Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia

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OBJECTIVE: Vitamin D deficiency has been linked to adverse pregnancy outcomes. The purpose of this investigation was to assess total 25-hydroxyvitamin D (25-OH-D) levels at diagnosis of early-onset severe preeclampsia (EOSPE).

STUDY DESIGN: After institutional review board approval, we enrolled subjects with EOSPE (<34 weeks' gestation with severe preeclampsia) in this case-control investigation in a 1:2 ratio with gestation-matched, contemporaneous control subjects. Demographic and outcome information was collected for each subject. Plasma total 25-OH-D levels were determined by radioimmunoassay and reported in nanograms per milliliter. Results were analyzed by Mann-Whitney *U* and multivariable regression.

RESULTS: Subjects with EOSPE (n = 50) were noted to have decreased total 25-OH-D levels relative to healthy control subjects (n = 100; $P <$ .001). This difference in total 25-OH-D remained significant after control for potential confounders.

CONCLUSION: Total 25-OH-D is decreased at diagnosis of EOSPE. Further study is needed to understand the impact of vitamin D deficiency on pregnancy outcomes.

Key words: 25-hydroxyvitamin D, adverse pregnancy outcome, preeclampsia, vitamin D

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With the resurgence of rickets in the 1990s, the scientific community has focused increased attention on vitamin D.[1] Vitamin D is a steroid hormone that is derived primarily from synthesis in the skin through exposure to ultraviolet B radiation. Vitamin D undergoes hydroxylation in the maternal liver to form 25-OH-vitamin D (25-OH-D) which is an inactive supply form of this hormone. The active form of vitamin D (1,25-$[OH]_2$-vitamin D) results from the activity of 1-α-hydroxylase in the maternal kidney or placenta.[2] Because the half-life of 1,25-$(OH)_2$-vitamin D is only several minutes, the more accurate assessment of an individual's vitamin D status is determined through measurement of 25-OH-D, which has a half-life of approximately 3 weeks.[3] An adequate 25-OH-D level has been determined to be ≥32 ng/mL. Vitamin D insufficiency and deficiency are diagnosed at levels of <32 ng/mL and <20 ng/mL 25-OH-D, respectively.[4] With these criteria, vitamin D deficiency is very common in pregnancy; up to 50% of the women are classified as vitamin D deficient.[5-8] Vitamin D deficiency has also been noted to have increased incidence among persons of African American race. This deficiency is likely the result of increased melanin content that prevents adequate exposure to ultraviolet B radiation for conversion of 7-dehydrocholesterol within the skin to vitamin D.[2,7,8] With an increased incidence of vitamin D deficiency documented in these populations, there is heightened awareness of the potential impact on pregnancy outcome.

★ EDITORS' CHOICE ★

Vitamin D deficiency has been linked to adverse perinatal outcomes in recent epidemiologic data. Rickets, a hypomineralization of the skeletal structure, is a well-described phenomenon of vitamin D deficiency.[1] More recently, data support associations of vitamin D deficiency and preterm birth, decreased birthweight, and hypertensive disease in pregnancy.[9-14] Authors speculate that these conditions may result from the lack of action of vitamin D in immunosuppression or placental development among deficient patients.[9,15-17] Thus, vitamin D deficiency may be involved in the pathophysiologic condition of preeclampsia.

Preeclampsia remains poorly characterized with regard to pathophysiologic elements involved in the development of hypertensive disease in pregnancy. Preeclampsia has been described as a 2-stage disease in which stage I is heralded by poor placental invasion, development, and remodeling. Stage II develops later and involves the clinical recognition of preeclampsia in the form of maternal hypertension, proteinuria, and end-organ

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disease.[18] Data that suggest an association between preeclampsia and vitamin D deficiency are now developing. In a recent investigation of 25-OH-D levels in pregnancy before the onset of preeclampsia, vitamin D levels that were assessed in early pregnancy were found to be lower among women who eventually experienced preeclampsia. In fact, these investigators noted a 2-fold increased risk for preeclampsia when serum vitamin D levels decreased by 20 ng/mL after adjusting for confounders.[10] Another population-based investigation in Norway among 23,423 nulliparous women found that vitamin D intake of 15-20 μg/day, relative to <5 μg/day, was associated with a 27% reduction in the risk for preeclampsia.[13] Both of these investigations suggest an association between vitamin D deficiency and the development of preeclampsia.

Early-onset severe preeclampsia (EOSPE) contributes 15% of the preterm births in the United States per annum and may also have ongoing increased risks for vascular disease in later life.[19,20] These women and their fetuses are also recognized to be at the greatest risk for adverse outcomes in pregnancy, with a 20-fold increased risk for maternal death and several-fold increased risk for neonatal morbidity or death, dependent on gestational age at delivery and presence of growth restriction in the fetus.[21] Thus, this group may serve as a target population for improving outcomes of preeclampsia.

The purpose of this investigation was to examine the maternal plasma level of 25-OH-D in cases of EOSPE relative to control subjects who experience a normal pregnancy outcome. The hypothesis for this investigation was that women who were diagnosed with EOSPE would have decreased 25-OH-D levels relative to control subjects who experience a normal pregnancy outcome. This association would further support the significance of vitamin D deficiency in preeclampsia.

Materials and Methods

The institutional review board at the Medical University of South Carolina approved this case-control investigation. Patients who were included in this investigation had to provide consent for the collection of demographic and outcome data and venipuncture for collection of plasma to be used in 25-OH-D analysis. Cases were recruited from the inpatient Labor and Delivery unit at the Medical University of South Carolina after confirmation of a diagnosis of EOSPE. EOSPE cases had to meet the American College of Obstetrics and Gynecology criteria for severe preeclampsia and have this diagnosis before 34 weeks of completed gestation.[22] Patients with EOSPE were excluded if they also had a diagnosis of chronic hypertension, pregestational diabetes mellitus, renal disease, lupus, or multifetal gestation. Contemporaneous control patients with a singleton gestation were recruited in a 2:1 match from the ambulatory care setting. Control patients were matched according to race and gestational age at the time of sample collection for the EOSPE case. Control subjects were followed through pregnancy to assess pregnancy outcomes in this cohort of patients. The control patients were excluded for the same exclusion diagnoses for EOSPE cases. Demographic data were collected on each case and control at the time of plasma collection, which included gestational age, maternal age, maternal prepregnancy body mass index, maternal systolic and diastolic blood pressure, and urine protein. Plasma was collected from EOSPE cases at the time of diagnosis. The 2 gestation-matched control samples were also obtained at a similar (within 1 week) gestational age for each EOPSE case. Plasma was collected in a ethylenediaminetetraacetic acid vacutainer tube (BD P100 v1.1; Becton Dickinson Labware, Franklin Lakes, NJ) that contained a protease inhibitor cocktail. Samples were processed and frozen in aliquots within 30 minutes of collection from each subject. The antepartum plasma sample that was collected at the time of diagnosis in EOSPE or matched gestational age for control subjects was assessed for total 25-OH-D in nanograms per milliliter with the use of a double antibody radioimmunoassay (DiaSorin, Stillwater, MN). In our laboratory, this assay has a <10% interassay and intraassay reliability. Vitamin D status was reported for both EOSPE and control groups according to the following 25-OH-D cutpoints: normal, >32 ng/mL; insufficient, ≥20 and ≤32 ng/mL; and deficient, <20 ng/mL.[2] The use of a sample size of 50 patients with EOSPE compared with 100 control subjects would allow detection of a 25% difference in 25-OH-D, with 80% power given an alpha of .05. After delivery, outcome data were collected on both EOSPE cases and control patients that included birthweight, gestational age at delivery, and an assessment of intrauterine growth restriction that was based on <10th percentile birthweight, as assessed by gestational age at delivery.[23]

Results of continuous and categoric variables were reported as median (25 percentile to 75 percentile) and percentage by case or control group, respectively. Bivariable analysis was conducted with the Mann Whitney *U* test for examination of continuous variables (maternal age, prepregnancy body mass index, gestational age at plasma sample collection, gestational age at delivery, mean arterial pressure at sample collection, birthweight, and plasma 25-OH-D levels) by case or control group. Proportions were compared by case or control group with the chi-square test. Unadjusted and adjusted odds ratios and associated 95% CIs were calculated for each covariate based on fitted simple and multiple logistic regressions for the outcome EOSPE. A multiple logistic regression was conducted to estimate the effect of plasma 25-OH-D level on the risk EOSPE with the following additional variables included in the model: prepregnancy body mass index, maternal age, African American race, and gestational age at plasma sample collection. Continuous variables were assessed for linearity in the logit and transformed as necessary. Model adequacy was assessed with the use of the Hosmer Lemeshow goodness-of-fit test. The area under the receiver operator characteristic curve was used to assess the predictive accuracy of the fitted multivariable model. All statistical tests were 2-sided with the alpha set at .05 to control for type I error. Data analysis was performed with SAS software (version 9.2; SAS Institute Inc,

TABLE 1
Demographics and outcomes of pregnancies complicated by early-onset severe preeclampsia vs control subjects

Variable	Group: Early-onset severe preeclampsia (n = 50)	Group: Control (n = 100)	*P* value
Maternal age, y[a]	24 (21–30)	28 (23–32)	.001[b]
Body mass index before pregnancy, kg/m^2[a]	34 (27–38)	28 (24–32)	< .00[b]
African American race, %	48	46	NS[c]
Nulliparous, %	54	47	NS[c]
Gestational age at sample collection, wk	29 (28–31)	29 (26–31)	NS[b]
Gestational age at delivery, wk	29 (28–32)	39 (37–40)	< .001[b]
Mean arterial pressure at collection of sample, mm Hg[a]	125 (116–134)	78 (71–86)	< .001[b]
Birthweight, g[a]	1170 (880–1420)	3260 (2960–3630)	<.001[b]
Intrauterine growth restriction, %[d]	42	10	< .001[c]
Vitamin D status			
Normal: 25-OH-D >32 ng/mL, %	24	47	
Insufficient: 25-OH-D 20-32 ng/mL, %	22	26	
Deficient: 25-OH-D < 20 ng/mL, %	54	27	.005[c]
Plasma 25-OH-D, ng/mL[a]	18 (13–31)	32 (20–44)	< .001[b]

25-OH-D, 25-hydroxyvitamin D; *NS*, not significant.

[a] Data are presented as median (Quartile 1 - Quartile 3); [b] Mann Whitney *U* test; [c] χ^2 test; [d] <10th percentile fetal growth for gestational age.

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Cary, NC). A secondary analysis of 25-OH-D level was examined by race and EOSPE status. Comparison of means by these groups was accomplished by Tukey-Kramer test.

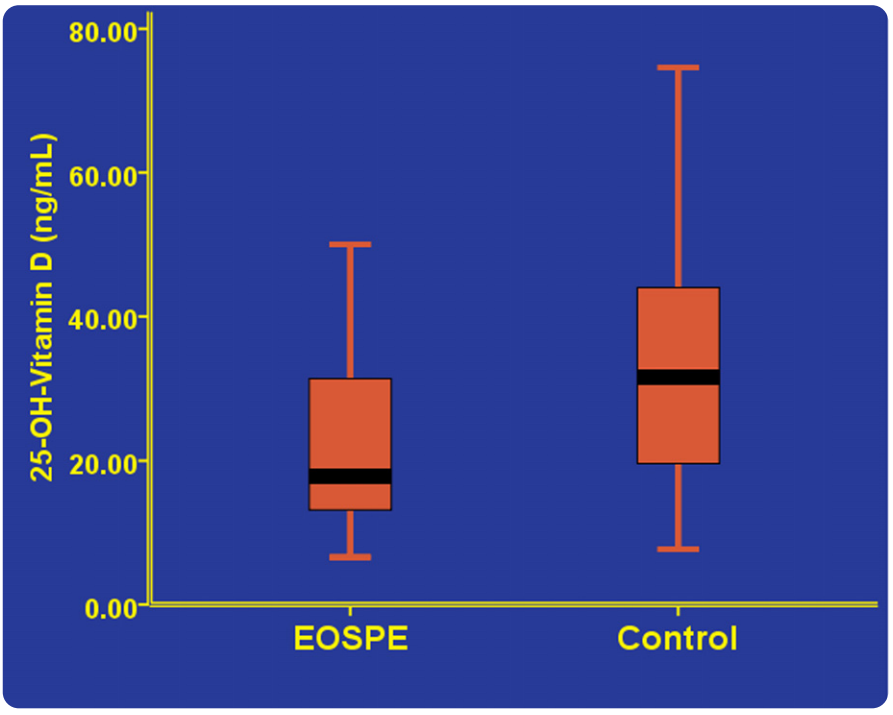
Results

Fifty patients with EOSPE and 100 matched control subjects gave consent and were included in this investigation. Pregnancy demographic and outcomes are summarized by EOSPE or control pregnancy in Table 1. Patients with EOSPE were noted to be younger with a greater prepregnancy body mass index. As expected, all cases of EOSPE were delivered preterm because of their disease with a median gestational age at delivery of 29 weeks, compared with 39 weeks in the control group. The hypertension that was encountered in the EOSPE group was severe, as noted by a median mean arterial pressure of 125 mm Hg, compared with 78 mm Hg at delivery for healthy control subjects. The incidence of intrauterine growth restriction in the EOSPE group was significantly greater compared with healthy control subjects (42% vs 10%; $P < .001$). Plasma 25-OH-D was significantly decreased among patients with EOSPE, compared with healthy control subjects (18 vs 32 ng/mL; $P < .001$) as shown in Figure 1.

To assess the effect of maternal plasma 25-OH-D on the odds of having a diagnosis of EOSPE, a multiple logistic regression analysis was performed. Model fit and predictive accuracy were determined to be adequate by Hosmer-Lemeshow goodness-of-fit ($P = .35$) and area under the receiver operator characteristic curve ($P = .82$), respectively. Results of this analysis are presented in Table 2. After adjustment for all covariates, the association of increased maternal plasma 25-OH-D levels and decreased risk for diagnosis of EOSPE remained statistically significant. Specifically, a 10-ng/mL increase in 25-OH-D yields a 63% decrease in the odds of EOSPE among these patients and would move 83% of this group into either the normal or insufficient category of vitamin D status. A 1-unit increase in the prepregnancy body mass index was also associated with an 8% increase in the odds of EOSPE. African American race was noted to carry a 12-fold increased odds of EOSPE in this investigation. There was no significant effect of the timing of plasma sampling on the odds of EOSPE as expected, given that patients were matched for this characteristic in this case-control investigation. On examination of the bottom quartile of maternal plasma 25-OH-D (≤19.6 ng/mL), there was a 3.6-fold increased odds of diagnosis of EOSPE among this group (odds ratio, 3.60; 95% confidence interval, 1.71–7.58; $P < .001$).

A secondary analysis of 25-OH-D plasma levels was also conducted by race, given the increased odds for EOSPE in African American women that was seen in the regression analysis. In Figure 2, both African American and white women who experienced EOSPE had decreased levels of 25-OH-D, compared with race-matched control subjects (Figure 2). However, African American women who had EOSPE were noted to

FIGURE 1
Maternal plasma 25-hydroxyvitamin D in early-onset severe preeclampsia (*EOSPE*) vs control subjects



Patients with early-onset severe preeclampsia had significantly decreased levels of 25-hydroxyvitamin D relative to healthy control pregnancies that were matched for gestational age at plasma sampling (median 25-hydroxyvitamin D, 18 vs 32 ng/mL; $P < .001$). The probability value was determined by Mann Whitney *U* test.

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have the lowest mean plasma level of 25-OH-D among groups.

Comments

In this investigation, we examined plasma 25-OH-D among those patients who experienced EOSPE, which is 1 of the most serious forms of hypertensive disease in pregnancy. This study found significantly decreased levels of 25-OH-D at the time of diagnosis of EOSPE. To our knowledge, this is the first report of maternal vitamin D status among patients with EOSPE. This is a significant strength of this investigation because future clinical trials will benefit this population most because it contributes 15% of the preterm births that are observed in the United States per annum and significant maternal-fetal morbidity and mortality rates.[19,21] The observations of decreased vitamin D status among African American women who experienced EOSPE and the 12-fold increased risk for diagnosis of EOSPE in the regression model suggests that vitamin D deficiency may be a factor in explaining their disproportionate incidence of adverse pregnancy outcomes. This observation is particularly important because these women also had the most decreased vitamin D levels among the population that was studied (Figure 2). In fact, their 25-OH-D levels in the control group were below that of white women who were diagnosed with EOSPE. This observation may suggest a threshold effect of vitamin D deficiency effect on EOSPE. Future investigations in longitudinal populations of African American women may assist in a better understanding of this observation. Alternatively, African American women may have unrecognized genetic or epigenetic factors that predispose them to EOSPE. Thus, this population may also benefit most from future prospective randomized trials of vitamin D supplementation for improved pregnancy outcomes.

The further examination of vitamin D deficiency and preeclampsia is warranted

TABLE 2
Unadjusted and adjusted odds ratios for early-onset severe preeclampsia

	Unadjusted analysis		Adjusted analysis	
Variable	**Odds ratio (95% CI)**	***P* value[a]**	**Odds ratio (95% CI)[b]**	***P* value[a]**
Maternal plasma 25-hydroxyvitamin D, per 10 ng/mL	0.58 (0.43–0.77)	< .001	0.37 (0.22–0.62)	< .001
Body mass index before pregnancy, kg/m^2 per 1 unit	1.08 (1.03–1.13)	.003	1.08 (1.02–1.15)	.01
Maternal age, y	0.89 (0.83–0.96)	.002	0.87 (0.80–0.96)	.004
African American race	0.93 (0.46–1.88)	NS	12.6 (3.1–50.4)	< .001
Gestational age at sample collection, wk	1.03 (0.82–1.31)	NS	0.94 (0.70–1.27)	NS

Hosmer-Lemeshaw probability value, .35; receiver operator characteristic curve area, 0.82.
CI, confidence interval; *NS*, nonsignificant.

[a] Wald χ^2 test; [b] Adjusted for maternal plasma 25-hydroxyvitamin-D level, body mass index, maternal age, African American race, and gestational age at sample collection.

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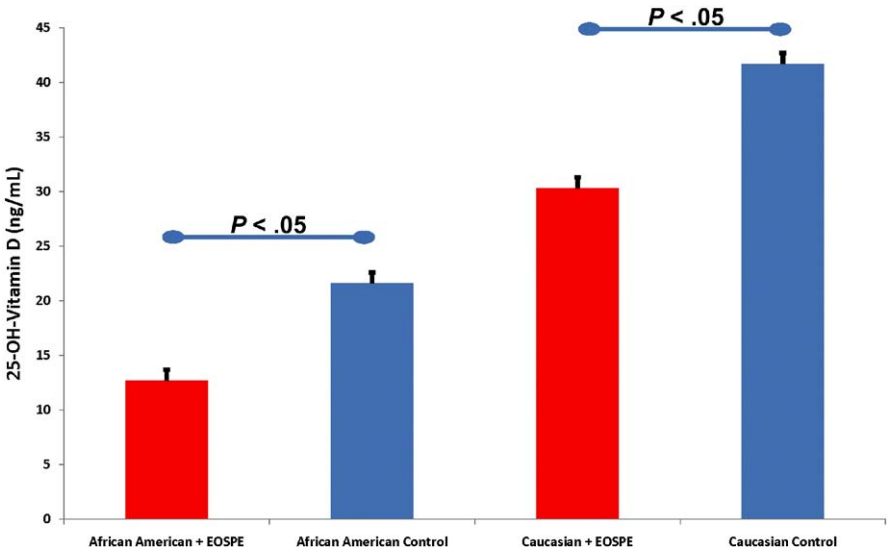
FIGURE 2
Plasma 25-OH-D by race and early-onset severe preeclampsia (*EOSPE*) status



African American and white women with early-onset severe preeclampsia were noted to have decreased plasma levels of 25-hydroxyvitamin D (25-OH-D) relative to matched-race, control pregnancies. African American women were noted to have the lowest plasma 25-OH-D levels when compared with white women. The *bars* represent mean 25-OH-D levels (mean ± SEM). The probability values were determined with the Tukey-Kramer test.

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because there are multiple potential mechanisms whereby a deficiency of vitamin D may contribute to the pathophysiologic condition of preeclampsia. Vitamin D has been implicated in providing critical signals in gene regulation and expression in early placental development among placental trophoblast models.[9,15,16,24,25] In vitamin D deficiency, there is concern that the lack of these signals may play a critical role in stage I of placental development that leads to the ultimate recognition of stage II and a diagnosis of preeclampsia.[15,18,25] It is not known exactly how these signals might lead to the ultimate diagnosis of preeclampsia; therefore, epidemiologic observations from incidence of preeclampsia that are associated with vitamin D deficiency currently lack fully defined pathways through which biomolecular mechanisms explain this relationship. However, based on the observations of this and other studies that link vitamin D deficiency and preeclampsia, vitamin D supplementation remains a possible target for intervention and possible improved pregnancy outcomes.[10,13]

This study has important limitations that should be noted and addressed in future investigations of these populations. There was no assessment of baseline dietary vitamin D intake or sun exposure. Also, given that prenatal vitamins contain 400 IU of vitamin D, compliance with prenatal vitamin use should be measured in populations in which vitamin D is considered. Alterations in these factors may result in effects not measured in our investigation.

This investigation cannot be used alone to assert a role for vitamin D supplementation in pregnancy to improve pregnancy outcomes. Given the collection of samples at the time of diagnosis of EOSPE, this investigation cannot determine the effect of this disease on levels of vitamin D at diagnosis. It is also important to point out that acute effects of preeclampsia on vitamin D physiology are not described in the literature and could also impact levels that are assessed at the time of disease. A longitudinal cohort study would best address the impact of hypertensive disease on vitamin D physiology. However, previous investigations have demonstrated low second-trimester maternal vitamin D levels as an independent predictor of future onset of term preeclampsia.[10,13] Thus, additional well-designed, prospective, randomized trials of supplementation that control for dietary exposure and race will be necessary to determine a potential role for vitamin D in the prevention of preeclampsia. ■

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