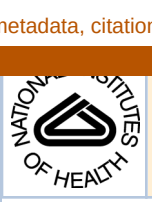

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Midgestation Maternal Serum 25-Hydroxyvitamin D Level and Soluble Fms-Like Tyrosine Kinase 1/Placental Growth Factor Ratio as Predictors of Severe Preeclampsia

Padmashree Chaudhury Woodham, **Julia E. Brittain**, **Arthur M. Baker**, **D. Leann Long**, **Sina Haeri**, **Carlos A. Camargo Jr**, **Kim A. Boggess**, and **Alison M. Stuebe**

Departments of Obstetrics and Gynecology (P.C.W., K.A.B., A.M.S.) and Biochemistry and Biophysics (J.E.B.), and Gillings School of Global Public Health, Department of Biostatistics (D.L.L.), University of North Carolina at Chapel Hill, Chapel Hill, NC; Savannah Perinatology Associates, Inc (A.M.B.), Savannah, GA; Department of Obstetrics and Gynecology (S.H.), Baylor College of Medicine, Houston, TX; Department of Emergency Medicine (C.A.C.), Massachusetts General Hospital, Boston, MA

Abstract

Recent studies have shown that low serum 25-hydroxyvitamin D (25[OH]D) level is a risk factor for preeclampsia. The clinical significance of in vitro findings that vitamin D regulates vascular endothelial growth factor production is unclear. We sought to determine whether there is an association between midgestation serum 25(OH)D levels and angiogenic factor activity and to compare their predictive value for the development of severe preeclampsia. We conducted a nested case-control study of women with severe preeclampsia (n=41) versus women with uncomplicated term birth (n=123) who had second trimester genetic screening (15–20 weeks). Using banked frozen serum, we measured levels of 25(OH)D, vascular endothelial growth factor, soluble fms-like tyrosine kinase 1, and placental growth factor and compared their correlations and predictive values. We found no correlation between serum 25(OH)D and angiogenic factors levels. 25(OH)D alone was comparable to vascular endothelial growth factor and soluble fms-like tyrosine kinase 1/placental growth factor ratio as a predictive marker for severe preeclampsia. A composite of both 25(OH)D level and soluble fms-like tyrosine kinase 1/placental growth factor ratio was more predictive than either alone (area under curve: 0.83 versus 0.74 and 0.67, respectively). In conclusion, combining midpregnancy 25(OH)D level with soluble fms-like tyrosine kinase 1/placental growth factor ratio provides a better prediction for the development of severe preeclampsia.

Keywords

25-hydroxyvitamin D; angiogenic factors; preeclampsia; sFLT-1/PlGF ratio; VEGF

Preeclampsia occurs in 2% to 5% of pregnancies and is a major cause of perinatal and maternal morbidity and mortality.[1,2] Furthermore, women with preeclampsia are at increased risk for long-term cardiovascular complications, such as chronic hypertension,

Correspondence to Padmashree Chaudhury Woodham, Departments of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, 3010 Old Clinic Building, CB#7516, Chapel Hill, NC 27599-7516. pchaudh@gmail.com.

Disclosures

None.

later in life.[3] Early identification of women at risk is essential for the development of preventive measures.

Although several theories regarding the pathogenesis of preeclampsia have emerged,[4,5] few early predictors have been identified. For example, low plasma vascular endothelial growth factor (VEGF) in the first trimester of pregnancy is a predictive marker for preeclampsia.[6,7] Increased mRNA concentrations of VEGF receptor 1, also known as soluble fms-like tyrosine kinase 1 (sFLT-1), and decreased concentrations of placental growth factor (PlGF) are present in women diagnosed with preeclampsia.[6 – 8] In addition, sFLT-1/PlGF ratio is increased in women who subsequently develop preeclampsia.[9] These angiogenic factors may play an important role in the pathogenesis of atherosclerosis and have a wide range of activity depending on genotype and phenotypic factors.[10]

We reported recently that low midgestation serum 25-hydroxyvitamin D (25[OH]D) is a risk factor for severe preeclampsia.[11] Its active form, 1,25-dihydroxyvitamin D(3) (1,25[OH][2]D[3]), stimulates VEGF expression in smooth muscle cells through a vitamin D response element in the VEGF promoter.[12] We hypothesized that low serum 25(OH)D may be associated with diminished VEGF production, leading to preeclampsia.

Our study had 2 objectives, to determine whether there is an association between early second trimester maternal serum 25(OH)D level and angiogenic factor activity and to compare the predictive value of serum 25(OH)D level alone versus in combination with angiogenic factor levels, for the later development of severe preeclampsia.

Methods

Study Design

We conducted a nested, case-control study in a cohort of 3992 women (Figure 1). All of the women who had previously given blood for routine genetic multiple marker screening and subsequently delivered at the University of North Carolina-Chapel Hill between January 2004 and November 2008 were eligible. All pregnant women, regardless of risk status or payer status, are offered this screening as part of routine prenatal care. Nonfasting blood samples were collected for routine genetic multiple marker screening between 15 and 20 weeks gestation, and serum aliquots were barcoded and frozen at −70°C. Maternal demographic and medical data were chart abstracted. This study was approved by the University of North Carolina at Chapel Hill Institutional Review Board before data collection, and permission was obtained to use banked serum from these women for research purposes.

Severe preeclampsia was defined as a systolic blood pressure of ≥160 mm Hg and/or a diastolic blood pressure of ≥110 mm Hg, recorded on ≥2 occasions 6 hours apart, plus proteinuria (≥300 mg in a 24-hour collection or 1+ on a urine dipstick) or a systolic blood pressure of ≥140 mm Hg and/or a diastolic blood pressure of ≥90 mm Hg, recorded on ≥2 occasions 6 hours apart plus 5 g of proteinuria in a 24-hour period. We further classified cases of preeclampsia as severe in the setting of pulmonary edema, seizures, oliguria (<500 mL/24 hours), elevated liver enzymes accompanied by right upper quadrant pain, thrombocytopenia (<100 000/mm^3), or persistent cerebral symptoms, such as headache or blurry vision.[13] Healthy women with term deliveries (≥37 weeks) were used as controls. We excluded from both cases and controls women with multiple gestation, major congenital fetal anomalies, pregestational hypertension, kidney disease, diabetes mellitus, known thrombophilias, or any other significant preexisting chronic medical disease. All of the patient records were reviewed by a single reviewer (A.M.B.); patients meeting the strict definitions described above were included in the study.

From the overall cohort of 3992 women, we identified 51 cases who met all of the inclusion and exclusion criteria. Ten did not have an adequate volume of serum available for analysis and, thus, were excluded. The 41 remaining cases were matched by race/ethnicity, in a 3:1 ratio, to a random, computer-generated control group of 123 healthy women delivering at term.

Laboratory Analyses

Serum aliquots for each enrolled subject were shipped on dry ice to Massachusetts General Hospital (Boston, MA) for serum 25(OH)D measurement by liquid chromatography-tandem mass spectrometry.[14] The method used is an isotope dilution, liquid chromatography-tandem mass spectrometry assay optimized in the Massachusetts General Hospital laboratory based on published procedures.[15] The limit of detection is 5.0 nmol for vitamin D2 and 7.5 nmol for vitamin D3. The between-run coefficient of variation for quality control serum containing a total vitamin D concentration of 57 nmol is 7.5%.

A second aliquot was transported to the University of North Carolina Department of Biochemistry and Biophysics for angiogenic factor analysis. VEGF, sFLT-1, and PlGF levels were assessed using ELISA, (R&D Systems, Minneapolis, MN) on undiluted single serum samples. Optical density was read on a VersaMax spectrophotometric plate reader at 450 nm, with λ correction at 570 nm. For sFLT-1 and PlGF assays, all of the samples were within the detectable limits of the assay, 4 to 3600 pg/mL and 7 to 800 pg/mL, respectively. The detectable limits for VEGF were 0.02 to 1200.00 pg/mL. Fourteen samples, 6 controls and 8 cases, fell below the minimum detection limit of VEGF. The interassay coefficients of variation of sFLT-1, PlGF, and VEGF are 8%, 4%, and 5%, respectively, consistent with other reported coefficients of variation for the assays.[16]

Statistical Analysis

Maternal demographic and medical characteristics were compared between cases and controls using Fisher exact test for categorical variables and Wilcoxon-Mann-Whitney test for continuous variables. Spearman correlations were calculated to determine whether VEGF, sFLT-1, PlGF, or sFLT-1/PlGF ratio were individually related to 25(OH)D. Logistic regression was used to calculate unadjusted estimated odds ratios of each predictor separately. To examine the best combination of analytes that predicted severe preeclampsia, logistic regression with backward selection was used, adjusting for age, body mass index (BMI), parity, season of blood draw, and gestational age at blood draw. For the backward selection procedure, the level of significance used was 0.05; that is, if a predictor had a P value >0.05, it was removed from the model. Receiver operator characteristic curves and their corresponding areas under the curve were generated to graphically compare the predictive abilities of logistic models. A 2-sided $P<0.05$ was considered statistically significant. All of the data analyses were carried out in SAS (version 9.2, SAS Institute, Cary, NC) and R (version 2.10.1, R Foundation for Statistical Computing, Vienna, Austria).

Results

We successfully measured 25(OH)D, VEGF, and PlGF levels from the 164 samples. To eliminate the influence of very extreme observations, 2 outlying values of VEGF >60 pg/mL (66 and 104 pg/mL) were excluded from analysis; 95% of all of the VEGF observations were <11 pg/mL. Three cases did not have enough serum to determine sFLT-1 levels, so their respective controls were removed from any analysis of this variable to maintain the matched case-control sample. Study group demographics and clinical characteristics are presented in Table 1. The groups only differed with respect to earlier gestational age at delivery among women with severe preeclampsia (32.6 versus 39.6 weeks; $P<0.001$).

As presented in Table 1, women with severe preeclampsia had significantly lower early pregnancy levels of 25(OH)D, VEGF, and PlGF compared with controls. sFLT-1 levels were not significantly different between cases and controls. Women with severe preeclampsia had higher sFLT-1/PlGF ratio than controls.

None of the angiogenic factors (VEGF, sFLT-1, PlGF, or sFLT-1/PlGF ratio) were significantly correlated with 25(OH)D levels using the Spearman rank-based method (Table 2). The unadjusted associations between each analyte and risk of severe preeclampsia were examined using logistic regression and are presented as odds ratio estimates in Table 3. Individually, both 25(OH)D level and sFLT-1/PlGF ratio were significant predictors of severe preeclampsia ($P<0.001$ and $P<0.003$, respectively).

During the model selection process, the ratio of sFLT-1/PlGF was determined to have more predictive value than either variable alone, thus it was used in the selection procedure. Leaving the confounders of age, BMI, parity, season of blood draw, and gestational age at blood draw in the logistic model, 25(OH)D, VEGF, and sFLT-1/PlGF ratio were compared in 1-, 2-, and 3- variable models for their joint predictive abilities for severe preeclampsia. VEGF alone was the least informative of these predictors ($P=0.08$) and provided limited additional information over 25(OH)D and sFLT-1/PlGF ratio. The areas under the curve, measures of each model's predictive ability, and the receiver operator characteristic curves of some of the potential models are presented in Figure 2. The model containing both 25(OH)D and sFLT-1/PlGF ratio was the most parsimonious model that best predicted severe preeclampsia. Table 4 presents the odds ratio estimates, 95% CIs and *P* values for this model. After adjusting for the confounders, both 25(OH)D and sFLT-1/PlGF ratio were highly significant independent predictors of severe preeclampsia (both $P<0.001$).

The odds ratio estimate of 0.95 for 25(OH)D means that each nanomole per liter increase in 25(OH)D level in the blood resulted in a 5% reduction in odds of developing severe preeclampsia, adjusted for age, BMI, parity, season, gestational age at blood draw, and sFLT-1/PlGF ratio. Alternatively, the odds ratio estimate for a 10-nmol/L increase in 25(OH)D level, a more clinically applicable value, is 0.62 (95% CI: 0.51–0.76), meaning that each 10-nmol/L increase in 25(OH)D level resulted in a 38% reduction in odds of developing severe preeclampsia, adjusted for the same variables. Similarly, the odds ratio estimate of 1.11 for sFLT-1/PlGF ratio implied that each 1-unit increase in the ratio resulted in an 11% increase in odds of developing severe preeclampsia, adjusted for age, BMI, parity, season, gestational age at blood draw, and 25(OH)D level.

On further analysis, when the total study population was divided into small for gestational age (SGA), defined as birthweight <10th percentile, and non-SGA infants, 25(OH)D and PlGF were both significantly lower in the SGA group ($P=0.006$ and $P=0.03$, respectively). We further examined the cases and controls separately with respect to SGA. 25(OH)D was again significantly lower in the SGA cases compared with the non-SGA cases ($P=0.01$). However, this difference was not noted in the control population.

Discussion

In the present study, we sought to determine whether there is an association between early second trimester maternal serum 25(OH)D level and angiogenic factor activity and to compare the predictive value of serum 25(OH)D level alone versus in combination with angiogenic factor levels for the later development of severe preeclampsia. We found no correlation between maternal serum 25(OH)D levels and angiogenic factors. We also found that combining circulating midgestation levels of 25(OH)D with sFLT-1/PlGF ratio yields better prediction of severe preeclampsia than either marker alone.

Our findings confirm and extend earlier work linking angiogenic factors with preeclampsia risk. Several studies have provided evidence that particular alterations in angiogenic factors are associated with preeclampsia.[6–9,17–20] However, much of this previous research measured analytes in serum samples obtained after the development of preeclampsia and, therefore, did not measure predictability of the disease. In addition, the relationship between angiogenic proteins and the development of preeclampsia is complex. Previous studies that did measure serum factors early in pregnancy usually considered single analytes when calculating predictive values. As our study demonstrates, a single angiogenic factor may not be the best early marker of development of severe preeclampsia.

We did not find evidence that 25(OH)D levels were associated with angiogenic factors, in contrast with in vitro studies. Previous studies have suggested that the active hormone 1,25(OH)(2)D(3) regulates VEGF production through a vitamin D response element in the VEGF promoter. Cardus et al[21] analyzed the effects of 1,25(OH)(2)D(3) in the proliferation of vascular smooth muscle cells. They found that 1,25(OH)(2)D(3) induces a dose-dependent increase in vascular smooth muscle proliferation in quiescent cells and in cells stimulated to grow. In addition, the investigators demonstrated that the effect of 1,25(OH)(2)D(3) on vascular smooth muscle cell proliferation is mediated by an increase of the expression of VEGF. This increase in proliferation was present both in vitro and in vivo in animals models. In a later study, this group performed promoter transactivation analysis and demonstrated binding of the vitamin D receptor to 2 response elements in the VEGF promoter and subsequent VEGF promoter activation.[12] The lack of association between vitamin D and VEGF in our study may be explained by the inherent differences between in vitro and in vivo studies, as well as animal versus human physiology.

Our study has several strengths. Maternal serum was collected in midpregnancy, before the presence of clinical manifestations of preeclampsia, reducing the likelihood that the disease process affected 25(OH)D and angiogenic factor levels. We excluded women with chronic medical illnesses, such as diabetes mellitus and chronic hypertension; these diseases have been associated with increased risk for preeclampsia and may cause preexisting alterations in 25(OH)D and angiogenic factor activity.[22] In addition, blood pressure at the time of serum blood draw was comparable between cases and controls.

Our findings must be interpreted in the context of the study design. The sample size, although comparable to others in the literature, is relatively small (n=41 cases). This limits our ability to detect smaller but clinically significant differences in analyte levels between the 2 groups. When using a case-control design, there is potential for selection bias, particularly in the selection of controls. However, our controls were nested within a large cohort of women who all provided serum samples as part of routine prenatal screening, reducing selection bias. The duration of storage of the samples ranged from 2 to 6 years. Evidence shows that long-term storage does not affect serum 25-OHD, PlGF, and sFlt-1 levels.[23,24] One study showed a possible effect of storage on VEGF levels.[25] Therefore, sample storage may be a potential confounder. Our cases of preeclampsia were heterogeneous, in that 44% of cases occurred in multiparous women. The underlying pathophysiology of severe preeclampsia may differ in primiparous versus multiparous women,[26] and these differences may reduce our power to detect underlying mechanisms. It is also possible that unmeasured confounding explains the apparent association between 25(OH)D deficiency, altered angiogenic factors, and severe preeclampsia. However, we found that adjustment for some known potential confounders strengthened the observed associations.

Perspectives

In summary, contrary to our hypothesis, we found no correlation between 25(OH)D and angiogenic factors in pregnant women. However, the combination of 25(OH)D level and sFLT-1/PlGF ratio is a better predictor of preeclampsia in midgestation than either marker alone. Our data suggest that 25(OH)D and angiogenic factors play independent roles in preeclampsia pathogenesis. Preeclampsia is a significant public health concern, and interventions aimed at reducing rates of preeclampsia are needed. Translational research identifying potentially modifiable risk factors, such as those identified in the present study, are essential to the success of targeting therapy. This study suggests the need for a randomized control trial of vitamin D supplementation to reduce the risk of severe preeclampsia.

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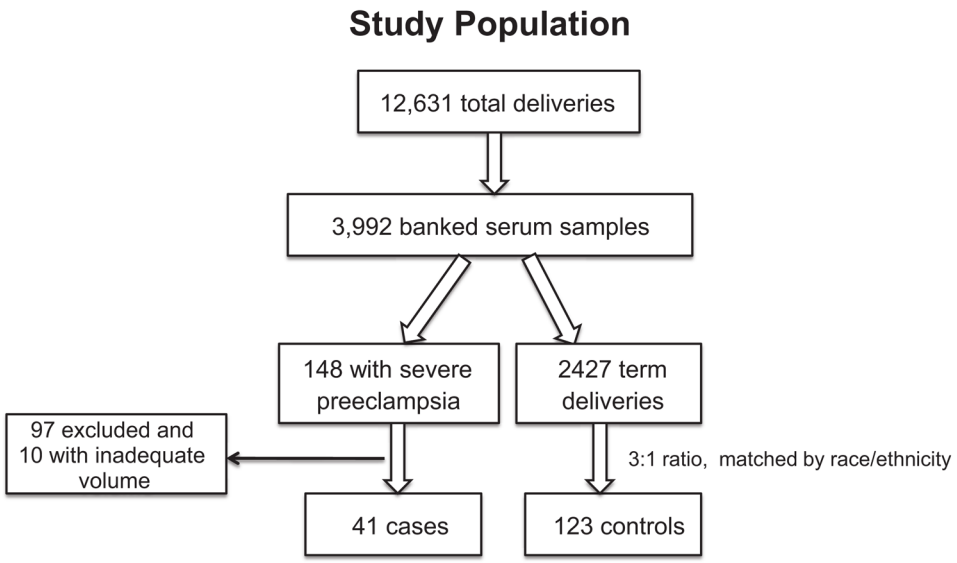

Figure 1.
Study population.

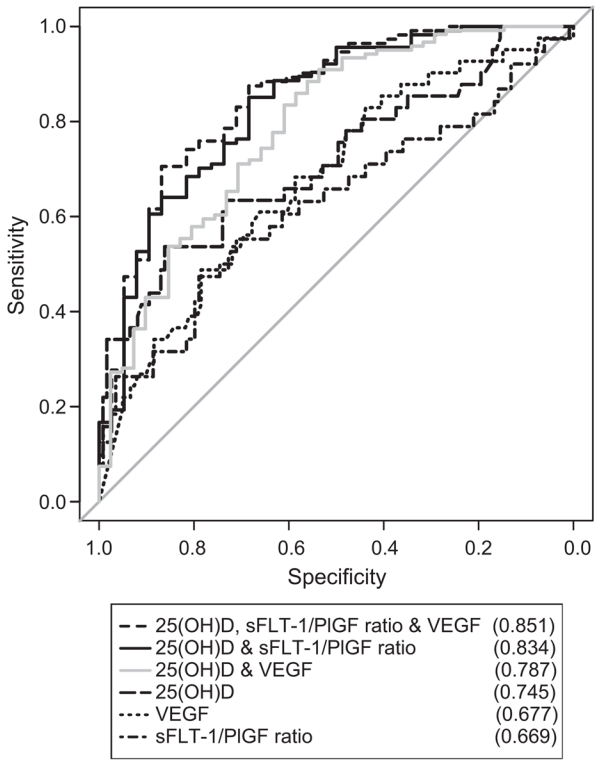

Figure 2.

Receiver operator characteristic (ROC) curve for adjusted logistic regression models. 25(OH)D indicates 25-hydroxyvitamin D; VEGF, vascular endothelial growth factor; sFLT-1, soluble fms-like tyrosine kinase 1; PlGF, placental growth factor. Gray line indicates no predictive ability. Area under curve is indicated in parenthesis.

Table 1

Clinical and Demographic Characteristics of Women Who Developed Severe Preeclampsia (Cases) and Race/Ethnicity-Matched Women Who Did Not (Controls)

Variables	Severe Preeclampsia (n = 41)	Controls (n = 123)	*P*
Age, y *	29 (25–34)	29 (25–33)	0.79
Race/ethnicity, n (%)			—
White	12 (29)	36 (29)	
Black	16 (39)	48 (39)	
Hispanic	11 (27)	33 (27)	
Asian	2 (5)	6 (5)	
Multiparous, n (%)	18 (44)	62 (50)	0.59
Body mass index *	30 (28–34)	30 (27–36)	0.89
Gestational age at serum collection, wk *	17.1 (16.1–18.9)	17.4 (16.4–18.3)	0.98
SBP at time of serum collection	119 (109–128)	124 (111–129)	0.49
DBP at time of serum collection	72 (65–80)	70 (61–81)	0.33
Gestational age at delivery, wk *	32.6 (30.4–34.7)	39.6 (39–40.6)	<0.0001
Season of blood draw, % (n)			0.42
Winter	12 (29)	25 (20)	
Spring	11 (27)	49 (40)	
Summer	10 (24)	29 (24)	
Fall	8 (20)	20 (16)	
25(OH)D, nmol/L *	75 (53–107)	107 (90–121)	<0.0001
VEGF, pg/mL *	1.9 (0.4–3.2)	3.2 (1.7–4.6)	0.0007
sFLT-1, pg/mL *†	1158 (763–1837)	1129 (819–1413)	0.46
PlGF, pg/mL *	84 (54–130)	99 (73–158)	0.03
sFLT-1/PlGF ratio *†	15.3 (8.4–27.3)	10.3 (6.4–15.9)	0.02

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; 25(OH)D, 25-hydroxyvitamin D; VEGF, vascular endothelial growth factor; sFLT-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor. Wilcoxon-Mann-Whitney test was used for continuous variables and Fisher exact test for categorical variables.

* Values are median (interquartile range).

† Because 3 cases had insufficient blood to determine sFLT-1, their matched controls were removed from any analysis pertaining to sFLT-1. Thus, the sample sizes here are 114 for the controls and 38 for the cases.

Table 2

Spearman Correlations of 25(OH)D With Angiogenic Factors

Variable	Correlation With 25(OH)D	*P*
VEGF	0.033	0.68
sFLT-1	0.138	0.09
PlGF	−0.085	0.28
sFLT-1/PlGF ratio	0.134	0.10

25(OH)D indicates 25-hydroxyvitamin D; VEGF, vascular endothelial growth factor; sFLT-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor.

Table 3

Unadjusted Logistic Regression Model of Each Analyte as a Predictor of Severe Preeclampsia

Analyte	Odds Ratio	95% CI	*P*
25(OH)D, nmol/L	0.97	0.96 – 0.98	<0.0001
sFLT-1/PlGF ratio	1.06	1.02–1.11	0.003
VEGF, pg/mL	0.86	0.73–1.02	0.08

25(OH)D indicates 25-hydroxyvitamin D; VEGF, vascular endothelial growth factor; sFLT-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor.

Table 4

Multivariable Logistic Regression Model of 25(OH)D Level and sFLT-1/PlGF Ratio as Independent Predictors of Severe Preeclampsia

Effect	Odds Ratio Estimate	95% CI	*P*
25(OH)D, nmol/L	0.95	0.94 – 0.97	<0.0001
sFLT-1/PlGF ratio	1.11	1.05–1.18	0.0003
Age, y	1.02	0.94–1.11	0.62
BMI, kg/m^2	0.96	0.90–1.03	0.23
Parity *	0.56	0.22–1.38	0.20
Gestational age at blood draw, wk	1.01	0.76–1.34	0.93
Spring *	0.73	0.20–2.67	0.63
Summer *	1.30	0.35–4.92	0.70
Fall *	2.32	0.55–9.86	0.26

25(OH)D indicates 25-hydroxyvitamin D; sFLT-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; BMI, body mass index.

* The reference groups for parity and season of blood draw are primigravida and winter, respectively.