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1,25-Dihydroxyvitamin D Deficiency Is Associated With Preterm Birth in African American and Caucasian Women

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Abstract

Vitamin (vit) D deficiency and preterm birth (PTB) are more prevalent among African American (AA) women compared to caucasian (Cau) women. Because vit D is important in regulating cell-mediated immune responses, vit D insufficiency or deficiency during pregnancy may enhance inflammation in pregnant women and increase the risk of PTB. In this study, circulatory levels of 25-hydroxy (OH) and 1,25-dihydroxy $(OH)_2$ vit D were measured using chemiluminescence and radioimmunoassay techniques, respectively, in AA ($n = 108$) and Cau ($n = 84$) women who delivered at term and preterm. The results from this study suggest that the serum levels of the 25-(OH) vit D concentrations tend to decrease ($P = .06$) in the Cau women who delivered at preterm compared to those delivering at term. However, the 25-(OH) vit D levels in Cau and AA between term and preterm deliveries were not significantly different. The serum levels of 1,25-$(OH)_2$ vit D were found to be significantly lower in AA women compared to Cau women ($P < .02$) at term, and in the Cau ($P < .01$) and AA ($P < .04$) women delivering at preterm compared to those delivering at term. One-way analysis of variance demonstrated that 1,25-$(OH)_2$ vit D levels were significantly lower in participants delivering at preterm (<34 weeks and between 34 and 37 weeks) compared to those delivering at term (>37 weeks).These results suggest that low levels of serum 1,25-$(OH)_2$ vit D are associated with PTB, and vit D can potentially be used as a novel diagnostic marker in the detection of PTB.

Keywords

preterm birth, African Americans, Caucasians, 25-(OH) vitamin D, 1,25-$(OH)_2$ vitamin D, serum levels

Introduction

A poor understanding of spontaneous preterm birth (PTB) or its risk factors and a lack of reliable biomarkers contribute to the difficulty in preventing, treating, and diagnosing PTB early.[1] Hypovitaminosis D and PTB are more prevalent among African Americans (AAs) than their Caucasian (Cau) counterparts.[2-5] Vitamin D (vit D) deficiency in AA is attributed to reduced UV light penetration through skin due to higher melanin pigmentation and consequent decrease in the cutaneous vit D synthesis.[6,7] Racial differences could also be attributable to varying dietary intake of vit D. However, studies to date failed to confirm a difference in dietary vit D3 intake between AA and Cau.[8] Vit D3 is produced primarily in the skin in response to sunlight; it is also obtained in small amounts from animal-based foods. Vit D2 is obtained from plant-based foods. Vit D2 and D3 are rapidly metabolized as follows: first into their respective inactive 25-hydroxyvitamin D (25-(OH) vit D) forms, 25-(OH) vit D2 and 25-(OH)vit D3, and then into active 1,25-$(OH)_2$ vit D2 and D3. In humans, both 1,25-$(OH)_2$ vit D2 and D3 bind to their receptors in the intestine, kidney, and bone; along with parathyroid hormone, 1,25-$(OH)_2$ vit D reportedly regulates plasma calcium and phosphate concentration.[9] In addition to its classical functions, 1,25-$(OH)_2$ vit D reportedly plays a role in cell proliferation,[10] innate,[11,12] and adaptive immunities.[13] The most common conditions that are associated with PTB are infection and inflammation.[14,15] Vit D is reported to inhibit the nuclear factor-κB (NFκB) pathway and reduce inflammation

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in decidua.[16] Therefore, women with lower levels of 25-(OH) vit D during pregnancy may be at risk for increased inflammation and PTB. However, to our knowledge there is no study attributing vit D deficiency to the incidence of PTB. We hypothesize that an insufficiency (21-29 ng/mL) or a deficiency ($\leq$20 ng/mL) of 25-(OH) vit D and a consequent decrease in 1,25-$(OH)_2$ vit D increase the risk of PTB. Serum levels of 25-(OH) vit D are the accepted means for determining the vit D status. Because 25-(OH) vit D is converted into the active form, 1,25-$(OH)_2$ vit D, which initiates physiological actions binding to the receptors, we measured 1,25-$(OH)_2$ vit D along with 25-(OH) vit D in term and preterm AA and Cau women from middle Tennessee and assessed whether hypovitaminosis D is a risk factor for PTB in these ethnicities.

Materials and Methods

Participants

Participants were women from 2 racial groups: Cau and AA who delivered at term (>37 weeks of gestation; AA = 65 and Cau = 48) or preterm (24-37 weeks of gestation; AA = 43 and Cau = 36). In this retrospective case–control study, women were recruited at Centennial Women's Hospital in Nashville, Tennessee, between September 2003 and December 2005. Race was identified using a self-report questionnaire that traced ancestry back to 3 generations as described earlier.[17] Participants included in the study had singleton deliveries and were between 18 and 40 years of age. Gestational age was determined by last menstrual period and corroborated by ultrasound dating. The study was approved by the Tristar Nashville Institutional Review Board (IRB) at Centennial Medical Center and IRB at Meharry Medial College, Nashville, Tennessee. All participants provided informed consent.

Exclusion Criteria

Women with multiple gestations, preeclampsia, placenta previa, fetal anomalies, gestational diabetes, poly- and oligohydramnios, and other pregnancy complications, such as surgery, were excluded. The control group comprised women with term deliveries with no medical, surgical, or obstetrical complications during pregnancy.

Sample Collection

Blood samples were collected at the time of delivery from Cau (term, n = 47; preterm, n = 35) and AAs (term, n = 64; preterm, n = 41) in tubes containing clot activator and gel. Serum was prepared by centrifuging the blood samples at 900*g* and 4°C for 20 minutes and stored at −20°C until analysis.

Hormone Estimation

The 25-(OH) vit D was measured by a Food and Drug Administration (FDA)-approved direct, competitive chemiluminescence immunoassay (CLIA) using the DiaSorin LIAISON 25-(OH) vit D total assay (Heartland Assays Inc, Ames, Iowa).[18] This assay is cospecific for 25-(OH)vit D2 and 25-(OH)vit D3 and henceforth will be referred to as 25-(OH) vit D. The assay utilizes a specific antibody (25-(OH) vit D) coated with magnetic particles (solid phase). A vit D analogue, 22-carboxy-23,24,25,26,27-pentanorvitamin D3, which was linked to an isoluminol derivative, competes with the serum 25-(OH) vit D for binding sites on the antibody. A set of known control samples were processed along with the samples to assess inter- and intra-assay variations. The final concentration of the 25-(OH) vit D is expressed as nanogram per milliliter (ng/mL). Inter- and intra-assay coefficients of variation are 11.2% and 8.1%, respectively.

The 1,25-$(OH)_2$ vit D levels in serum samples were measured by radioimmunoassay (RIA; Heartland Assays Inc, Ames, Iowa).[19] The extraction and pretreatment of the hormone from serum were performed using acetonitrile and sodium metaperiodate and isolated on a C18-OH cartridge. The samples were incubated with a polyclonal antibody that is specific for both 1,25-$(OH)_2$ vit D2 and 1,25-$(OH)_2$ vit D3 in the presence of a tracer for 2 hours at 20°C to 25°C. Phase separation was accomplished after incubating with a second antibody and after centrifugation. The bound fraction remaining in the pellet was counted in a gamma counter. A set of known control samples were processed along with the samples to assess inter- and intra-assay variations. The final concentration of the 1,25-$(OH)_2$ vit D is expressed as picogram per milliliter (pg/mL). Inter- and intra-assay coefficients of variation were 12.6% and 9.8%, respectively. This assay is cospecific for 1,25-$(OH)_2$ vit D2 and D3 and henceforth will be referred to as 1,25-$(OH)_2$ vit D.

Statistical Analysis

The Shapiro-Wilks test of normality was performed for maternal age, gestational age, weight, and Appearance, Pulse, Grimace, Activity, Respiration (APGAR) score at 1 and 5 minutes. The APGAR score is a simple method used to assess the health of newborns and is achieved by evaluating each criterion on a scale of 0 to 2 and summing the criteria values at 1 and 5 minutes of birth. All measurements deviated significantly from normality, with the exception of maternal age; as a result Mann-Whitney 2-sample rank sum tests were used to test for statistical differences between medians of preterm and term deliveries; 2-tailed Student *t* tests were used to test for statistical differences in maternal age, body mass index (BMI), annual income, and marital status between term and preterm deliveries. To determine whether 25-(OH) and 1,25-$(OH)_2$ vit D levels differ between preterm and term births, 2-tailed Student *t* tests were performed on combined data stratified by race. To determine whether vit D concentrations were associated with the risk of PTB, the pooled data from both races were dichotomized at the median and logistic regression (LR) models, with PTB as the outcome (term birth as the reference value), were computed after adjusting for potential confounders including race, gravidity, parity, education level, and income level. The odds ratio obtained reflects the association between vit D

Table 1. Serum Vitamin D Data Set: Demographic and Clinical Variables in Term and Preterm Women.

Demographic	Preterm, n = 79	Term, n = 113	*P* Value[a]	Total, N = 192
Age (maternal) in years, mean (SD)[b]	26.91 (6.50)	26.65 (5.52)	.77	26.75 (5.92)
Body mass index (BMI), mean (SD)[b]	29.03 (7.71)	27.29 (7.17)	.28	28.27 (7.38)
Income, n (%)[b]				
US$0-50 000	68 (88.32)	96 (86.48)	.71	164 (87.23)
>US$50 000	9 (11.68)	15 (13.52)		24 (12.77)
Marital status, n (%)				
Single or divorced	42 (53.16)	73 (64.03)	.13	115 (59.59)
Married	37 (46.84)	41 (35.97)		78 (40.41)
Race, n (%)[b]				
Caucasian	36 (45.57)	48 (42.48)	.67	84 (43.75)
African American	43 (54.43)	65 (57.52)		108 (56.25)
Clinical				
APGAR score, mean (SD)[b]				
At 1 minute	6.80 (2.52)	8.13 (1.39)	<.01	7.59 (2.03)
At 5 minutes	8.05 (2.14)	8.79 (1.22)	<.01	8.50 (1.68)
Preterm deliveries, n (%)				
<27 weeks	9.0 (11.40)		<.01	
28-36.5 weeks	70.0 (88.60)			

Abbreviations: APGAR, Appearance, Pulse, Grimace, Activity, Respiration; SD, standard deviation; 25-(OH), 25-hydroxy; 1,25-$(OH)_2$, 1,25-dihydroxy.
[a] The *P* values are derived using a 2-tailed Student *t* test.
[b] Missing observations for: age (1 case and 1 control); BMI (9 cases, 3 controls); income (2 cases and 3 controls); race (1 control); APGAR score 1 minute (4 cases and 2 controls), APGAR score 5 minutes (5 cases and 2 controls); 1,25-(OH) vitamin D (3 cases and 2 controls); 25-(OH) vitamin D (3 cases and 3 controls).

deficiency and the PTB, with term birth as the reference value. One-way analysis of variance (ANOVA) was performed to assess longitudinal gestational changes in vit D levels in term (>37 weeks) and preterm deliveries (<34 weeks and between 34 and 37 weeks).

Results

Demographic Variables Were Not Different Between Term and Preterm Women

Analysis of the demographic variables maternal age, income, marital status, and BMI suggested that there were no significant differences between the term and preterm deliveries or between races (Table 1). The APGAR scores at 1 and 5 minutes were significantly ($P < .01$) low for babies born at preterm compared to term (Table 1).

Serum 25-(OH) Vit D Levels Were Not Significantly Different Between Women Who Delivered at Preterm and Term

We measured serum levels of 25-(OH) vit D by chemiluminescence immunoassay in women who delivered at term and preterm in both races. No significant differences were observed between women delivering at term and preterm in the data combined from both races (Table 2). Concentrations of 25-(OH) vit D tended to be lower ($P = .06$) in Cau women who delivered at preterm compared to those who delivered at term (Table 2). However, the differences observed were not significant. Serum 25-(OH) vit D levels measured in term and preterm AA women were not significantly different. Serum 25-(OH) vit D levels measured in term AA were significantly ($P< .01$) low compared to term Cau (Table 2) and are in agreement with the published literature.

Serum 1,25-$(OH)_2$ Vit D Levels Were Significantly Lower in Women Who Delivered at Preterm Compared to Term

The 25-(OH) vit D gets converted into 1,25-$(OH)_2$ vit D before it binds to the receptor and initiate its actions. Because serum 25-(OH) vit D levels have been reported to be significantly lower in AA women compared to Cau women,[4,5] we measured the differences in the levels of 1,25-$(OH)_2$ vit D in serum collected from Cau and AA participants using radioimmunoassays. A 2-tailed Student *t* test performed demonstrated that the serum 1,25-$(OH)_2$ vit D levels measured in AA women were significantly lower ($P < .01$) when compared to the levels measured in Cau women (Table 3). In addition, we measured the levels of 1,25-$(OH)_2$ vit D in serum obtained from women who delivered at term and preterm. The levels of 1,25-$(OH)_2$ vit D were significantly lower ($P < .01$) in women delivering at preterm compared to women delivering at term in the data pooled from both the races and in the data obtained from Cau (Table 4). A regression analysis showed that decreases in concentrations of 1,25-$(OH)_2$ vit D were associated with PTB in the pooled data unadjusted and adjusted for gravidity, income level, parity, education, and race in LR models ($P < .01$; Table 4). In the data stratified by race, the levels of 1,25-$(OH)_2$ vit D in Cau women were significantly low ($P < .01$; Table 4) in preterm women compared to term women when the data were unadjusted and adjusted for gravidity, income level, parity, and education. However, in

Table 2. Serum 25-(OH) Vitamin D Concentrations in Caucasian and African American Women Delivered at Term and Preterm.

25-(OH) Vitamin D	N	Mean (±SEM), ng/mL[a]	*P* Value[b]
Combined race			
Term	110	20.59 (0.86)	.81
Preterm	76	19.32 (2.21)	
Caucasian			
Term	47	25.21 (1.20)	<.06
Preterm	35	22.02 (1.10)	
African American			
Term	63	17.24 (1.91)	.33
Preterm	41	18.81 (2.55)	
Term			
Caucasian	47	25.21 (1.39)	<.01
African American	35	17.24 (2.37)	

Abbreviations: SEM, standard error of the mean; 25-(OH), 25-hydroxy.
[a] Data are represented as the mean (±SEM).
[b] The *P* values are derived using a 2-tailed Student *t* test.

Table 3. Serum 1,25-$(OH)_2$ Vitamin D Levels in Caucasian and African American Women Delivered at Term and Preterm.

1,25-$(OH)_2$ Vitamin D	N	Mean (±SEM)[a], pg/mL	*P* Value[b]
Combined race			
Term	111	71.50 (5.07)	<.01
Preterm	76	43.22 (2.73)	
Caucasian			
Term	47	87.33 (8.60)	<.01
Preterm	35	36.02 (2.54)	
African American			
Term	64	60.48 (7.37)	.13
Preterm	41	49.37 (4.38)	
Term			
Caucasian	47	87.33 (8.60)	<.01
African American	35	60.48 (7.37)	

Abbreviation: SEM, standard error of the mean; 1,25-$(OH)_2$, 1,25-dihydroxy.
[a] Data are represented as the mean (±SEM).
[b] The *P* values are derived using a 2-tailed Student *t* test.

AA the differences were significant ($P < .04$) only for the data adjusted for gravidity, income level, parity, and education. The odds ratio given in Table 4 reflects the association between vit D deficiency and the PTB, with term birth as the reference value. It does not equate the relative risk but is used as an association with PTB. Furthermore, 1-way ANOVA performed to assess longitudinal gestational changes showed that 1,25-$(OH)_2$ vit D concentrations were significantly lower in preterm deliveries occurring at <34 weeks ($F = 6.4$; $P < .01$) and those occurring between 34 and 37 weeks ($F = 16.19$; $P < .01$) in comparison to normal term deliveries (>37 weeks; Table 5). However, no significant differences were observed between preterm deliveries occurring at <34 and between 34 and 37 weeks ($F = 0.19$; $P = .67$) of gestation.

Discussion

In this study, we assessed whether circulatory levels of vit D affected pregnancy outcomes in Cau and AA in the Middle Tennessee region. The results from this study showed that in addition to having low levels of serum 25-(OH) vit D,[4,5] AA women also have lower levels of 1,25-$(OH)_2$ vit D (the active form of vit D) compared to Cau women. Our studies further revealed that the levels of 1,25-$(OH)_2$ vit D were significantly lower in women who delivered at preterm compared to their respective term counterparts, for both races. In Cau, 25-(OH) vit D concentrations tended to be lower ($P = .06$) in women who delivered at preterm compared to women who delivered at term. However, the differences observed in 25-(OH) vit D levels between term and preterm were not significantly different in either race. These results indicate that hypovitaminosis D, particularly low levels of the active form of vit D, 1,25-$(OH)_2$ vit D, is a risk factor for PTB in AA and Cau women.

In our study, an analysis of socioeconomic factors and BMI revealed no differences between term and preterm participants or between races. Similar findings were reported in a case-control study involving 1491 pregnant AA and Cau women,[20] suggesting that socioeconomic conditions may not be the primary cause of PTB. Low levels of 25-(OH) vit D that were observed in AA women compared to Cau women in our study (Table 2) agreed with the data reported from other laboratories.[4,5] Low levels of 25-(OH) vit D in AA are because of the variation in cutaneous vit D synthesis due to the differences in the degree of melanin pigmentation.[7] Racial differences could also be attributable to varying dietary intake of vit D among AA and Cau women. However, studies conducted so far failed to confirm a difference in dietary vit D intake between AA and Cau women, which suggests that there may be differences in steps leading to the synthesis of 25-(OH) vit D rather than the amount of intake.[8]

Deficiency of 25-(OH) vit D is a global phenomenon[4,21]; however, to our knowledge, there is no study linking vit D deficiency to the PTB. Because vit D is important in regulating cell-mediated immune responses, [11,12] a deficiency or insufficiency in 25-(OH) or 1,25-(OH) vit D during pregnancy may enhance the inflammatory response to clinical and subclinical infections and increase the risk of PTB. Circulatory levels of 25-(OH) vit D are used to determine the vit D status in humans. Therefore, we measured 25-(OH) vit D in term and preterm participants from both ethnicities. The 25-(OH) vit D levels measured in Cau women in our study showed a decreasing trend in women who delivered at preterm compared to term, however, the differences observed were not significant ($P = .06$). Analysis of the pooled data and data from the AA in the present study did not suggest any difference in the 25-(OH) vit D levels between term and preterm deliveries. Recent findings suggest that 25-(OH) vit D is significantly low in Cau women delivering at preterm.[22] Therefore, the decreasing trend ($P = .06$) observed in Cau women who delivered at preterm warrants additional studies using large PTB cohorts to verify the levels of serum 25-(OH) vit D in Cau women delivering at preterm.

Because 25-(OH) vit D gets converted into 1,25-$(OH)_2$ vit D, which in turn binds to the receptors and initiates physiological functions, we measured the differences in the levels of 1,25-

Table 4. Regression Analysis of Serum 25-(OH) and 1,25-$(OH)_2$ Vitamin D Levels in Term and Preterm Deliveries.

	Combined Race			Caucasians			African American		
	Term		Preterm	Term		Preterm	Term	Preterm	
N	111		76	47		35	64	41	
Mean ± SEM	71.50 ± 5.07		43.22 ± 2.73	87.33 ± 8.6		36.02 ± 2.54	60.48 ± 5.8	49.37 ± 4.38	
	OR[a]	95% CI	*P* Value	OR	95% CI	*P* Value	OR	95% CI	*P* Value
25-(OH) vit D[b]	1.04	0.59-1.85	.89	1.40	0.57-3.47	.47	0.92	0.42-2.06	.85
1,25-$(OH)_2$ vit D[b]	4.02	2.17-7.44	<.01	15.06	4.86-46.67	<.01	1.84	0.84-4.00	.13
25-(OH) vit D[c]	1.55	0.80-2.99	.20	1.67	0.64-4.37	.30	1.67	0.64-4.37	.30
1,25-$(OH)_2$ vit D[c]	5.45	2.72-10.95	<.01	16.70	5.06-55.15	<.01	2.55	1.03-6.32	<.04

Abbreviations: 25-(OH) vit D, 25-hydroxyvitamin D; 1,25-$(OH)_2$ vit D, 1,25-dihydroxyvitamin D; OR, odds ratio; CI, confidence interval; SEM, standard error of the mean.
[a] OR reflect association between vitamin D deficiency and preterm birth with term birth as the reference value.
[b] Unadjusted.
[c] Adjusted for gravidity, income level, parity, education, and race for combined estimates and gravidity, income level, parity, and education for data stratified by race.

Table 5. Vitamin D Levels Across the Gestation Length in a Cohort of Caucasian and African American Women Delivered at Term and Preterm.

Gestational Age	*F* Value	*P* Value[a]
1,25-$(OH)_2$ vitamin D		
<34 weeks (preterm) versus >37 weeks (term)	6.40	<.01
<34 weeks (preterm) versus 34-37 weeks (preterm)	0.19	.67
34-37 weeks (preterm) versus >37 weeks (term)	16.19	<.01
25-(OH) vitamin D		
<34 weeks (preterm) versus >37 weeks (term)	0.17	.68
<34 weeks (preterm) versus 34-37 weeks (preterm)	0.60	.44
34-37 weeks (preterm) versus >37 weeks (term)	0.33	.57

Abbreviations: 25-(OH), 25-hydroxy; 1,25-$(OH)_2$, 1,25-dihydroxy; ANOVA, analysis of variance.
[a] The *P* values derived using a 1-way ANOVA.

$(OH)_2$ vit D in serum collected from Cau and AA participants using immunoassays. Serum levels of 1,25-$(OH)_2$ vit D measured at term delivery are lower in AA compared to Cau women. This difference in 1,25-$(OH)_2$ vit D levels can be attributed to the lower levels of 25-(OH) vit D observed in AA compared to Cau. In contrast, Hollis and Pittard[23] reported that there were no differences in total 1,25-$(OH)_2$ vit D levels between AA and Cau term pregnant women in a study involving 12 Cau and 10 AA women. But they reported differences in the synthesis of 1,25-$(OH)_2$ vit D2 and D3 levels in full-term pregnant AA and Cau women. These differences could be caused by variation in certain steps in the vit D metabolism.[24] We reported serum levels of 1,25-$(OH)_2$ vit D as total levels because the antibody used binds to both 1,25-$(OH)_2$ vit D2 and D3. In our study, the serum levels of 1,25-$(OH)_2$ vit D were found to be significantly low in AA and Cau women who delivered at preterm compared to those who delivered at term, suggesting that hypovitaminosis D, particularly low levels of 1,25-$(OH)_2$ vit D, is a risk factor for PTB. A regression analysis showed that the decrease in 1,25-$(OH)_2$ vit D levels was associated with PTB in unadjusted and adjusted linear regression (LR) models in combined race and in the Cau and in adjusted LR models in AA women. The regression analysis suggested that women with 1,25-$(OH)_2$ vit D concentrations below the median displayed 4 times the risk of having a preterm infant than those whose concentrations were above the median; this difference suggests a critical role for vit D during pregnancy. The 1-way ANOVA performed to assess longitudinal gestational changes in vit D levels showed a significant ($P < .01$) decrease in the serum levels of 1,25-$(OH)_2$ vit D in preterm deliveries occurring at <34 and between 34 and 37 gestational weeks compared to term deliveries (≥37 weeks). No significant differences were observed in the serum levels of 1,25-$(OH)_2$ vit D in preterm deliveries between <34 weeks and 34- <37 weeks of gestation. This variation suggests that there are some differences in steps involved in the metabolism of vit D in women proceeding to preterm delivery. These results support the available literature and suggest that the longitudinal changes in serum 1,25-$(OH)_2$ vit D levels in term pregnant women do not show any significant changes within the third trimester. However, the differences in serum 1,25-$(OH)_2$ vit D levels between first and third trimesters are significant.[25,26] Because the majority (70 of 79) of the preterm deliveries observed in our study were in the third trimester (between 28 and <37 weeks) of pregnancy, we believe that low levels of 1,25-$(OH)_2$ vit D observed in preterm deliveries between 28 and <37 weeks of gestation could be caused by differences in the steps involved in the vit D metabolism. These results further suggest that vit D levels in the third trimester of pregnancy can potentially be used as a diagnostic biomarker in the detection of PTB.

Because 25-(OH) vit D levels measured in Cau, in our study, showed a decreasing trend in preterm compared to term deliveries, additional studies using large PTB cohorts are required to further confirm the decreases in serum 25-(OH) vit D levels in Cau women delivering at preterm. Women recruited in our study were free from pregnancy complications, but we did not

have information regarding clinical and subclinical infections and inflammation from them. Therefore, additional studies are required to assess whether PTB is associated with low levels of vit D in women having clinical infections. Because dietary status in prepregnancy and early pregnancy has been reported to affect the length of gestation,[27,28] further studies are warranted to assess the levels of vit D in prepregnancy and early pregnancy in women who delivered at preterm. This will help in understanding the role of vit D during pregnancy and PTB. In conclusion, our study suggests that hypovitaminosis D, particularly low levels of 1,25-$(OH)_2$ vit D, the active form of vit D, is a risk factor for PTB in both races. Decreased levels of 1,25-$(OH)_2$ vit D in preterm women compared to term women suggest some differences in the synthesis of vit D in term and preterm women in both races and warrant further studies. Furthermore, replication of serum vit D data in other PTB cohorts will enhance our understanding of the role of vit D in human pregnancy and PTB. Measuring vit D levels during prepregnancy and early pregnancy in women with spontaneous PTB and in women delivering at preterm because of infections, such as bacterial vaginosis and chorioamnionitis, leading to the preterm premature rupture of membranes, will help assess the role of vit D in spontaneous and in infection-induced PTBs.

Authors' Note

The authors CT and AA designed research; RM and SF provided samples necessary for the study; CT conducted research; CT, AA, RM, LB and JEL analyzed data; CT wrote the paper. CT and AA have primary responsibility for the final content. The authors wish to thank Ms Archana Laknaur, Dr Sangeeta Nair, and Dr Veera Rajaratnam for critical reading and scientific editing of the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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