studies are necessary.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

Potential Conflicts of Interest: None

Competing Interest: None

Acceptance Date: 29 May 2026

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