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A Nested Case-Control Study of First-Trimester Maternal Vitamin D Status and Risk for Spontaneous Preterm Birth

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Abstract

We assessed if first-trimester vitamin D deficiency is more prevalent in women who experienced a spontaneous preterm birth compared with women who delivered at term. We conducted a nested case-control study of pregnant women who had previously given blood for first-trimester combined screening for trisomy 21 and subsequently delivered at a tertiary hospital between November 2004 and July 2009. From an overall cohort of 4225 women, 40 cases of spontaneous preterm birth (23 0/7 and 34 6/7 weeks) were matched by race/ethnicity with 120 women delivering at term (37 0/7 weeks) with uncomplicated pregnancies. Banked maternal serum was used to measure maternal 25-hydroxyvitamin D [25(OH)D]. The prevalence of first-trimester maternal vitamin D deficiency [25(OH)D <50 nmol/L] was comparable among women who subsequently delivered preterm compared with controls (7.5% versus 6.7%, $p=0.90$). The median 25(OH)D level for all subjects was 89 nmol/L (interquartile range, 73 to 106 nmol/L). Seventy-three percent (117/160) of the cohort had sufficient vitamin D levels [25(OH)D ≥ 75 nmol/L]. In a cohort of pregnant women with mostly sufficient levels of first-trimester serum 25(OH)D, vitamin D deficiency was not associated with spontaneous preterm birth.

Keywords

Preterm birth; vitamin D; 25-hydroxyvitamin D

Prematurity is the most common cause for mortality among nonanomalous infants born in the United States. Surviving premature infants have significant morbidity.¹ Unfortunately, efforts to prevent, predict, or delay preterm birth have had limited success.^{2,3} In addition to

the morbidity of prematurity for the neonate, mothers who deliver preterm infants may have an elevated risk for cardiovascular disease later in life. Several large epidemiological studies have demonstrated a two- to threefold higher risk for cardiovascular death in women who delivered preterm compared with women delivering at term.^{4,5} The mechanism underlying this increased risk is not well understood. Evaluating pathophysiological changes associated with preterm birth that are amenable to therapy could therefore impact both neonatal and maternal outcomes.

The etiology of spontaneous preterm birth is multifactorial. Contributing factors include uterine over-distention, abnormal fetal endocrine activation, and uterine infection and inflammation.⁶ An emerging area of study that has garnered significant attention is the role of vitamin D and its active metabolite, 1,25(OH)₂D. Increased production of inflammatory cytokines, such as tumor necrosis factor- α , has been reported in pregnancies complicated by vitamin D deficiency.⁷ Furthermore, 1,25(OH)₂D stimulates the activity of T-regulatory cells, which support placental implantation through immune tolerance.⁸ These data support a possible role for vitamin D in preventing spontaneous preterm birth through anti-inflammatory and immunomodulatory effects. Consequently, our objective was to assess if first-trimester vitamin D deficiency is more prevalent in women delivering preterm. We hypothesized that early pregnancy levels of vitamin D are lower among women who later experience a spontaneous preterm birth, compared with healthy women who later deliver at term.

MATERIALS AND METHODS

Study Design

We conducted a nested case-control study in a cohort of 4225 women. All women who had previously given blood for first-trimester combined screening (measurement of nuchal translucency, pregnancy-associated plasma protein A, and the free β subunit of human chorionic gonadotropin) for trisomy 21 and subsequently delivered at the University of North Carolina–Chapel Hill, between November 2004 and July 2009 were eligible. Nonfasting blood samples were collected for routine genetic multiple marker screening between 11 and 14 weeks' gestation, and serum aliquots were bar-coded and frozen at -70°C . This study was approved by the Institutional Review Board at the University of North Carolina–Chapel Hill prior to data collection, and permission was obtained to use banked serum from these women for research purposes.

Using a standard data collection sheet, two investigators (A.B. and S.H.) abstracted demographic characteristics and obstetric and neonatal outcomes from prenatal and inpatient medical records. The following maternal characteristics were based on self-report: height, prepregnancy weight, and date of last menstrual period. Gestational age was determined by menstrual dating. In cases of uncertain menstrual dates, ultrasound estimates of gestational age were used. Maternal body mass index was calculated from the patient's self-reported height and prepregnancy weight. Other abstracted variables included race/ethnicity, maternal health insurance type, and chronic maternal illness such as pregestational hypertension, diabetes, liver or kidney insufficiency, or rheumatologic disorders.

Preterm birth was defined as spontaneous non-iatrogenic delivery at 23 0/7 and 34 6/7 weeks' gestation. We used this gestational age range to minimize inclusion of pregnancy losses associated with cervical incompetence. In addition, the upper limit of gestational age was chosen to exclude term deliveries (< 37 weeks) that may have been assigned an incorrect gestational age. Exclusion criteria included medically indicated preterm delivery, multiple gestation, major congenital fetal anomalies, placenta previa, preeclampsia, pregestational hypertension, kidney disease, diabetes mellitus, known thrombophilias, or any other significant preexisting chronic medical disease. Women who experienced preterm premature rupture of membranes and who were later delivered for chorioamnionitis, nonreassuring fetal testing, or completion of 34 weeks' gestation were included.

From the total cohort of 4225 women, 118 women delivered between 23 0/7 and 34 6/7 weeks, and 43 met all criteria listed above. Of the 43 cases, 40 had an adequate volume of serum available for analysis. These cases were matched by race/ethnicity, in 3:1 ratio, to a random computer-generated referent group of 120 healthy women delivering at term (< 37 weeks) using the same exclusion criteria. Assuming a 25% rate of vitamin D deficiency in our control group, an increase in vitamin D deficiency of 25% in our case group (50%), and α of 0.05, we needed a sample size of at least 148 patients (cases: 37, controls: 111) to achieve 80% power.

Laboratory Analyses

Serum aliquots of cases and controls were shipped on dry ice to Massachusetts General Hospital (MGH, Boston, MA) for serum 25-hydroxyvitamin D [25(OH)D] measurement by liquid chromatography-tandem mass spectrometry (LC-MS).⁹ The method used is an isotope dilution, LC-MS assay optimized in MGH laboratory based on published procedures.¹⁰ The limit of detection is 5 nmol/L for D2 and 7.5 nmol/L for D3. The between-run coefficient of variation for a quality control serum containing a total vitamin D concentration of 57 nmol/L is 7.5%. Based on definitions of vitamin D status in the literature,^{11,12} we categorized 25(OH)D $\geq$ 75 nmol/L as sufficient and 25(OH)D 50 to 74.9 nmol/L as insufficient. Vitamin D deficiency was defined as 25(OH)D <50 nmol/L.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics software (version 19.0, SPSS, Inc., Chicago, IL) and summarized using descriptive statistics. We performed unadjusted analyses using Wilcoxon-Mann-Whitney and Fisher exact tests to compare differences between cases and controls. All *p* values were two-tailed, with *p* < 0.05 considered statistically significant. Multivariable logistical regression was performed to evaluate independent predictors of spontaneous preterm birth with results reported as odds ratios (ORs) with 95% confidence intervals (CIs).

RESULTS

We successfully analyzed 25(OH)D levels from all 160 samples (40 cases; 120 controls). The median gestational age of serum collection was similar for the two groups (13 weeks). As shown in Table 1, demographic and clinical characteristics between the groups in early

pregnancy were similar. The incidence of spontaneous preterm birth as defined in this study was 2.8%. Women with a spontaneous preterm birth delivered on average 10 weeks earlier than controls (29 versus 39 weeks, $p < 0.001$). The median 25(OH)D level for all subjects was 89 nmol/L (interquartile range, 73 to 106 nmol/L). Seventy-three percent (117/160) of the cohort had sufficient vitamin D levels [25(OH)D $\geq$ 75 nmol/L].

The prevalence of first-trimester maternal 25(OH)D deficiency (<50 nmol/L) was low both among women later experiencing a spontaneous preterm birth and among women who delivered at term (7.5% versus 6.7%, respectively; $p = 0.90$). In unadjusted models, first-trimester maternal 25(OH)D <50 nmol/L was not associated with spontaneous preterm birth (OR 1.14; 95% CI, 0.31 to 4.26) compared with first-trimester levels of $\geq$ 75 nmol/L (Table 2). We similarly found no association after adjustment for maternal age, insurance status, body mass index, gestational age at serum collection, and season of blood draw (adjusted OR, 0.82; 95% CI, 0.19 to 3.57).

DISCUSSION

In this study, we measured the association between first-trimester vitamin D deficiency and spontaneous preterm birth. We found no difference in prevalence of early pregnancy vitamin D deficiency [defined as 25(OH)D <50 nmol/L] between women delivering preterm or at term. However, the prevalence of vitamin D deficiency in our study cohort was only 6.9%, with 11 of 160 women falling into this category. This is much lower than recently published data reporting a 33% rate of vitamin D deficiency in a nationally representative sample of pregnant women in the United States.¹³

There are sparse data on vitamin D status in pregnancy and its association with spontaneous preterm birth. Morley et al examined maternal serum 25(OH)D in relation to gestational length. They reported that 25(OH)D <28 nmol/L at 28 to 32 weeks' gestation was associated with a 0.7-week shorter total gestational length.¹⁴ However, this study was limited in its ability to assess this outcome as only 4% (14/374) of these pregnancies delivered before 36 weeks. Mehta et al studied a large cohort of HIV-infected pregnant women in Tanzania to see if vitamin D status affected perinatal outcomes.¹⁵ The rate of preterm delivery <37 weeks in their study was high at 23% (204/884), and they did not find an association between low 25(OH)D at 12 to 27 weeks and risk of preterm birth.

Our findings must be interpreted in the context of the study design. A surprisingly high number of women in our cohort had sufficient vitamin D status. There are several possible explanations for this finding. Perhaps the most significant factor is that the majority of the patients in this study were privately insured (88%) and thus more likely to be taking nutritional supplements and be physically active.¹⁶ Unfortunately, we do not have information regarding supplement use or exposure to sunlight in this study. Although there is no uniform agreement as to "optimal" vitamin D status, the vitamin D levels necessary to prevent rickets are much lower than levels that have been associated with non-bone-related outcomes.¹⁷ For examples, 25(OH)D <75 nmol/L is associated with periodontal disease,¹⁸ and levels <50 nmol/L are associated with severe preeclampsia.¹⁹ We cannot determine an

optimal cut point for spontaneous preterm birth given our small sample size and small number of women with 25(OH)D <75 nmol/L.

Our study population also may not be generalizable to all pregnant women. The prevalence of vitamin D deficiency in pregnancy is highest among African-American women.²⁰ Our study population was more than 50% Caucasian, which limits extrapolation to ethnically diverse groups. Furthermore, the incidence of spontaneous preterm birth was low, with 2.8% of women delivering at 23 to 35 weeks' gestation.

Selection bias is also a concern in any case-control study, especially in the selection of controls. It is challenging to match for all potential covariates, and it is possible that unmeasured differences exist between the two study groups. However, our controls were nested within a large cohort of women who provided serum samples as part of routine prenatal screening, reducing selection bias. An important strength of our study is that maternal serum was collected well before delivery at a time when no clinical manifestations of preterm labor were evident, reducing the likelihood that subclinical disease affected vitamin D levels.

In conclusion, in a cohort of women with a median serum 25(OH)D of 89 nmol/L and low levels of spontaneous preterm birth (2.8%), first-trimester vitamin D status was not associated with spontaneous preterm birth. Future studies in high-risk, racially diverse, and low-income populations are needed to further address the association of vitamin D status and spontaneous preterm birth.

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Table 1

Clinical and Demographic Characteristics of Women Who Delivered Preterm and Race/Ethnicity-Matched Women Who Did Not (Controls)

Variables	Controls (<i>n</i> =120)	Preterm Birth (<i>n</i> =40)	<i>p</i>
Age (y)*	33 (30–36)	35 (30–37)	0.50
Race/ethnicity, <i>n</i> (%)			-
White	63 (53)	21 (53)	
Black	39 (33)	13 (33)	
Hispanic	12 (10)	4 (10)	
Other	6 (5)	2 (5)	
Multiparous, <i>n</i> (%)	64 (53)	21 (53)	0.99
Private insurance, <i>n</i> (%)	105 (88)	36 (90)	0.78
Body mass index *	25 (22–28)	29 (25–34)	0.26
Gestational age at serum collection (wk)*	13 (12–13)	13 (12–13)	0.53
Gestational age at delivery (wk)*	39 (39–40)	29 (26–33)	<0.001
Season of blood draw, <i>n</i> (%)			0.06
Winter	36 (30)	6 (15)	
Spring	26 (22)	8 (20)	
Summer	28 (23)	18 (45)	
Fall	30 (25)	8 (20)	
Serum 25(OH)D level, <i>n</i> (%)			0.91
<50 nmol/L	8 (6.7)	3 (7.5)	
50–74.9 nmol/L	24 (20)	8 (20)	
75 nmol/L	88 (73.3)	29 (72.5)	

* Values are median (interquartile range).

Wilcoxon-Mann-Whitney test for continuous variables; Fisher exact test for categorical variables. 25(OH)D, 25-hydroxyvitamin D.

Table 2

Unadjusted and Adjusted ORs for Preterm Birth According to Vitamin D Status

Serum 25(OH)D Level (nmol/L)	Controls (n)	Preterm Birth (n)	Unadjusted OR (95% CI)	p Value	Adjusted OR (95% CI)*	p Value
75	88	29	1.00 (Reference group)	—	1.00 (Reference group)	—
50–74.9	24	8	1.01 (0.42–2.46)	0.61	0.87 (0.34–2.25)	0.77
<50	8	3	1.14 (0.31–4.26)	0.99	0.82 (0.19–3.57)	0.79

* Adjusted for maternal age (continuous), insurance status (dichotomous), body mass index (continuous), gestational age at serum collection (continuous), and season of blood draw (winter, spring, summer, fall).

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio.