Published in final edited form as:

Am J Perinatol. 2014 June ; 31(6): 541–546. doi:10.1055/s-0033-1356483.

Is Midtrimester Vitamin D Status Associated with Spontaneous Preterm Birth and Preeclampsia?

Luisa A Wetta, MD¹, Joseph R Biggio, MD¹, Ms. Suzanne Cliver¹, Adi Abramovici, MD¹, Stephen Barnes, PhD², and Alan TN Tita, MD, PhD¹

¹Center for Women's Reproductive Health and Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, Alabama

²Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, Alabama

Abstract

Objective—To evaluate whether midtrimester maternal vitamin D is associated with preeclampsia <37 weeks or spontaneous preterm birth (SPTB) <35 weeks.

Study Design—Nested case-control comprising 2 case-subsets: a) 100 women with preeclampsia <37 weeks and b) 100 women with SPB <35 weeks. Controls consisted of 200 women delivered between 39–40 weeks. Stored maternal serum obtained between 15–21 weeks was tested for total 25-hydroxy vitamin D levels (25-OH D) using liquid chromatography-tandem mass spectrometry. Mean 25-OH D levels and prevalence of vitamin D insufficiency (25-OH D < 30 ng/mL) and deficiency (25-OH D <15 ng/mL) were compared.

Results—89 preeclampsia, 90 spontaneous preterm birth cases, and 177 controls had valid measurements. Mean midtrimester vitamin D was not significantly different between women with preeclampsia (27.4 ng/mL±14.4) and controls (28.6±12.6) (p=0.46), or SPTB (28.8±13.2) and controls (p=0.92). After adjusting for potential cofounders, neither vitamin D insufficiency (aOR 1.1; 95% CI 0.6–2.0) nor deficiency (aOR 1.4; 95% CI 0.7–3.0) was significantly associated with preeclampsia. Likewise, SPTB was not significantly associated with either vitamin D insufficiency or deficiency (aOR 0.8; 95% CI 0.4–1.4, aOR 1.3; 95% CI 0.6–3.0, respectively).

Conclusion—Midtrimester maternal vitamin D was not significantly associated with preeclampsia <37 weeks or SPTB <35 weeks.

Keywords

Adverse; 25-hydroxy vitamin D; Preeclampsia; Preterm birth

Corresponding Author/Request for Reprints: Luisa Wetta, MD, 176 F 10270T, 619 19th Street South, Birmingham, AL 35249-7333, Phone 205-934-9482, Fax: 205-975-4375, lwetta@uabmc.edu.

Disclosure: The authors report no conflict of interest.

Presented in part as a poster at the Society for Maternal-Fetal Medicine 32nd Annual Meeting, February 6–12, 2012, Dallas, Texas

Introduction

Renewed interest in examining the association between vitamin D status and adverse health outcomes¹ extends to the potential relationship with adverse pregnancy outcomes. Studies have examined the relationships between maternal vitamin D status and preterm birth, gestational diabetes, preeclampsia, and fetal growth abnormalities with contradictory findings.^{2,3,4, 5, 6, 7}

Specifically, low maternal vitamin D levels prior to 22 weeks have been associated with an increased risk of preeclampsia, especially early onset severe preeclampsia in some studies.^{8,9} Intake of vitamin D supplements during pregnancy has also been reported to decrease a subsequent risk for preeclampsia.¹⁰ However, other studies failed to show the same association.⁴ Yet other studies have associated maternal vitamin D status with bacterial vaginosis, a strong correlate of spontaneous preterm birth.¹¹ Different methodologies available for measurement of serum vitamin D, as well as the known variations in vitamin D status by race/ethnicity, season, and location probably account for some of the discrepancy in findings.¹² The objective of our investigation was to further evaluate the relationship between maternal vitamin D status and a) preeclampsia prior to 37 weeks gestation and b) spontaneous preterm birth prior to 35 weeks.

Materials and Methods

We conducted two related nested case-control studies involving women with singleton pregnancies who received prenatal care and delivered at our institution between 2007 and 2008. This cohort was identified using our electronic obstetric database. A random sample of 200 healthy women (without any documented medical or obstetric complications) who delivered at 39 0/7–40 6/7 completed weeks was selected and comprised the control group. Among women with preeclampsia prior to 37 0/7 weeks gestation or with spontaneous preterm birth prior to 35 0/7 weeks, 100 women in each group were also randomly selected to form the corresponding case groups. Gestational age was determined from the EDC determined by the earliest ultrasound. Controls and cases were not individually matched. Women with multiple gestations, fetal anomalies or maternal medical complications (chronic hypertension, pregestational diabetes, or renal disease were excluded). Preeclampsia was defined as new onset hypertension ($\geq 140/90$) and proteinuria (urine dipstick of 1+ or greater or a urine protein to creatinine ratio of greater than 0.3, or a 24 hour urine protein ≥ 300 mg). The diagnosis of preeclampsia was validated by chart review. Spontaneous preterm birth was defined as any birth due to either progressive preterm labor or preterm premature membrane rupture leading to spontaneous or induced delivery.

Maternal vitamin D status was based on serum 25-hydroxy vitamin D (25-OH D) levels determined by the gold standard for measurement, liquid chromatography-mass spectrometry method.¹³ Cases and controls were matched to their stored serum remnant specimens that had been obtained from a midtrimester sample drawn between 15–21 weeks gestation for maternal serum aneuploidy screening.

The specimens were remnants of blood used for maternal serum screening that were originally obtained in tiger top tubes. The specimens were sent to the lab and the remnant aliquots from the maternal serum screening were stored in polyallomer non-toxic storage vials and frozen at -80°C until analyzed. Specimens had not previously been thawed. Total 25-OH D levels in the stored specimens available were measured utilizing a validated liquid chromatography-tandem mass spectrometry method. 25-OH D2 (Sigma 17937-1MG; Sigma-Aldrich; St. Louis, MO) and 25-OH D3 (Sigma H4014-1MG; Sigma-Aldrich; Saint Louis, MO) were each dissolved in 1 mL ethanol for a 1 mg/mL stock solution. The internal standard was deuterated (9)-tetrahydrocannabinol, purchased as a 100 $\mu\text{g/mL}$ solution (Cerrilant T-003, Cerilliant Corporation; Round Rock, TX). All dilutions were carried out in methanol. Mobile phase A consisted of 0.1% formic acid in water. Mobile phase B consisted of methanol + 0.1% formic acid. The initial condition of the gradient was 80% B, increasing to 100% B at 10 minutes. The gradient was held at 100%B from 10 to 12 minutes, returning to initial conditions (80%B) at 13 minutes. The gradient was then held at 80%B until 20 minutes to allow equilibration of the column. The column was a 2 x 250 mm Synergi Hydro-RP 80A with a guard column assembly (Phenomenex; Torrance, CA). The flow rate was 0.4 mL/min, the column temperature was 60°C and the samples were stored in the autosampler at 4°C . Injection volume was 20 μL . Each sample was extracted by adding 1.2 mL of diethyl ether to 0.2 mL serum. Samples were then vortexed and centrifuged at 3200 rpm for 5 minutes at 4°C . The organic layer was removed and dried under nitrogen gas.

Mean 25-OH D levels were compared between each case group and controls using the Student t-test. Frequency of vitamin D insufficiency (defined as a 25-OH D level less < 30 ng/mL) and deficiency (defined as a 25-OH D level of <15 ng/mL) were also compared to the controls. Similar cut-offs for insufficiency and deficiency have been previously used in the literature.⁴ Logistic regression was used to control for multiple variables including age, race/ethnicity, parity, history of a prior preterm birth (both spontaneous preterm birth and indicated preterm birth for preeclampsia), maternal weight at first prenatal visit, smoking status, gestational age at which the sample was drawn and time of year specimen obtained (since vitamin D synthesis varies by the amount of sunlight exposure, this was categorized into October–April with less sunlight vs. May to September). Institutional review board approval was obtained at the University of Alabama at Birmingham.

Results

The cases and controls were selected from an eligible cohort of women who received prenatal care and delivered at our institution from 2007 to 2008, comprising 200 healthy women delivering at 39–40 weeks, 100 with preeclampsia at less than 37 weeks and 100 with spontaneous PTB prior to 35 weeks.

A total of 177 controls (88.5%), 89 cases of preeclampsia less than 37 weeks (89%) and 90 cases with spontaneous preterm birth prior to 35 weeks (90%) had remnant specimens available for study. The descriptive characteristics of these women are presented in Table 1. Compared with controls, preeclampsia cases were more likely to be African American (less likely Hispanic) and nulliparous with higher mean maternal weight; there were no

differences in maternal age and period of collection. As expected, mean gestational age and birth weight were significantly lower among preeclampsia cases. Similar findings were noted when comparing spontaneous preterm birth cases with controls, except maternal race/ethnicity and weight were not significantly different. Smoking status did differ between cases and controls and those women with a spontaneous preterm birth less than 35 weeks were more likely to be smokers.

Comparison of vitamin D status between cases and controls is presented in Table 2. Mean midtrimester values of 25-OH D were not different between controls and preeclampsia cases (28.6 ± 12.6 versus 27.4 ± 14.4 , $p=0.46$). Similarly, mean midtrimester 25-OH D levels were not different between controls and spontaneous preterm birth less than 35 weeks (28.6 ± 12.6 versus 28.8 ± 13.2 , $p=0.92$).

We conducted analyses stratified by period of specimen collection for vitamin D status. Among women with specimens drawn between May and September when there is ample sunlight, preeclampsia was significantly associated with vitamin D deficiency, OR 3.8 (95% CI 1.08–13.60). This association was not observed in women who had specimens drawn between October and April, OR 1.1 (95% CI 0.46–2.46). However, a Breslow-Day test of heterogeneity between subgroups was not significant ($p=0.09$). Spontaneous preterm birth less than 35 weeks was not significantly associated with vitamin D deficiency whether the specimen was drawn between May and September or October and April (OR 3.14; 95% CI 0.83–11.92, OR 1.02; 95% CI 0.45–2.31, respectively). A Breslow-Day test of heterogeneity between subgroups was not significant ($p=0.15$).

After controlling for differences in age, race, parity, weight, history of a prior preterm birth, smoking status, gestational age at which specimen was drawn and season of specimen collection, preeclampsia <37 weeks was neither associated with vitamin D insufficiency (Adjusted Odds Ratio 1.1; 95% CI 0.6–2.0) nor vitamin D deficiency (Adjusted OR 1.4; 95% CI 0.7–3.0)(Table 3). In the adjusted model evaluating vitamin D insufficiency, as expected, preeclampsia was associated with increasing maternal age (OR 1.1; 95% CI 1.0–1.2), nulliparity (OR 6.8; 95% CI 3.2–14.5) and a history of a prior preterm birth (OR 5.6; 95% CI 2.4–13.0).

Similarly, after controlling for age, race, parity, weight, prior preterm birth and season of specimen collection, spontaneous preterm birth less <35 weeks was associated with neither vitamin D insufficiency (Adjusted OR 0.8; 95% CI 0.4–1.4) nor vitamin D deficiency (Adjusted OR 1.3; 95% CI 0.6–3.0)(Table 3). As expected, spontaneous preterm birth less than 35 weeks was associated with nulliparity (OR 4.1; 95% CI 2.0–8.4) and a history of a prior preterm birth (OR 8.1; 3.5–18.3) in the adjusted model evaluating vitamin D insufficiency.

Comment

In our population, midtrimester vitamin D status was not significantly associated with the development of preeclampsia at less than 37 weeks gestation or spontaneous preterm delivery less than 35 weeks. Specifically, the results were the same when vitamin D status was based on mean levels or when cut-offs defining vitamin D insufficiency (<30ng/mL) or

deficiency (<15ng/mL) were used. It remains feasible that with a larger sample size providing greater power to detect a difference, preeclampsia may be modestly associated with vitamin D deficiency (OR 1.4; 95% CI 0.7–3.0).

The findings of our study are consistent with those from 2 recent smaller case-control studies examining similar adverse outcomes.^{14,15} Cumulatively, vitamin D levels or cut-offs defining insufficiency or deficiency were not associated with spontaneous preterm birth or preeclampsia.^{14,15} It is reassuring that the levels of vitamin D are generally consistent with observations from prior studies and the relationships between preterm birth or preeclampsia and factors such as nulliparity or prior preterm birth reflect current knowledge. Our results, however, differ from those of other studies that have associated low vitamin D levels with early-onset preeclampsia less than 34 weeks.⁹

A potential reason for the discrepant findings is the fact that vitamin D levels were measured at time of delivery in studies that showed an association compared with early pregnancy measurement in ours and studies with similar negative results.^{4,8,9,11,14,15} Furthermore, we examined preeclampsia less than 37 weeks whereas a positive study examined preeclampsia less than 34 weeks.⁹ The results of the subgroup analysis by period of specimen collection may be viewed by some to support the potential for an association between vitamin D deficiency and preeclampsia – patients who are deficient in the summer months likely represent those with the lowest vitamin D levels at any time. This is a question for future studies.

Relating first and midtrimester levels, as well as delivery levels of vitamin D to adverse pregnancy outcomes is more desirable in order to account for a potential cause and effect relationship (preexistent temporal association) and the relevant induction period. Furthermore, the majority of prior studies have evaluated mostly women of Caucasian background and not women of African American race who, because of melanin inhibition of UVB-mediated synthesis of vitamin D, are at increased risk for insufficiency or deficiency.¹⁶ Vitamin D differences have been proposed as a possible explanation for the racial disparity in preterm birth and preeclampsia that is observed.¹⁷ However, our study, which consisted of mostly African American women, does not support a major role for vitamin D insufficiency in spontaneous preterm birth and preeclampsia up to 37 weeks.

The limitations of our study include its retrospective nature, the relatively small sample size (although larger compared with many similar studies) and potential for residual confounding. Our sample size may have limited our ability to fully ascertain potential associations between preeclampsia and a role for deficiency or lower vitamin D thresholds. We did, however, perform a thorough chart review to confirm the diagnosis of preeclampsia in our cohort. Other strengths of our study include adequate representation of the high risk sub-group of African Americans, and the use of the gold standard method of vitamin D quantification.

According to ACOG, there is currently a lack of information and data to draw any definitive conclusions regarding vitamin D's role in adverse pregnancy outcomes.¹⁸ Overall our study adds to the accumulating data to clarify this issue. Several problems arise in trying to

compare data across multiple different studies. First, there are several different assays available to measure vitamin D. These include ELISA, RIA, EIA, and HPLC-MS.¹³ Though HPLC is considered to be the gold standard, not all studies have used this assay, and there is known to be a significant difference in inter-assay performance.^{12,13} Secondly, levels of vitamin D across studies have been measured at different times during pregnancy. Also, there is currently no consensus on a cutoff for vitamin D deficiency in the pregnant population, as most studies extrapolate cutoffs from the non-pregnant population.¹⁸ Perhaps most importantly are differences in outcome and gestational age examined. Robinson et al. reported an aOR of 3.6; 95% CI 1.71–7.58 for the association of preeclampsia less than 34 weeks and low vitamin D.⁹ We report an aOR of 1.4; 95% CI 0.70–3.00 for vitamin D deficiency and preeclampsia less than 37 weeks. Therefore it remains possible that with a large sample our findings may support such an association. However, all the differences in ethnicity, geographic location, and gestational age at vitamin D measurement make it difficult to generalize across different studies with any high degree of accuracy. Future studies may focus on well-defined subgroups evaluating those who have vitamin D deficiency or more extreme, especially during the sunlight periods when levels are supposed to be optimal, and the impact on early onset preeclampsia.

Acknowledgments

Funded in part by a UAB Faculty Development Grant award to Dr. Tita

We would like to acknowledge Mr. Doyle Ray Moore, Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, Alabama for his assistance with 25-hydroxy vitamin D (25-OH D) measurements. Measurements were performed in the Targeted Metabolomics and Proteomics Laboratory at the University of Alabama at Birmingham. Funds for the operation of the Targeted Metabolomics and Proteomics Laboratory come in part from the UAB O'Brien Acute Kidney Injury Center (P30 DK079337), the UAB Skin Disease Research Center (P30 AR50948), the UAB Lung Health Center and the UAB Center for Free Radical Biology.

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Table 1

Patient characteristics and outcomes

Characteristic	Controls N=177	Preeclampsia <37 weeks N=89	P-value*	SPTB <35 weeks N=90	P-value [†]
Race/Ethnicity (N)					
African American	97 (54.8%)	60 (67.4%)	0.02	62 (68.9%)	0.05
Hispanic/Other	49 (27.7%)	11 (12.4%)		14 (15.6%)	
Caucasian	31 (17.5%)	18 (20.2%)		14 (15.6%)	
Nulliparous (N)	57 (32.2%)	44 (49.4%)	0.01	44 (48.9%)	0.01
History of a prior preterm birth	20 (22.5%)	16 (9.0%)	0.00	25 (27.8%)	<0.001
Smoking[‡]	47 (27.0%)	23 (27.4%)	0.95	34 (39.5%)	0.04
Maternal age-yrs	25.2 ± 5.6	26.1 ± 5.5	0.20	23.8 ± 5.6	0.05
Maternal weight (at first prenatal visit) – kg	71.7 ± 18.0	77.2 ± 21.1	0.03	73.2 ± 20.7	0.55
Gestational age specimen drawn -wks	17.3 ± 3.4	17.2 ± 2.4	0.87	17.3 ± 2.2	0.87
Specimen collected (Oct–April)	106 (59.9%)	46 (51.7%)	0.20	52 (57.8%)	0.74
Birthweight – gm	3355 ± 476	1874 ± 673	<0.001	1546 ± 524	<0.001
GA at birth - wks	39.4 ± 0.5	33.2 ± 3.1	<0.001	30.1 ± 3.2	<0.001

* Preeclampsia less than 37 weeks versus controls, all values are N(%), except where mean ± SD

[†] Spontaneous preterm birth (SPTB) less than 35 weeks versus controls[‡] Smoking status only available on 174 controls, 84 women with preeclampsia <37 weeks, and 86 women with spontaneous preterm birth <35 weeks

Table 2

Vitamin D status between cases and controls

Vitamin D Status	Controls N=177	Preeclampsia <37 weeks N=89	P-value*	SPTB < 35 weeks N=90	P-value†
Mean 25-OH D (ng/mL)	28.6 ± 12.6	27.4 ± 14.4	0.46	28.8 ± 13.2	0.92
Normal: 30ng/mL	76 (42.9%)	33 (37.1%)	0.45	39 (43.3%)	0.62
Insufficient: <30ng/mL	75 (42.3%)	38 (42.7%)		34 (37.8%)	
Deficient: <15ng/mL	26 (14.7%)	18 (20.2%)		17 (18.9%)	

* Preeclampsia less than 37 weeks versus controls, all values are N(%), except where mean ± SD

† Spontaneous preterm birth (SPTB) less than 35 weeks versus controls

Table 3

Unadjusted and Adjusted OR for Vitamin D cases and controls

Vitamin D Status	Unadjusted OR (95% CI)	Adjusted OR (95%CI)
Preeclampsia <37weeks		
Normal: 30ng/mL	----Referent----	----Referent----
Insufficient: <30ng/mL	1.3(0.8–2.2)	1.1(0.6–2.0)
Deficient: <15ng/mL	1.5(0.8–2.9)	1.4(0.7–3.0)
SPTB<35 weeks		
Normal: 30ng/mL	----Referent----	----Referent----
Insufficient: <30ng/mL	1.1 (0.6–2.2)	0.8(0.4–1.4)
Deficient: <15ng/mL	1.5 (0.7–3.5)	1.3 (0.6–3.0)