Abnormal expression and clinical significance of 25-hydroxyvitamin D and sFlt-1 in patients with preeclampsia

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Abstract

Objective: To determine the association between levels of serum 25-hydroxyvitamin D (25[OH]D) and soluble fms-like tyrosine kinase 1 (sFlt-1) in patients with preeclampsia.

Methods: Clinical and demographic data were collected from patients with preeclampsia and healthy pregnant controls. Serum 25(OH)D and sFlt-1 levels were evaluated by enzyme-linked immunosorbent assay and their correlations were determined using Spearman's rank correlation coefficient. Associations between serum 25(OH)D and sFlt-1 levels and disease severity and clinical parameters were evaluated.

Results: Significantly lower serum 25(OH)D and higher sFlt-1 levels were observed in patients with preeclampsia ($n = 100$) versus controls ($n = 100$), and 25(OH)D was inversely correlated with sFlt-1 in patients with preeclampsia. Serum 25(OH)D levels were reduced, while sFlt-1 concentration was increased in patients with severe versus mild preeclampsia. Serum 25(OH)D levels were reduced in late-onset versus early-onset severe preeclampsia. Patients with pre-eclampsia who had lower serum 25(OH)D or elevated sFlt-1 levels showed significantly higher blood pressure indexes versus those with higher 25(OH)D or lower sFlt-1.

Conclusions: Low serum 25(OH)D and high sFlt-1 may be candidate biomarkers for preeclampsia diagnosis and prognosis.

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Introduction

Preeclampsia is a pregnancy-specific disorder resulting in hypertension and multiorgan dysfunction,[1] and is a leading cause of maternal and neonatal morbidity and mortality, affecting patients' long-term quality of life.[2] Low-dose administration of aspirin before 16 weeks of gestation has been reported to effectively reduce preterm preeclampsia.[3] Therefore, novel biomarkers with high sensitivity and specificity are needed to contribute to early screening of high-risk patients, in order to effectively prevent or delay the occurrence and development of preeclampsia.

Vitamin D deficiency is a notable risk factor for preeclampsia development, due to its regulation of the transcription and activity of genes associated with placental function, including placental invasion, normal implantation, and angiogenesis.[4,5] A metabolic intermediate of vitamin D, 25-hydroxyvitamin D (25[OH]D), plays a vital role in maintaining the balance of calcium and phosphate metabolism by promoting the absorption and utilization of calcium.[6] In its active form as 1, 25-dihydroxyvitamin D, it significantly inhibits vascular endothelial growth factor (VEGF)-induced endothelial cell activation and vascular formation.[6] Vitamin D status is determined by measuring serum levels of 25(OH)D, and low concentrations of 25(OH)D have been suggested to increase the risk of severe and mild forms of preeclampsia.[7]

One of the most potent proangiogenic factors, VEGF, has received attention due to its involvement in vascular remodelling, and in endothelial repair and regeneration.[8] Conversely, soluble fms-like tyrosine kinase 1 (sFlt-1), also known as soluble VEGF receptor-1 (sVEGFR-1), functions as an inhibitor of angiogenesis by competing with angiogenic factors such as VEGF and placental growth factor (PLGF).[9] Indeed, competitive binding of sFlt-1 with VEGF has been shown to abolish the biological functions of VEGF and PLGF, leading to endothelial dysfunction.[10] In patients with preeclampsia, the ratio of sFlt1 to PLGF may be useful as a biomarker to predict preeclampsia. For instance, urinary sFlt-1/PLGF ratio is reported to be effective in differentiating women with severe preeclampsia from healthy pregnant women,[11] and patients with preeclampsia have been shown to have increased serum levels of sFlt-1, and decreased circulating PLGF.[12,13] Nevertheless, the association between 25(OH)D and sFlt-1 in preeclampsia remains unclear. Thus, the aim of the present study was to investigate the prognostic value and correlation between serum 25(OH)D and sFlt-1, in patients with preeclampsia.

Patients and methods

Study population and data collection

Patients with preeclampsia who were admitted to Wuhu First People's Hospital, were recruited into the present observational study between April 2015 and August 2016. All patients meeting the inclusion

criteria during this period were enrolled. Preeclampsia diagnosis was confirmed according to clinical findings including increased blood pressure (BP), i.e., systolic (S)BP ≥ 140 mmHg; or diastolic (D)BP ≥ 90 mmHg on two or more measurements at least 6 h apart, and proteinuria ≥ 0.3 g/ 24 h or $\geq +1$ on a urine dipstick after 20 weeks of gestation. Patients were further divided into early- and late-onset preeclampsia subgroups according to gestational age of disease onset, either before 34 weeks of gestation (early onset preeclampsia) or ≥ 34 weeks of gestation (late-onset preeclampsia). Severe preeclampsia was defined as severe hypertension (SBP >160 mmHg or DBP >110 mmHg) or severe proteinuria (>2.0 g in a 24 h urine collection or $>3+$ by dipstick test). In the absence of an existing diagnosis of preeclampsia, patients were diagnosed with preeclampsia if they met any of the following criteria: (1) platelet count $<100,000/\mu l$; (2) serum creatinine concentration >1.1 mg/ dl or a doubling of the serum creatinine concentration in the absence of other renal disease; (3) elevated blood concentrations of liver transaminases to twice normal concentration; (4) pulmonary oedema; or (5) cerebral or visual symptoms, according to the American College of Obstetricians and Gynaecologists 2013 recommendations.[14] The criteria for study exclusion were as follows: (1) essential hypertension prior to pregnancy; (2) cholestasis and/or other serious diseases during pregnancy; (3) artificial impregnation; (4) gestational diabetes or diabetes mellitus; (5) severe liver or kidney disease prior to pregnancy; and (6) anaemia. Female patients who developed preeclampsia after study enrolment were also excluded. Additionally, healthy pregnant female patients, who attended a clinic for routine examination in the department of Obstetrics and Gynaecology of Wuhu First People's Hospital, were enrolled into a control group, according to the following criteria: good glycaemic control and normal blood pressure throughout the pregnancy.

Baseline demographic and clinical data were collected, comprising age, number of pregnancies, gestational age, prepregnancy body mass index (BMI), urine protein, resting heart rate (HR), and resting BP (mean arterial pressure [MAP], SBP and DBP), measured by routine laboratory methods. Urine (10 ml) was collected during 24 hours for proteinuria determination using colorimetry. All experimental protocols were approved by the Ethics Committee of Wuhu First People's Hospital and written informed consent was obtained from all participants.

Measurement of serum 25(OH)D and sFlt-1

Venous blood samples (5 ml) were collected from each participant at hospital admission, into tubes without anticoagulant, and allowed to stand for 30 min at room temperature to achieve complete clotting. Samples were then centrifuged at $3\,000 \times g$ for 10 min at $4\,°C$ to acquire serum, and the serum samples were stored at $-80°C$ until use. Serum levels of 25(OH)D and sFlt-1 were evaluated using enzyme-linked immunosorbent assay (ELISA) kits (Human Total 25-OH Vitamin D IVD ELISA Kit/ Cat No. RDKAP1971 and Human VEGFR1/Flt-1 Quantikine ELISA Kit/ Cat No. DVR100C, respectively; R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. The resultant optical density (OD) values at 450 nm were obtained using a Multiskan MK3 ELISA microplate reader (Thermo Scientific, Shanghai, China).

Statistical analyses

All data are presented as mean $\pm$ SD. Student's t-test was used for intergroup comparisons of clinical parameters and

serum levels of 25(OH)D and sFlt-1. Correlations between two variables were evaluated using Spearman's rank correlation coefficient. Statistical analyses were performed using SPSS software, version 22.0 (SPSS Inc., Chicago, IL, USA) and a P value < 0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 100 female patients with preeclampsia and 100 healthy pregnant controls were enrolled into this study (Table 1). No statistically significant differences were observed between these two groups regarding maternal age, prepregnancy BMI, and number of pregnancies (excluding aborted pregnancies) at the time of serum sampling (all $P > 0.05$). Patients with preeclampsia had a longer gestational age compared with controls ($P = 0.014$), and heart rates of patients

with preeclampsia were higher than those of healthy pregnant controls ($P = 0.033$). MAP, SBP and DBP were also higher in patients with preeclampsia versus healthy pregnant controls (all $P < 0.001$). Moreover, patients with preeclampsia displayed decreased gestational week at birth and birth weight compared with the control group (both $P < 0.05$).

Correlation between serum 25(OH)D and sFlt-1 levels

For the serum 25(OH)D ELISA kit used in the present study, the intra-assay and inter-assay coefficients of variation (CVs) were 4.66–8.86%, and 8.50–9.60%, respectively, and the sensitivity and specificity values were 56.5% and 95.8%, respectively. For the serum sFlt-1 ELISA kit, the intra-assay and inter-assay CVs were 6.51–7.28%, and 3.12–4.05%, respectively, and the sensitivity and specificity values were 79.2% and 89.6%, respectively. Parameters were determined using three

Table 1. Demographic and clinical characteristics of pregnant female patients with preeclampsia and healthy pregnant controls.

Variable	Study group		Statistical significance
	Control	Preeclampsia	
Patients, n	100	100	–
Age, years	28.45 ± 4.35	29.46 ± 4.89	NS
Prepregnancy BMI, kg/m^2	22.20 ± 2.81	21.51 ± 2.42	NS
Number of pregnancies	1.15 ± 0.33	1.26 ± 0.35	NS
Gestational age, weeks	31.15 ± 4.58	33.84 ± 3.57	$P = 0.014$
Heart rate, beats/min	87.05 ± 10.04	90.15 ± 12.45	$P = 0.033$
Urine protein, g/24 h	3.42 ± 1.19	<0.3	–
MAP, mmHg	87.59 ± 3.28	129.28 ± 10.54	$P < 0.001$
SBP, mmHg	109.90 ± 5.00	156.53 ± 7.51	$P < 0.001$
DBP, mmHg	58.67 ± 5.96	99.51 ± 8.85	$P < 0.001$
Gestational age at birth, weeks	39.41 ± 2.41	38.67 ± 2.98	$P = 0.031$
Birth weight, g	3412.30 ± 653.20	324.30 ± 653.2	$P = 0.024$

Data presented as mean ± SD.
BMI, body mass index; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.
NS, no statistically significant between-group difference ($P > 0.05$; Student's t-test).

samples in any experiment and by testing three replicates per sample.

In patients with preeclampsia, the concentration of serum 25(OH)D was significantly lower than in healthy pregnant controls ($P < 0.05$; Figure 1A). In contrast, the serum concentration of sFlt-1 was significantly elevated in patients with preeclampsia compared with healthy pregnant controls ($P < 0.05$; Figure 1B). Further analysis revealed a statistically significant inverse correlation between serum levels of 25(OH)D and sFlt-1 in patients with preeclampsia ($P < 0.01$ [Spearman's rank correlation coefficient]; Figure 1C).

Association between 25(OH)D and sFlt-1 and disease severity in patients with preeclampsia

Serum concentrations of 25(OH)D were shown to be decreased in patients with severe preeclampsia ($n = 70$) versus those with mild preeclampsia ($n = 30$) ($P < 0.05$; Figure 2A). Conversely, patients with severe preeclampsia ($n = 70$) displayed higher serum levels of sFlt-1 compared with the mild preeclampsia group ($n = 30$) ($P < 0.05$; Figure 2B). Levels of 25(OH)D and sFlt-1 in patients classified as early-onset or late-onset severe preeclampsia
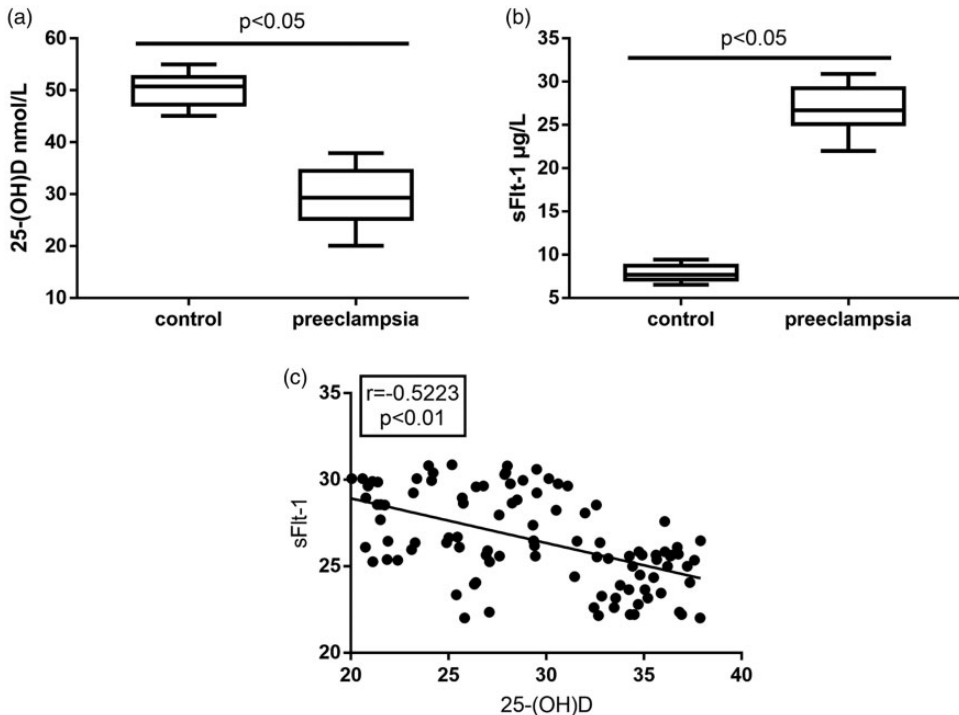

Figure 1. Box-whisker plots showing serum levels of (a) 25-hydroxyvitamin D (25[OH]D) and (b) soluble fms-like tyrosine kinase-1 (sFlt-1) in patients with preeclampsia ($n = 100$) and healthy pregnant controls ($n = 100$) determined by enzyme-linked immunosorbent assay ($P < 0.05$; Student's *t*-test); and (c) scatter plot showing an inverse correlation between serum 25(OH)D and sFlt-1 levels in patients with preeclampsia ($r = -0.5223$, $P < 0.01$; Spearman's rank correlation coefficient). Data in box-whisker plots presented as mean (black horizontal line), 25th and 75th percentiles (extremities of the box), and minimum/maximum outliers (error bars).
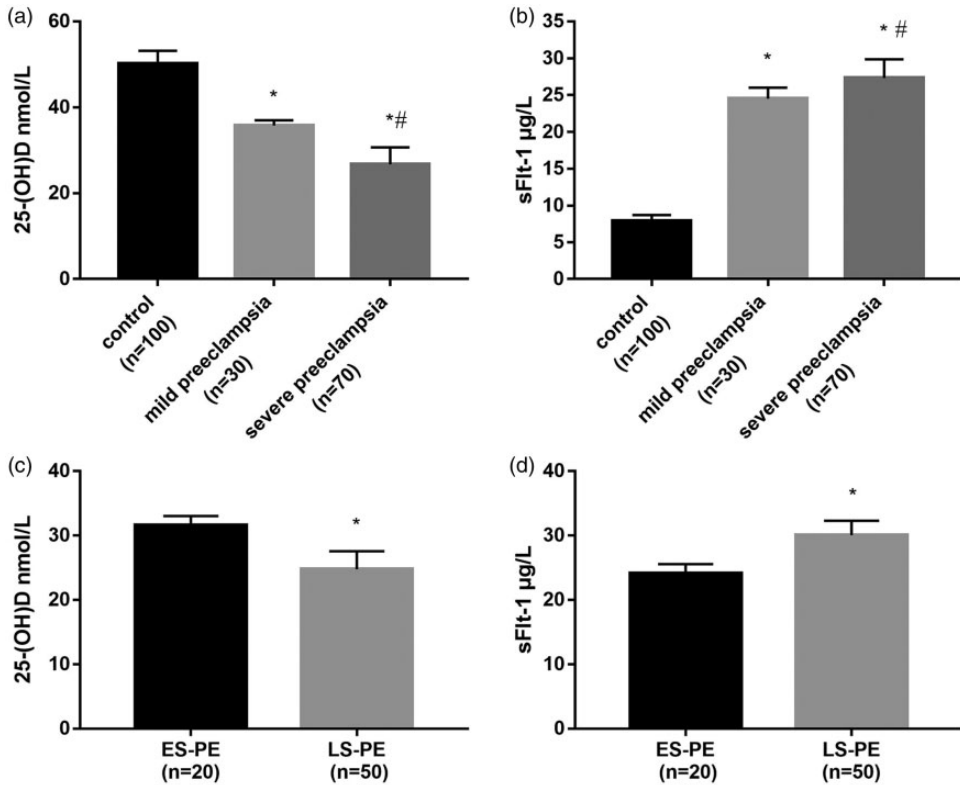
Figure 2. Association between 25-hydroxyvitamin D (25[OH]D) and soluble fms-like tyrosine kinase-1 (sFlt-1) and disease severity in patients with preeclampsia: serum levels of (a) 25(OH)D and (b) sFlt-1 in healthy pregnant controls and patients with mild or severe preeclampsia (*$P < 0.05$ versus the control group; #$P < 0.05$ versus the mild preeclampsia group); and serum levels of (c) 25(OH)D and (d) sFlt-1 in patients with early-onset severe preeclampsia (ES-PE) and late-onset severe preeclampsia (LS-PE); *$P < 0.05$ versus ES-PE group.

were then evaluated. The results showed that serum 25(OH)D was significantly lower in patients with late-onset severe pre-eclampsia versus those with early-onset severe preeclampsia ($P < 0.05$; Figure 2C), and serum sFlt-1 was significantly increased in patients with late-onset severe pre-eclampsia versus those with early-onset severe preeclampsia ($P < 0.05$; Figure 2D).

Relationship between serum 25(OH)D and sFlt-1 levels and clinical parameters

To further determine the association between serum 25(OH)D and sFlt-1 levels

and clinical parameters of patients with pre-eclampsia, all patients with preeclampsia were sub-grouped into 25(OH)D or sFlt-1 high/low group according to the mean levels of serum 25(OH)D (28.6 ± 5.5) or sFlt-1 (26.8 ± 2.5): for 25(OH)D, low values were <28.6 and high values were ≥ 28.6; for sFlt-1, low values were <26.8 and high values were ≥ 26.8. The MAP, SBP and DBP levels were increased in patients with lower serum 25(OH)D levels than those with higher serum 25(OH)D levels (all $P < 0.05$; Table 2). However, patients with elevated levels of sFlt-1 exhibited higher MAP, SBP and DBP values

Table 2. Comparison of clinical data in pregnant female patients with pre-eclampsia, grouped according to high or low 25(OH)D level.

Variable	Study group		Statistical significance
	High 25(OH)D level	Low 25(OH)D level	
Patients, n	49	51	–
MAP, mmHg	119.72 ± 5.71	138.11 ± 8.50	$P = 0.006$
SBP, mmHg	153.83 ± 2.32	163.23 ± 3.55	$P = 0.004$
DBP, mmHg	97.95 ± 1.71	102.96 ± 1.26	$P = 0.034$

Data presented as mean ± SD.
25(OH)D, 25-hydroxyvitamin D; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Statistically significant between group-differences ($P < 0.05$; Student's t-test).

Table 3. Comparison of clinical data in pregnant female patients with pre-eclampsia, grouped according to high or low sFlt-1 level.

Variable	Study group		Statistical significance
	low sFlt-1 level	high sFlt-1 level	
Patients, n	40	60	–
MAP, mmHg	117.78 ± 4.43	136.63 ± 6.37	$P = 0.018$
SBP, mmHg	152.90 ± 1.64	162.37 ± 3.36	$P < 0.001$
DBP, mmHg	97.35 ± 1.40	102.56 ± 1.89	$P = 0.049$

Data presented as mean ± SD.
sFlt-1, soluble fms-like tyrosine kinase 1; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Statistically significant between-group differences ($P < 0.05$; Student's t-test).

than those with lower serum sFlt-1 levels (all $P < 0.05$; Table 3).

Discussion

Preeclampsia is a pregnancy-related disease, affecting 2–8% of all pregnancies,[15] however, the aetiology of preeclampsia has not been fully elucidated. The pathophysiological process of preeclampsia is widely accepted to include two stages: an initial stage of abnormal placenta formation and a decrease in the invasiveness of trophoblast cells, which leads to insufficient infiltration of the endometrium and uterine spiral artery remodelling disorder that results in decreased placental perfusion secondary to placental ischaemia. The ischaemic placenta then produces extensive inflammatory cytokines that initiate generalized endothelial dysfunction.[16] Therefore, there is an urgent need for effective biomarkers for the early detection of preeclampsia.

Placental protein-13 (PP13), a galectin specifically located at the brush border membrane of the syncytiotrophoblast, has been identified as a potential predictive biomarker of preeclampsia, as it is significantly down-regulated in the first trimester of pregnancies that develop preeclampsia.[17,18] Other potential biomarkers of preeclampsia include vitamin D and serum sFlt-1 levels.

Active vitamin D is involved in placental implantation and angiogenesis, and

vitamin D levels of more than 30 ng/ml in early pregnancy, and retained in late pregnancy, have been shown to significantly lower the risk of preeclampsia compared with insufficient vitamin D levels.[19] There are several mechanisms by which the progression of preeclampsia may be prevented, such as cardio-protective effects via the renin-angiotensin-aldosterone system, and regulation of effector T cells and calcitriol.[20–23] Emerging evidence has confirmed that decreased serum 25(OH)D level, the active form of vitamin D, is associated with the occurrence and development of preeclampsia. For example, in 1993, Cruikshank and colleagues found that baseline maternal levels of 25(OH)D were lower in those with preeclampsia than in normal pregnant females.[24] Plasma 25(OH)D levels were shown to be significantly decreased in patients with early-onset severe preeclampsia compared with controls with a singleton gestation.[25] In another study involving 560 cases of mild preeclampsia and 157 severe cases, patients with 25(OH)D >50 nmol/l before the 26th week of pregnancy had a 40% reduction in severe preeclampsia risk compared with those with 25(OH)D < 50 nmol/l.[26] Whether or not decreased serum 25(OH)D levels are associated with the occurrence and development of preeclampsia at different times during pregnancy remains controversial. Alvarez-Fernandez et al.[27] suggested that low 25(OH)D status may constitute a biomarker for the imminent late onset of preeclampsia rather than an early predictor of the disease. The authors did not find any significant difference in 25(OH) D levels between controls and patients with preeclampsia, either in the first trimester or in the early onset of preeclampsia (24–34 gestational weeks), and only describe significant differences between controls and late onset preeclampsia.[27] In the present study, serum levels of 25(OH)D were significantly decreased in patients with preeclampsia

compared with healthy pregnant females, while patients with severe preeclampsia, particularly those with late-onset severe preeclampsia, had the lowest serum levels of 25(OH)D. In addition, patients with preeclampsia and lower serum of 25(OH)D showed higher blood pressure indexes (MAP, SBP and DBP) versus those with higher serum 25(OH)D levels.

The usefulness of serum sFlt-1 in identifying female patients with preeclampsia has been largely reported, and during pregnancy, the placenta is the main source of sFlt-1 in the maternal circulation.[28] In the present study, and consistent with previous reports,[29,30] serum sFlt-1 levels were demonstrated to be significantly increased in patients with preeclampsia compared with healthy pregnant controls. However, higher serum sFlt-1 concentration was observed in patients with late-onset severe preeclampsia versus those with early-onset severe preeclampsia. A previously published study reported no statistically significant difference between early and late-onset severe preeclampsia, but early-onset was defined as <37 weeks, rather than <34 weeks in the present study.[31] In addition, unlike the present study, other reports have shown that circulating sFlt-1 levels were significantly higher in early-onset preeclampsia than in late-onset preeclampsia.[32–34] In the present study, serum sFlt-1 levels in patients with severe preeclampsia were significantly higher than those in the mild preeclampsia group, which was comparable to a previous report.[35] Finally, to the best of the authors' knowledge, the present study demonstrated for the first time that serum 25(OH)D was inversely correlated with sFlt-1 levels in female patients with preeclampsia. In addition, compared with those with low sFlt-1 levels, blood pressure levels were increased in patients with preeclampsia who had higher sFlt-1 levels. Similarly, Hirokoshi et al.[30] suggested a positive correlation between serum sFlt-1

levels and mean blood pressure in patients with preeclampsia.

In summary, the findings of the present study suggest that reduced serum levels of 25(OH)D and elevated levels of sFlt-1 are associated with disease severity and blood pressure indices in patients with preeclampsia, indicating that 25(OH)D and sFlt-1 may serve as potential biomarkers for the diagnosis and prognosis of preeclampsia.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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