Published in final edited form as:

Ann Epidemiol. 2016 December ; 26(12): 853–857.e1. doi:10.1016/j.annepidem.2016.09.015.

Low maternal 25-hydroxyvitamin D concentration increases the risk of severe and mild preeclampsia

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Abstract

Purpose: The objective of this case-cohort study was to evaluate the relationship between maternal 25-hydroxyvitamin D (25(OH)D) concentration and preeclampsia overall and by severity.

Methods: From an eligible cohort of 12,861 women who had serum banked from aneuploidy screening in Pittsburgh, Pennsylvania from 1999 to 2010, we randomly sampled a subcohort of 2327 pregnancies and all remaining preeclampsia cases ($n = 650$ cases). Preeclampsia (defined as new-onset hypertension and proteinuria) and its mild and severe forms were identified using ICD-9 codes. Maternal serum collected at 20 weeks or less gestation was measured for 25(OH)D. We used log-binomial regression with restricted cubic splines to estimate the association between 25(OH)D and preeclampsia after adjusting for confounders.

Results: Approximately 21% of the randomly selected sample had 25(OH)D less than 50 nmol per L. We found that the adjusted risk of preeclampsia declined as serum 25(OH)D increased to 50 nmol per L and then plateaued (test of nonlinearity $P < .05$). The adjusted preeclampsia risk ratios (95% confidence intervals) for 25(OH)D less than 25 nmol per L, 25 to 49.9 nmol per L, and 50 to 74.9 nmol per L were 2.4 (1.2–4.8), 1.1 (0.69–1.7), and 1.3 (0.89–1.8), respectively, compared with those with 25(OH)D 75 nmol per L and over. Similar associations were observed with severe and mild preeclampsia.

Conclusions: Vitamin D deficiency increases risks of severe and mild forms of preeclampsia.

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We have no conflicts of interest to disclose.

Keywords

Pregnancy; Preeclampsia; Hypertension; Vitamin D deficiency; 25-Hydroxyvitamin D

Introduction

Preeclampsia is a pregnancy-specific, multisystemic condition that is defined by new-onset hypertension and either proteinuria or end-organ dysfunction at 20 weeks of gestation or later. Complicating 3%–5% of pregnancies in the United States [1,2], preeclampsia is a leading cause of maternal and infant morbidity and mortality [3]. The origins of preeclampsia likely lie in abnormal placental development, which induces oxidative stress and maternal systemic inflammation that lead to the clinical symptoms seen in preeclampsia [4,5].

Vitamin D may play a role in the etiology of preeclampsia by regulating the transcription and function of genes associated with placental function, including placental invasion, normal implantation, and angiogenesis [6,7]. Vitamin D also modulates immune function and inflammatory response [8]. Many [9-17] but not all [18-20] observational studies suggest vitamin D deficiency before disease onset is a risk factor for preeclampsia. Some of the uncertainty in the literature may be because preeclampsia has not been studied separately by subtype [21,22]. Preeclampsia is a heterogeneous disease, therefore classifying preeclampsia cases into more homogenous subgroups based on severity may enhance our understanding of specific exposures in the pathogenesis of preeclampsia [23]. Maternal vitamin D concentration may be influenced by several factors, including diet, supplementation, sun exposure, skin pigmentation, and genetics; therefore, vitamin D deficiency is a potentially modifiable risk factor for preeclampsia risk. Our objective was to evaluate the relationship between maternal 25-hydroxyvitamin D (25(OH)D) concentration at 20 weeks gestation or less and the risk of preeclampsia and subtypes based on symptom severity.

Methods

EVITA is a case-cohort study of vitamin D and adverse pregnancy outcomes. The parent study and these secondary analyses were approved by our institution review board. EVITA used data and banked serum samples from women who had aneuploidy screening at 20 weeks or less gestation and who subsequently delivered live born infants at Magee-Womens Hospital of UPMC in Pittsburgh, Pennsylvania. Data came from a detailed and validated electronic perinatal database at the hospital, described in detail previously [24], which was merged with a database of all clinical genetics encounters and laboratory results performed by the Center for Medical Genetics and Genomics. Data are populated from various electronic sources (e.g., procedure coding) and medical chart abstractors. A data administrator reviews and cleans these data regularly.

There were 65,867 deliveries at our hospital in 1999, 2000, 2001, 2003, 2009, and 2010 (when aneuploidy samples were banked). This eligible cohort contained only one pregnancy per individual. Of these, 12,861 received aneuploidy screening at the Center for Medical

Genetics and Genomics at or before 20 weeks and were therefore eligible. For this case-cohort design, we randomly selected 2327 of the eligible pregnancies and augmented this subcohort with all remaining preeclampsia cases from the eligible pregnancies (n = 650 total cases).

Preeclampsia was defined as new-onset hypertension and proteinuria for the first time after 20-week gestation on the basis of *International Classification of Diseases-9 codes* (ICD-9; 642.4–642.6). Mild preeclampsia without mention of preexisting hypertension was identified by ICD-9 code 642.4. We excluded ICD-9 code 642.7 because those individuals had preexisting hypertension. Severe preeclampsia was defined as severe preeclampsia or eclampsia with no preexisting hypertension (ICD-9 codes 642.5–642.6). Severe forms of preeclampsia are identified by symptoms that suggest end-organ damage. We performed a validation study of preeclampsia diagnosed by ICD code versus adjudication by clinical experts (gold standard) using case-control data from our population [25]. We found that preeclampsia diagnosed by ICD-9 codes in our perinatal database have a sensitivity of 78% and specificity of 96%.

Maternal sera at 20 weeks gestation or less were used because this time period generally precedes the clinical onset of preeclampsia. Samples were stored at −80° C for up to 12 years. Serum was assayed for total 25-hydroxyvitamin D (25(OH)D) (25(OH) D_2+25(OH)D_3) using liquid chromatography–tandem mass spectrometry [26]. We categorized serum 25(OH)D concentration as less than 25 nmol per L, 25 to 49.9 nmol per L, 50 to 74.9 nmol per L, and 75 nmol per L and greater [27]. To model flexible nonlinear relations between 25(OH)D and preeclampsia, we used restricted cubic splines with three knots located at 25, 50, and 75 nmol per L [28-30].

The perinatal database provided information on potential confounders: maternal self-reported race/ethnicity (black, white, and other), prepregnancy body mass index (BMI) (self-reported weight [kg] divided by height [m^2]) (<18.5, 18.5–24.9, 25.0–24.9, >30 kg per m^2), maternal age (<20, 20–29, 30–34, >35 years), prepregnancy diabetes (yes, no), education (less than high school, high school, some college, college), marital status (unmarried, married), smoking (yes, no), insurance (Medicaid, insured/self-pay), provider type (clinic, private), parity (0 or more), gestational age of blood draw (<15 weeks, >15 weeks), season of blood draw (winter, spring, summer, fall), year of delivery (1999, 2000, 2001, 2003, 2007, 2008, 2009, and 2010), and laboratory batch number (1, 2, and 3).

We used multiple imputation to address missing data on height (n = 950 missing, because the perinatal database did not collect height data until 2003), prepregnancy weight (n = 12), education (n = 379), diabetes (n = 136), smoking (n = 4), or parity (n = 2). The data were imputed to create 22 imputed data sets that assumed a multivariable normal distribution with a Markov Chain Monte Carlo approach [31,32]. The number of imputed data sets was based on variance and unrestricted fraction of missing information [33]. A previous publication described the multiple imputation methods in further detail [24].

We used multivariable log-binomial regression to calculate risk ratios (RRs) and 95% confidence intervals (CIs) for associations between maternal 25(OH)D and preeclampsia as

well as its subtypes. To account for the case-cohort design, we used robust standard errors [34]. Subjects in the subcohort were weighted by the inverse of their sampling probability (1 for cases and 5.52 for noncases). We used substantive knowledge to inform our choice of confounders to consider in the full model. These include year of delivery, laboratory batch number, gestational age of sample, season of blood draw, race/ethnicity, maternal age, parity, smoking status, prepregnancy BMI, insurance, marital status, and maternal education. We used the synergy index (S) [35] to test for effect modification on the additive scale by race and/or ethnicity, parity, and gestational age of blood sampling. The synergy index is a test of interaction that evaluates whether the joint effect is greater than the sum of the independent effects of the single factors [35]. In the sensitivity analyses, we excluded women with prepregnancy hypertension or diabetes, we repeated the analyses using subjects with only complete data (replacing BMI with maternal prepregnancy weight in our model), and we repeated the analyses using only the years of enrollment that collected data on height (years 2003 to 2010).

Results

The subcohort was predominately non-Hispanic white, college graduates, married, normal weight, nulliparous, and nonsmokers and had private health insurance (Table 1). Compared with the subcohort, preeclampsia cases were older and more likely to be Black, nulliparous, and recipients of Medicaid. They also completed fewer years of education and had a higher prepregnancy BMI. When separated into mild and severe preeclampsia cases, a greater proportion of severe cases were Black, >30 years, obese, unmarried, recipients of Medicaid, chronic hypertensives, and multiparous as compared with mild cases.

The geometric mean 25(OH)D was 64.6 (95% CI, 64.4–64.8) nmol/L in the subcohort and 57.8 (95% CI, 57.3–58.3) nmol/L among the cases. In the subcohort, 3.3%, 18.0%, 36.8%, and 41.9% of women had 25(OH)D concentration less than 25 nmol per L, 25 to 49.9 nmol per L, 50 to 74.9 nmol per L, and 75 nmol per L and greater, respectively. The weighted incidence of preeclampsia in the cohort was 5.0%. The unadjusted weighted incidence of preeclampsia was 9.1%, 5.8%, 5.4%, and 3.9% among women with 25(OH)D less than 25, 25 to 49.9, 50 to 74.9, and 75 nmol per L and greater, respectively.

After adjusting for confounders, there was a curvilinear association between maternal vitamin D status and the risk of preeclampsia (Fig. 1). As serum 25(OH)D concentration increased, preeclampsia risk declined, then plateaued at approximately 50 nmol per L. Relative to 25(OH)D of 75 nmol per L, women with serum 25(OH)D of 20, 30, or 40 nmol per L had 2.0-, 1.6-, and 1.3-fold increases in risk of preeclampsia, respectively (Table 2). Similar results were found in the categorical analysis, which assumes a constant risk within groups defined by 25(OH)D (Table 2). For example, after confounder adjustment, there was a 2.4-fold increased risk of preeclampsia for women with 25(OH)D less than 25 nmol per L compared to those with 25(OH)D 75 nmol per L or greater. However, the confidence intervals were wide for these estimates.

The weighted incidences of severe and mild preeclampsia were 1.5% and 3.5%, respectively, and the incidence of each subtype increased as 25(OH)D category increased (Table 2).

Confounder-adjusted associations between categories of 25(OH)D and risk of severe and mild preeclampsia were similar to results observed for overall preeclampsia. The spline analysis indicating high risk with low 25(OH)D and a plateau at approximately 50 nmol per L were also similar for mild and severe disease (Appendix Figures 1-2).

None of these results varied by race/ethnicity (S = 8.5, $P = .897$), parity (S = 3.4, $P = .249$), or gestational age of blood draw (S = 17, $P = .102$). After excluding women with prepregnancy hypertension or diabetes, the associations were somewhat attenuated. Adjusted RRs (95% CI) for 25(OH)D of 20, 30, or 40 nmol per L were 2.0 (1.1–3.7), 1.6 (1.0–2.4), and 1.3 (0.95–1.6) compared with 75 nmol per L. Estimates were similar in the sample with the complete data and when the data were restricted to individuals that enrolled after 2002 (Appendix Table 1).

Discussion

In this large contemporary cohort of pregnancies electing aneuploidy screening, low maternal 25(OH)D at ≤20 weeks of gestation was associated with an increased risk of preeclampsia. The analysis of 25(OH)D in categories suggested that the effect was statistically significant only among 25(OH)D <25 nmol per L compared with 25(OH)D of 75 nmol per L or greater. However, this approach assumes that the risk of preeclampsia is constant within each category. The spline analysis, which smooths effects to produce more biologically plausible risk curves, revealed that the risk declined with increasing 25(OH)D with a plateau at 50 nmol per L. Serum 25(OH)D over 50 nmol per L was not associated with preeclampsia risk. We observed similar findings when preeclampsia cases were separated into severe and mild subtypes. Association remained after adjusting for confounders.

We are aware of only one previous study that analyzed vitamin D in relation to both mild and severe forms of preeclampsia. One large study ($n = 560$ mild and $n = 157$ severe cases) found that mothers with 25(OH)D 50 nmol per L or greater at ≤26 weeks gestation had a 40% reduction in severe preeclampsia risk compared with those with 25(OH)D <50 nmol per L (RR, 0.65; 95% CI, 0.43–0.98) after adjusting for confounders [21]. Contrary to our findings, they found no association for mild preeclampsia. This may be due to differences in case definition or population characteristics because these pregnancies were from the 1960s. All other studies only examined severe forms of preeclampsia and tended to find a positive association between 25(OH)D in early pregnancy and risk of the disease [22,36,37].

Our results examining preeclampsia risk overall are consistent with four meta-analyses of observational studies [16,17,38,39]. These meta-analyses revealed that women with 25(OH)D <50 nmol per L in pregnancy experienced an increased risk of preeclampsia compared to women with 25(OH)D 75 nmol per L or more, with pooled odds ratios ranging from 1.6 to 2.3 [16,17,38,39]. Our observation of a threshold at 50 nmol per L 25(OH)D may support these findings. However, the numbers of women with 25(OH)D <30nmol per L were too small for these meta-analyses to report meaningful estimates that we could compare with ours. Our use of splines suggested a possible threshold where risk of

preeclampsia does not further decline as 25(OH)D increases about 50 nmol per L. Other studies are needed to confirm or refute our findings.

Our results suggest that vitamin D deficiency may impact pathophysiological changes found in both severe and mild subtypes of preeclampsia, including an inappropriate inflammatory response, endothelial dysfunction, and high blood pressure [40]. The active form of vitamin D has a role in maintaining an appropriate inflammatory response in the maternal-fetal interface [41]. Endothelial function is maintained via vitamin D by improving proliferation, migration, and tubule formation [42,43]. Furthermore, there is evidence that vitamin D metabolites protect endothelial cells from oxidative stress and minimize the effects of exposure to preeclampsia-related factors [44,45]. In addition, active vitamin D influences the renin-angiotensin-aldosterone system, including the regulation of blood pressure [46].

Our reliance on correct assignment of ICD-9 codes for preeclampsia may contribute to some misclassification. Again, sensitivity and specificity of preeclampsia in our database were 78% and 96%, respectively [25]. However, we could not separate these by mild and severe preeclampsia; therefore, we do not know if the accuracy varies by severity subtype in our cohort as it has been shown in other cohorts [47]. Because preeclampsia might manifest before proteinuria is induced, clinicians may be categorizing potential preeclamptic cases as gestational hypertension. Therefore, the American Congress of Obstetricians and Gynecologists recommend gestational hypertension and preeclampsia be classified with preeclampsia with or without severe characteristics [48]. We were unable to test the relationship between 25(OH)D and gestational hypertension because our perinatal database used ICD-9 codes which lack this detailed information.

Women who elect prenatal aneuploidy screening may be different than those who do not elect screening. However, we have previously demonstrated that there are no major differences in the eligible EVITA subcohort compared with the full cohort [24]. As with any observational study, there is potential for unmeasured confounding; lack of data on socioeconomic status, diet, physical activity, supplement use, or genetics may have biased our results, but without a formal quantitative bias analysis, it is difficult to predict direction and magnitude of the bias [49]. Our study population may have limited generalizability to more diverse populations. In addition, our case-cohort contained few women with 25(OH)D <25 nmol per L which led to imprecise estimates. We also did not have other biomarkers of vitamin D, such as the vitamin D binding protein, which controls the bioavailability of free 25(OH)D [50].

Major strengths of this study were the large number of preeclamptic cases and our ability phenotype preeclampsia based on severity. In addition, we used serum samples collected before onset of symptoms, which is important to establish temporality. These findings further develop our understanding of the role of vitamin D in preeclampsia.

These results have important implications. The Institute of Medicine recommended that pregnant women achieve serum 25(OH)D concentrations of 50 nmol per L to reduce adverse skeletal outcomes [51]. If others confirm our findings of a threshold effect for preeclampsia, this concentration may be adequate for optimizing pregnancy outcomes as well.

Acknowledgments

Supported by Centers for Disease Control and Prevention cooperative agreement U01 DP003177.

Appendix

Appendix Table 1

Sensitivity analyses on the association between maternal serum 25(OH)D concentration and the risk of preeclampsia

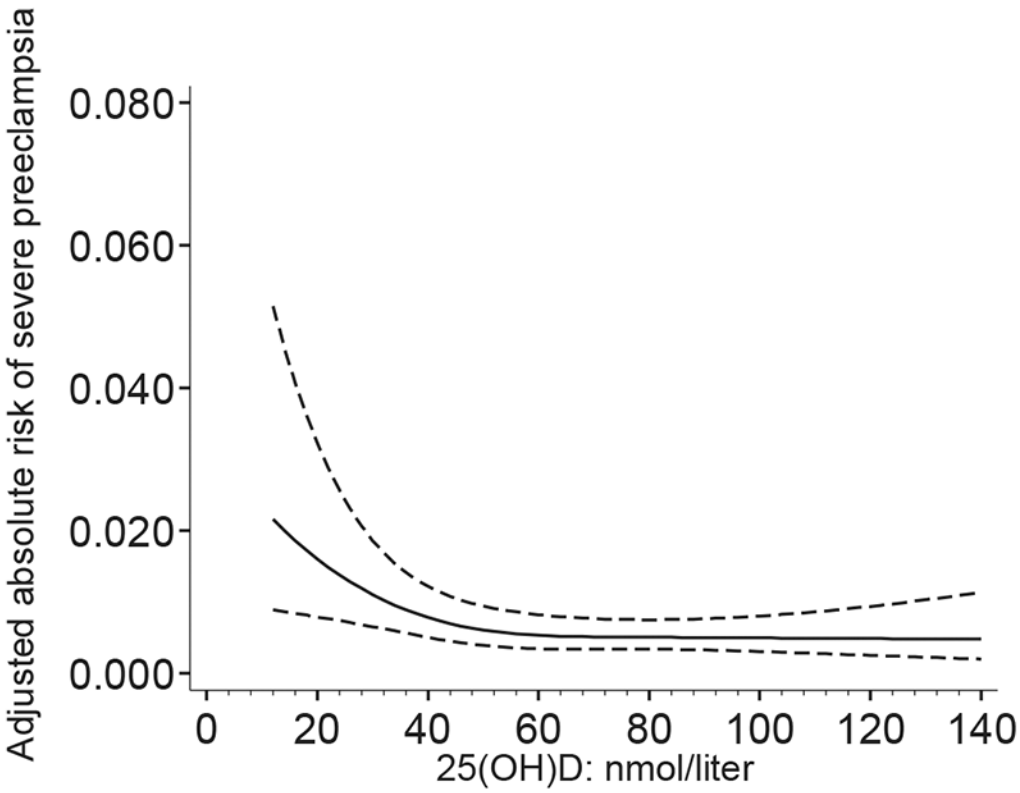
Among the entire cohort after 2002[*], *n* = 1946	Unadjusted relative risk (95% CI)	Adjusted relative risk[†] (95% CI)	Among those with complete data[‡], *n* = 2580	Unadjusted relative risk (95% CI)	Adjusted relative risk[†] (95% CI)
Splines[§]					
20	2.2 (1.5–3.3)	2.1 (1.1–4.1)		2.0 (1.5–2.7)	2.8 (1.5–5.0)
30	1.8 (1.4–2.3)	1.6 (1.1–2.6)		1.6 (1.3–2.0)	1.9 (1.3–2.9)
40	1.4 (1.2–1.6)	1.3 (0.95–1.7)		1.3 (1.2–1.5)	1.4 (1.1–1.8)
50	1.2 (1.0–1.3)	1.1 (0.88–1.3)		1.2 (1.1–1.3)	1.1 (0.91–1.3)
75	Ref	Ref		Ref	Ref
90	0.96 (0.86–1.1)	1.0 (0.86–1.2)		0.97 (0.89–1.0)	1.0 (0.91–1.2)
100	0.93 (0.77–1.1)	1.0 (0.78–1.3)		0.94 (0.83–1.1)	1.1 (0.85–1.4)

[*] Data were restricted to pregnancies that occurred after 2002.

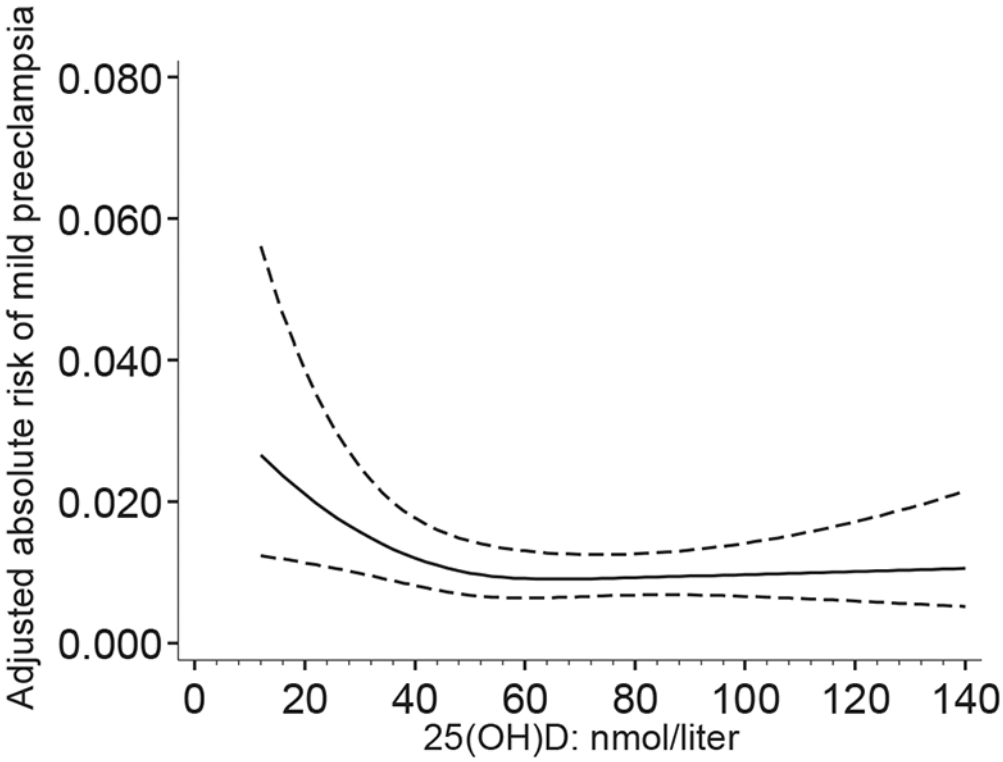
[†] Adjusted for year of delivery, laboratory batch number, gestational age at blood drawn, season of blood draw, race/ethnicity, maternal age, smoking status, prepregnancy BMI, insurance, education, marital status, and parity.

[‡] Data were restricted to the complete data set, using weight instead of BMI in the analysis.

[§] Restricted cubic spline model with three knots.



Appendix Fig. 1.
Risk of severe preeclampsia with increasing 25(OH)D concentration (nmol/L) using restricted cubic spline analysis with three knots.



Appendix Fig. 2.
Risk of mild preeclampsia with increasing 25(OH)D concentration (nmol/L) using restricted cubic spline analysis with three knots.

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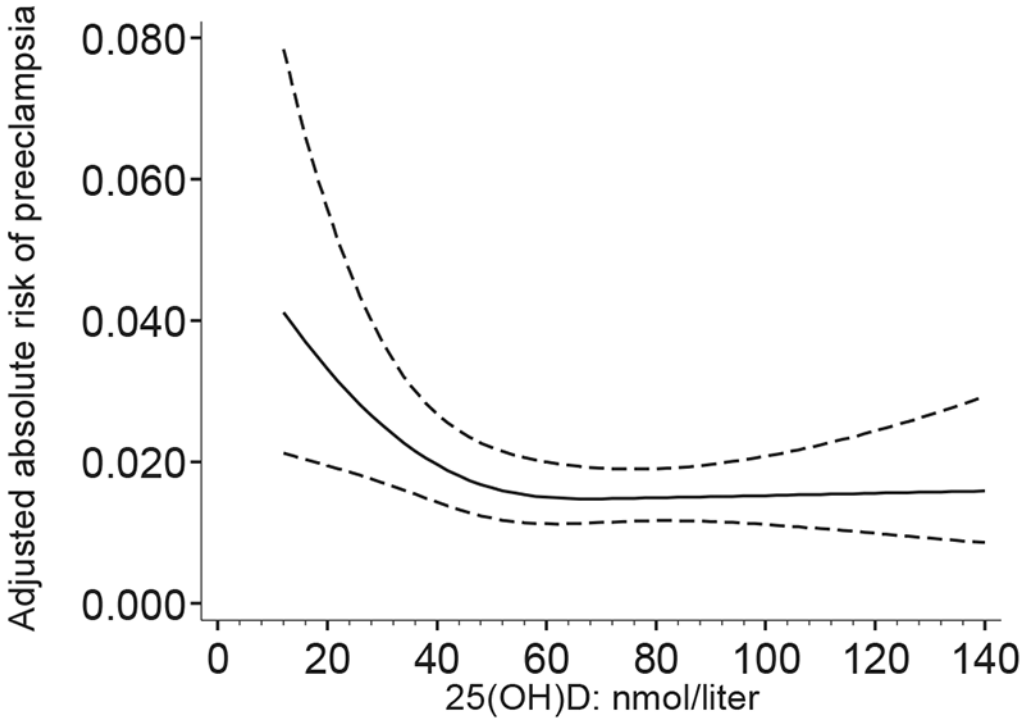

Fig. 1.

Risk of preeclampsia with increasing 25(OH)D concentration (nmol/L) using restricted cubic spline analysis with three knots.

Table 1

Characteristics of the randomly selected subcohort and preeclampsia cases

Characteristic	Subcohort (*n* = 2327) %	Preeclampsia cases (*n* = 650) %	Severe preeclampsia cases (*n* = 201)%	Mild preeclampsia cases (*n* = 449) %
Maternal race/ethnicity				
White	74	65	61	67
Black	19	30	35	27
Other race	7	5	3	6
Parity				
0	68	66	60	68
1 or more	32	34	40	32
Maternal age (y)				
<20	6	9	7	10
20–29	41	43	37	46
30–34	32	24	24	23
>35	21	24	31	21
Prepregnancy body mass index (kg/m^2)				
<18.5	3	1	1	2
18.5–24.9	49	32	27	33
25–29.9	28	32	33	32
≥30	21	35	39	33
Marital status				
Unmarried	36	47	49	45
Married	64	53	51	55
Maternal education				
Less than high school	7	9	8	9
High school	23	30	26	30
Some college	23	22	26	20
College	47	40	39	41
Smoking during pregnancy				
Yes	11	9	8	9
No	89	91	92	91

Characteristic	Subcohort (*n* = 2327) %	Preeclampsia cases (*n* = 650) %	Severe preeclampsia cases (*n* = 201)%	Mild preeclampsia cases (*n* = 449) %
Gestational age of blood sample (wk)				
<15	44	53	59	50
≥15	56	47	41	50
Season of blood sampling				
Winter	21	22	18	23
Spring	28	26	28	24
Summer	25	28	26	28
Fall	26	25	28	24
Type of provider				
Clinic	21	25	20	27
Private	79	75	80	72
Type of insurance				
Medicaid	37	41	44	40
Insured/self-pay	63	59	56	60

Table 2

Association between maternal serum 25(OH)D concentration and the risk of preeclampsia

Serum 25(OH)D (nmol/L) at ≤20 weeks gestation	Number of cases	Unadjusted incidence†	Unadjusted relative risk (95% CI)	Adjusted relative risk‡ (95% CI)
Preeclampsia				
Categories				
less than 25	42	0.091*	2.5 (1.7–3.8)	2.4 (1.2–4.8)
25 to 49.9	138	0.058	1.5 (1.2–2.0)	1.1 (0.69–1.7)
50 to 74.9	261	0.058	1.4 (1.2–1.8)	1.3 (0.89–1.8)
75 or greater	209	0.039	Ref	Ref
Splines§				
20			2.0 (1.5–2.7)	2.4 (1.4–4.3)
30			1.6 (1.3–2.0)	1.8 (1.2–2.7)
40			1.3 (1.2–1.5)	1.4 (1.2–1.7)
50			1.2 (1.0–1.3)	1.1 (0.92–1.3)
75			Ref	Ref
90			0.96 (0.88–1.1)	1.0 (0.89–1.2)
100			0.94 (0.81–1.1)	1.0 (0.82–1.3)
Severe Preeclampsia				
less than 25	15	0.035*	3.2 (1.7–5.9)	3.2 (1.3–7.9)
25 to 49.9	49	0.021	1.9 (1.3–2.9)	1.3 (0.70–2.4)
50 to 74.9	78	0.017	1.5 (1.1–2.2)	1.3 (0.78–2.1)
75 or greater	59	0.011	Ref	Ref
Mild Preeclampsia				
less than 25	27	0.061*	2.2 (1.4–3.6)	2.4 (1.1–5.1)
25 to 49.9	89	0.038	1.4 (1.0–1.8)	1.1 (0.62–1.8)
50 to 74.9	183	0.039	1.4 (1.1–1.8)	1.2 (0.82–1.8)
75 or greater	150	0.028	Ref	Ref

*$P < .05$.

†Based on weighted samples.

‡Adjusted for year of delivery, laboratory batch number, gestational age at blood drawn, season of blood draw, race/ethnicity, maternal age, smoking status, prepregnancy BMI, insurance, education, marital status, and parity.

§Restricted cubic spline model with three knots.