Maternal Vitamin D Status and Risk of Pre-Eclampsia: A Systematic Review and Meta-Analysis

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Background: Although several studies have investigated the association between maternal serum vitamin D levels and risk of pre-eclampsia, findings are inconsistent. This systematic review and meta-analysis of published observational studies was conducted to summarize the evidence on the association between maternal serum vitamin D levels and risk of pre-eclampsia.

Methods: PubMed, ISI (Web of science), SCOPUS, SCIRUS, Google Scholar, and EMBASE databases were searched to identify related articles published through December 2012. For systematic review, we found 15 articles that assessed the association between maternal serum vitamin D levels and risk of pre-eclampsia. The meta-analysis was done on 8 studies that reported odds ratios or relative risks for pre-eclampsia. Between-study heterogeneity was examined using Cochran's Q test and I^2. Subgroup analysis and meta-regression were used to find possible sources of heterogeneity.

Results: The meta-analysis on 8 relevant papers revealed an overall significant association between vitamin D deficiency and risk of pre-eclampsia; however, there was significant between-study heterogeneity ($I^2 = 52.7\%$; $P = .039$). In the subgroup analysis, we found that the overall effect was significant for studies that defined vitamin D deficiency as 25(OH)D $\leq$ 50 nmol/L (20 ng/mL), but not for those that considered it as $<$38 nmol/L (15.2 ng/mL). The association was seen for "cohort or nested case-control studies" as well as for "cross-sectional or case-control studies" (2.78; 1.45–5.33; $P = .002$). When the analysis was done by study location, the associations remained significant only for studies that came from the United States.

Conclusion: There was a significant relationship between vitamin D deficiency and increased risk of pre-eclampsia. Further studies are required, particularly in developing countries. **(*J Clin Endocrinol Metab* 98: 3165–3173, 2013)**

Pre-eclampsia is a major complication in pregnant women, affecting 3–10% of all pregnancies worldwide. It is associated with maternal morbidity and mortality (1). Pregnant women with pre-eclampsia are susceptible to pulmonary edema, coagulation defects, and renal failure (2–6). They are also at elevated risk of hypertension and cardiovascular disease later in life (7, 8). In the past 2 decades, several studies have examined the association between maternal lifestyle and risk of developing pre-eclampsia (9, 10); however, the etiology of pre-eclampsia still remains unknown. There is an increasing interest in the role of vitamin D in pregnancy (11, 12). Some studies have shown increased proinflammatory cytokines such as TNF-α, IL-6, and interferon-γ among vitamin D-deficient pregnant women (13–16). In addition, hypertension is highly prevalent among individuals with vitamin D deficiency (14, 17–20). Kidneys and placenta, major organs for conversion of 25-hydroxyvitamin D [25(OH)D] to its biologically active form 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], are affected physiologically and metabol-

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Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; RR, relative risk.

ically during pregnancy (21–23). Therefore, vitamin D has received great attention as a possible etiological factor in pre-eclampsia. Vitamin D deficiency can affect the health of both mother and fetus by increasing the production of inflammatory cytokines and stimulating the activity of T-regulatory cells (14, 15). It results in poor bone mineralization in infants (24, 25), low birth weight (26), and other adverse pregnancy outcomes (27, 28).

Although several studies have assessed the association between maternal vitamin D status and risk of developing pre-eclampsia during pregnancy, results are inconsistent (29–35). Almost all cross-sectional studies have reported a significant association between vitamin D deficiency and risk of pre-eclampsia (36–38). Findings from case-control studies have revealed that serum vitamin D concentrations in pre-eclamptic women were lower than non-pre-eclamptic women (31, 32, 35, 39–41); however, some other studies failed to reach such associations (29, 33). In a prospective cohort study, Shand et al (30) reported no association between vitamin D deficiency and risk of developing pre-eclampsia. In another cohort study (34) that included a large sample size, it was shown that low serum 25(OH)D concentrations (<50 nmol/L or <20 ng/mL) at weeks 24–26 of gestation was associated with 3.2 times greater risk of pre-eclampsia. Despite several published papers, we are aware of no systematic review and meta-analysis of studies that examined the relationship between vitamin D status and risk of pre-eclampsia. Given the high prevalence of vitamin D deficiency across the globe and inconsistent findings about the association between vitamin D status and risk of pre-eclampsia, this study aimed to review the current observational studies about the association between maternal vitamin D status and risk of pre-eclampsia and to perform a meta-analysis of published studies.

Materials and Methods

Search strategy

A systematic search of the literature published earlier than December 2012 was conducted in PubMed, ISI (Web of science), SCOPUS, SCIRUS, Google Scholar, and EMBASE databases by 2 independent investigators (Marj.T. and Mary.T.) to identify related articles. The following keywords were used in our search strategy: "vitamin D" OR "25-hydroxy vitamin D" OR "25(OH)D" OR "calcidiol" OR "calcitriol" OR "cholecalciferol" OR "hydroxy cholecalciferol" in combination with "EPH complex" OR "EPH gestosis" OR "EPH toxemias" OR "pregnancy toxemias" OR "hypertension edema proteinuria gestosis" OR "preeclampsia." All keywords were selected from the Medical Subject Headings (MeSH) database. No language and time restrictions were done. No attempt was made to include unpublished studies. Duplicate citations were then removed. In addition, a manual search of references was performed to find other relevant articles. The full-text of some articles was obtained through contacting the corresponding author. This study was registered in www.crd.york.ac.uk/Prospero and www.prisma-statement.org (registration no.: CRD42012002662).

Eligibility criteria

Published articles were included in this systematic review if they: 1) were observational studies; 2) had examined the association of maternal serum vitamin D levels with pre-eclampsia; and 3) had reported mean and standard deviation (or median and interquartile range) of maternal 25(OH)D or $1,25(OH)_2D$ concentrations in pre-eclamptic and normal pregnant women. Of the publications we included in systematic review, those that had reported odds ratios (ORs) or relative risks (RRs) for pre-eclampsia were included in the meta-analysis (29–31, 33, 34, 42, 43). Information on study design, sample size, participants' ages, duration of follow-up, and form of serum vitamin D levels assessed was extracted by 2 independent reviewers (Marj.T. and A.S.-A.). In case of disagreements, a third investigator (A.E.) was consulted. Discrepancies were resolved by consensus.

Excluded studies

In total, 1058 articles were found in our initial search. After elimination of duplicates, 768 articles remained. We excluded 702 articles through reading the title and abstract. Another 51 papers were excluded because they did not meet the inclusion criteria: 19 articles were not observational studies, 2 studies had examined vitamin D in the umbilical cord instead of maternal serum, 26 articles had not reported sufficient data on maternal vitamin D status or incidence of pre-eclampsia, 2 papers had evaluated dietary intake of vitamin D instead of assessing serum levels, 1 paper had reported the association between maternal serum vitamin D levels and risk of high blood pressure in pregnancy, and another study had reported multiple of the median instead of OR (44). Seven studies (35, 36–41) that had not reported ORs or RRs were included in the systematic review, but not in the meta-analysis. Finally, 15 observational studies, including 2 cohort studies (30, 34), 4 cross-sectional studies (36–38, 42), 5 nested case-control studies (29, 31–33, 35), and 4 case-control studies (39–41, 43) were considered for inclusion in the systematic review, and 8 studies (out of 15 papers included in systematic review) including 2 cohort studies (30, 34), 1 cross-sectional study (42), 1 case-control study (43), and 4 nested case-control studies (29, 31–33) were included in the meta-analysis (Figure 1).

Data extraction

The following information was extracted by 2 independent reviewers: the first author's last name, date of publication, sample size, study design, duration of follow-up, participants' ages, form of serum vitamin D levels assessed, mean and standard deviation of maternal serum 25(OH)D or $1,25(OH)_2D$ levels, method of vitamin D measurement, ORs or RRs for pre-eclampsia, as well as covariates used in the study. In case of a cross-sectional study that had separately reported the number of pre-eclamptic women in vitamin D-deficient and vitamin D-sufficient groups, we manually calculated the OR (42). One study had assessed the association of 25(OH)D concentrations with pre-eclampsia twice—at 12–18 and 24–26 week gestation (34). Therefore, findings of this study were included as 2 separate studies in the meta-analysis.

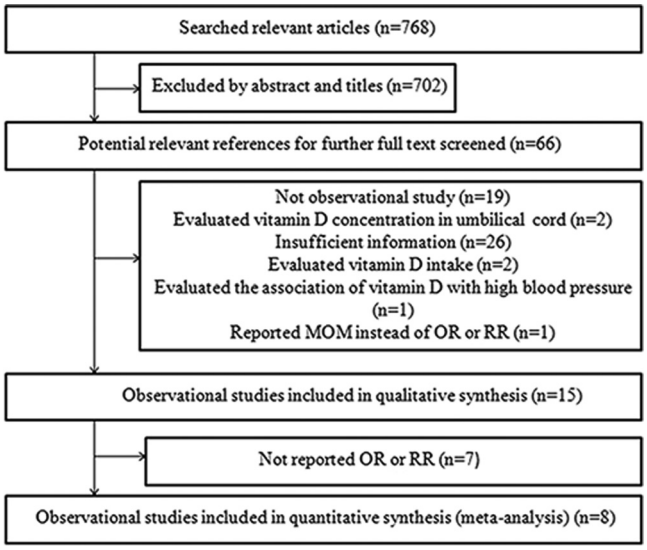

Figure 1. The flow diagram of search strategy and study selection process.

Statistical analysis

All reported RRs and ORs (and their 95% confidence intervals) were used to calculate log RR and its standard error. Using a random effects model that takes between-study variation into account, the overall effect size was calculated. Between-study heterogeneity was assessed using Cochran's Q test and I^2. In case of significant between-study heterogeneity, we used subgroup analysis and meta-regression to find out possible sources of heterogeneity. Between-subgroup heterogeneity was examined through fixed effects model. Sensitivity analysis was done to explore for the extent to which inferences might depend on a particular study. Publication bias was assessed by looking over Begg's funnel plots. Formal statistical assessment of funnel plot asymmetry was done by Egger's regression asymmetry test. Statistical analyses were conducted using STATA version 11.2 (STATA Corp., College Station, Texas). *P* values less than .05 were considered statistically significant.

Results

Of the 768 retrieved papers, 15 observational studies including 2 cohort (30, 34), 4 cross-sectional (36–38, 42), 5 nested case-control (29, 31–33, 35), and 4 case-control studies (39–41, 43) were included in this systematic review. Of these 15 papers, 2 cohort, 1 case-control, 1 cross-sectional, and 4 nested case-control studies that reported ORs or RRs for pre-eclampsia were included in the meta-analysis (29–34, 42, 43). The studies included in this systematic review are summarized in Table 1. In total, 3007 participants aged ≥18 years were included in 15 studies we considered for systematic review. One study assessed maternal serum concentrations of vitamin D at first trimester (33), 5 studies at second trimester (30–32, 34, 35), and 5 studies at the third trimester of pregnancy (29, 36, 38, 41, 43). One study quantified maternal vitamin D concentrations both at second and third trimesters of pregnancy (42). Others did not describe the time of blood sampling for vitamin D assessment (37, 39, 40). In 3 studies, participants were followed up until the delivery (30, 32, 42). Ten studies were carried out in the United States (31–33, 35, 36, 38–41, 43), and others were in Canada (30, 34), Denmark (37), United Kingdom (29), and Spain (42).

Assessment of vitamin D status

Nine studies considered maternal serum 25(OH)D levels as a marker of vitamin D status, whereas 5 studies considered serum $1,25(OH)_2D$ levels (36, 38–41), and 1 study evaluated both 25(OH)D and $1,25(OH)_2D$ levels (37). Most studies assessed serum vitamin D levels by using the RIA method (30, 39, 41, 43) or chromatography (29, 31, 33, 37). Some studies used ELISA (32) or chemiluminescence immunoassay (34, 42) or microassay (40). Others (35, 36, 38) did not report the method of vitamin D measurement.

Assessment of pre-eclampsia

Most included studies in this systematic review defined pre-eclampsia as a blood pressure of ≥140/90 mm Hg after 20 weeks gestation, accompanied by proteinuria of ≥0.3 g in a 24-hour urine collection or a urine dipstick result of +2 or greater (29, 32–34, 39–41). In 2 studies, pre-eclampsia defined as mentioned above but a urine dipstick result of +1 or greater had been considered (30, 42). One study (31) used 2 definitions for pre-eclampsia: 1) blood pressure ≥160/110 mm Hg accompanied by proteinuria; or 2) systolic blood pressure of at least 90 mm Hg plus 5 g proteinuria in a 24-hour urine sample. The definition of pre-eclampsia was not stated in other studies (35–38, 43).

Adjustment for confounders

Nearly all studies that reported ORs or RRs used multivariable logistic or Cox regression analysis. However, the number and type of potential confounders controlled for was different between studies. Most studies controlled for maternal age, body mass index, season, and gestational trimester at sample collection. Two studies did further adjustment for smoking (30, 34) and 2 other studies additionally controlled for parity (29, 30). One study adjusted for multiparty (31) and another for education (32). In an attempt to control for the effect of possible confounders, some studies excluded women with pre-existing medical conditions including diabetes mellitus (31–34, 43), chronic hypertension (31, 32, 34, 43), kidney disease (31, 43), lupus (43), thrombophilias (42), major congenital fetal abnormalities (31), those with a history of pre-eclampsia (34), and those with a history of small-for-ges-

Table 1. Summary of Studies That Assessed the Association Between Vitamin D Status and Risk of Pre-Eclampsia

First Author (Ref.)	Year	Study Design	n	Time of Blood Sampling	Age, y
Wei (34)	2012	Cohort	697	12–18, 24–26 wk	P, 30.9; non-P, 30.3
Fernandez-Alonso (42)	2012	Cross-sectional	466	11–14, 36–39 wk	—
Shand (30)	2010	Cohort	221	10–20 wk	≥18
Baker (31)	2010	Nested case-control	241; case, 43; control, 198	15–20 wk	P, 28 (23–32); non-P, 30 (25–34)
Bodnar (32)	2007	Nested case-control	274; case, 55; control, 219	<22 wk	14–44
Robinson (43)	2010	Case-control	150; case, 50; control, 100	<34 wk	P, 24 (21–30); non-P, 28 (23–32)
Powe (33)	2010	Nested case-control	170; case, 39; control, 131	First trimester	P, 28.9 ± 6.4; non-P, 30.4 ± 6.0
Wetta (29)	2012	Nested case-control	266; case, 89; control, 177	<37 wk	—
Halhali (40)	2000	Case-control	48; case, 24; control, 24	Third trimester	P, 24.5 ± 5.6; non-P, 22.7 ± 3.9
Frolich (37)	1992	Cross-sectional	53	30–36 wk	P, 26 (20–41); non-P, 28 (19–45)
Halhali (39)	2007	Case-control	52; case, 26; control, 26	Third trimester	P, 26.6 ± 6.4; non-P, 25 ± 6.1
Halhali (41)	2004	Case-control	50; case, 10; control, 40	Third trimester	—
August (36)	1992	Cross-sectional	32	Third trimester	—
Halhali (38)	1995	Cross-sectional	52; case, 26; control, 26	26.7 and 39.7 wk	—
Woodham (35)	2011	Nested case-control	164; case, 41; control, 123	Second trimester	—

Abbreviations: CI, confidence interval; P, pre-eclamptic; non-P, non-pre-eclamptic; —, lack of information. All measurements are on maternal serum. 25 (OH)D and 1,25$(OH)_2$ D units are nmol/L.

tational-age delivery (33). Furthermore, three studies included singleton pregnant women (31, 34, 43), whereas two others recruited nulliparous women (32, 34). To further adjust for potential confounders, investigators of one study (31) matched cases and controls in terms of race. Others also matched for gestational age in addition to race (43). Bodnar et al (32) and Wei et al (34) did not match cases and controls for any covariate. Besides the above-mentioned factors that were taken into account in earlier studies, it seems that additional adjustment for some other confounders like physical activity (45), dietary calcium intake (46), and vitamin D-binding proteins (47) are required to reach an independent association between serum vitamin D levels and pre-eclampsia.

Findings from systematic review

Four studies in the systematic review including one cross-sectional (42), one cohort (30), and two nested case-control studies (29, 33) found no association between maternal 25(OH)D levels and pre-eclampsia. One cohort study (34) reported that maternal 25(OH)D levels of <50 nmol/L (20 ng/mL) at 24–26 weeks gestation was associated with an increased risk of pre-eclampsia. Four other studies showed a significant association between lower levels of maternal 25(OH)D and increased risk of pre-eclampsia (31, 32, 35, 43). Six studies reached a significant association between lower levels of 1,25$(OH)_2$D concentrations and increased risk of pre-eclampsia (36–41).

Findings from meta-analysis

Of the 15 studies included in the systematic review, 7 had not reported ORs or RRs; therefore, we included the 8 remaining papers in our meta-analysis (29–34, 42, 43). In total, 2485 women aged ≥18 years were studied in these 8 studies. In the meta-analysis, we found a significant association between vitamin D deficiency and risk of pre-eclampsia (Figure 2); however, significant between-study heterogeneity was found (I^2 = 52.7%; P = .03). To find

Table 1. Continued

Follow-Up	Country	Latitude	Biomarker	Comparisons	RR (95% CI)
—	Canada	45.30	25(OH)D	<50 vs ≥50 nmol/L (<20 vs ≥20 ng/mL)	Wk 12–18, 1.24 (0.58–2.67); wk 24–26, 3.24 (1.37–7.69)
Until delivery	Spain	36.84	25(OH)D	<50 vs ≥50 nmol/L (<20 vs ≥20 ng/mL)	Wk 11–14, 1.35 (0.26–7.02)
Until delivery	Canada	49.15	25(OH)D	<37.5 vs ≥37.5 nmol/L (<15.2 vs ≥15.2 ng/mL), and <50 vs ≥50 nmol/L (<20 vs ≥20 ng/mL)	For <37.5, 0.91 (0.31–2.62); for <50, 1.39 (0.54–3.53)
—	United States	42.21	25(OH)D	<50 vs ≥50 nmol/L (<20 vs ≥20 ng/mL)	5.41 (2.02–14.52)
Until delivery	United States	42.21	25(OH)D	<37.5 vs ≥37.5 nmol/L (<15.2 vs ≥15.2 ng/mL)	5.00 (1.74–14.40)
—	United States	33.50	25(OH)D	<50 vs ≥50 nmol/L (<20 vs ≥20 ng/mL)	3.17 (1.56–6.46)
—	United States	42.21	25(OH)D	<37.5 vs ≥37.5 nmol/L (<15.2 vs ≥15.2 ng/mL)	1.35 (0.40–4.50)
—	UK	33.53	25(OH)D	<37.5 vs ≥37.5 nmol/L (<15.2 vs ≥15.2 ng/mL)	1.10 (0.60–2.01)
—	United States	19.24	$1,25(OH)_2D$	—	—
—	Denmark	55.41	$1,25(OH)_2D$ and 25(OH)D	—	—
—	United States	19.24	$1,25(OH)_2D$	—	—
—	United States	19.24	$1,25(OH)_2D$	—	—
—	United States	40.71	$1,25(OH)_2D$	—	—
—	United States	19.24	$1,25(OH)_2D$	—	—
—	United States	42.21	25(OH)D	—	—

the source of heterogeneity, we performed a subgroup analysis based on cutoff points used to define vitamin D deficiency in different studies as well as analysis based on study design. Subgroup analyses according to study location and trimester of pregnancy (time of blood sampling) were also done.

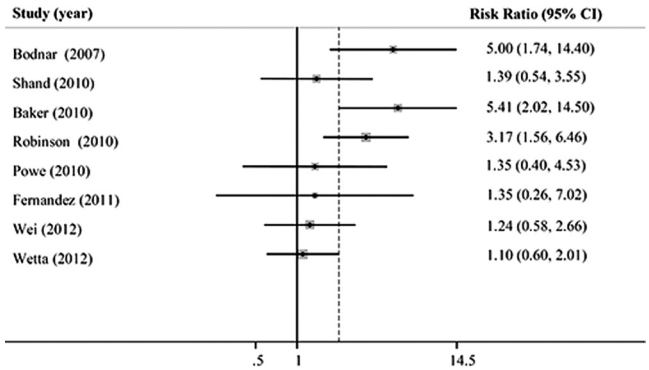

Figure 2. Forest plot of observational studies that assessed the association between serum vitamin D levels and pre-eclampsia for all eligible studies.

Out of 8 studies, 3 reports considered vitamin D deficiency as ≤38 nmol/L (15.2 ng/mL) (29, 32, 33), and 4 papers defined the deficiency state as ≤50 nmol/L (20 ng/mL) (31, 34, 42, 43); 1 paper (30) defined vitamin D deficiency based on both cut-points, and we therefore included this paper in both categories. Meta-analysis on 4 papers (29, 30, 32, 33) including 931 participants, which considered 25(OH)D levels of <38 nmol/L (15.2 ng/mL) as deficiency, revealed no significant association between vitamin D deficiency and risk of pre-eclampsia (Figure 3). Meta-analysis of 5 articles (30, 31, 34, 42, 43), including 1775 participants, that defined the deficiency as 25(OH)D levels of ≤50 nmol/L (20 ng/mL) showed a significant association between vitamin D deficiency and risk of pre-eclampsia (Figure 4). We also did the meta-analysis separately by study designs. Cohort and nested case-control

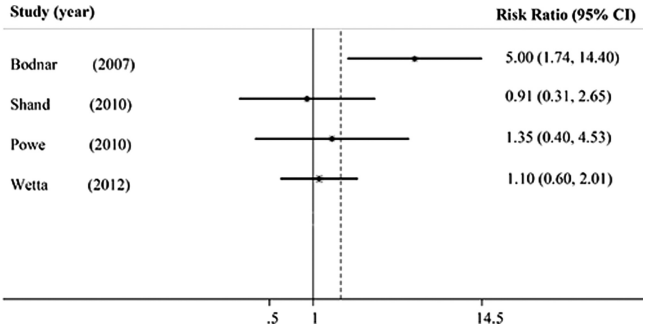

Figure 3. Forest plot of observational studies that assessed the association between serum vitamin D levels and pre-eclampsia for 4 studies that considered 25(OH)D <38 nmol/L as deficiency.

studies were considered together in the first group and cross-sectional and case-control studies in the second group. Data from "cohort and nested case-control studies" showed that maternal deficiency of vitamin D was significantly associated with risk of developing pre-eclampsia (Figure 5). Meta-analysis of "cross-sectional and case-control studies" revealed the same association (2.78; 1.45–5.33; $P = .002$). We do not have enough information to reject our null hypothesis about between-study heterogeneity for all mentioned analyses.

Subgroup analysis based on study location showed a significant association between vitamin D deficiency and increased risk of pre-eclampsia in the United States, but no significant association in other countries. We do not have enough information to reject our null hypothesis about between-study heterogeneity either for studies done in the United States (Q test, $P = .304$; $I^2 = 17.4\%$) or for those done in other regions (Q test, $P = .978$; $I^2 = 0.0\%$) (Figure 6).

To examine whether the time of blood sampling (trimester of pregnancy) can contribute to the between-study heterogeneity, we used meta-regression with time of blood sampling as a covariate. This analysis showed that time of blood sampling could not be a possible source of heterogeneity (B = 0.11; $P = .79$).

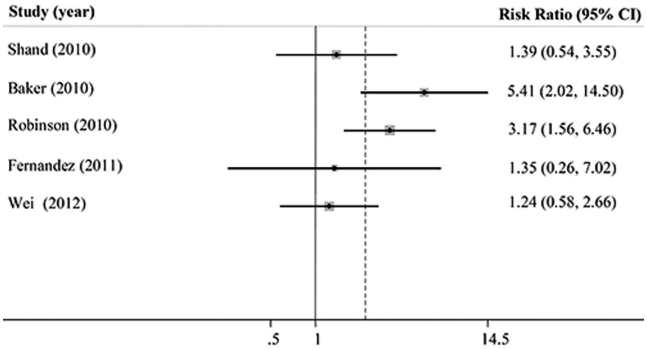

Figure 4. Forest plot of observational studies that assessed the association between serum vitamin D levels and pre-eclampsia for 5 studies that considered 25(OH)D ≤50 nmol/L as deficiency.

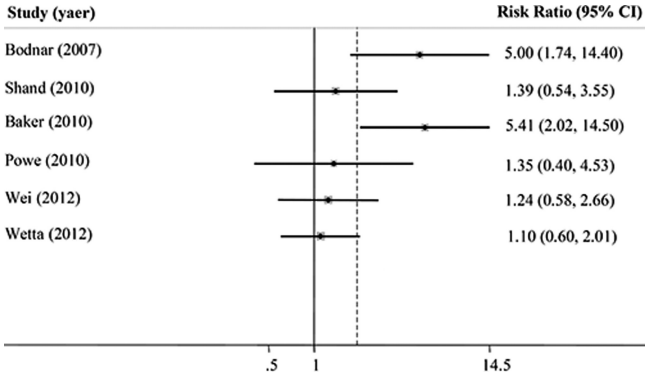

Figure 5. Forest plot of "cohort and nested case-control" for the association between serum vitamin D levels and pre-eclampsia.

Findings from sensitivity analysis revealed that no particular study significantly affected the summary effects. Although a slight asymmetry was seen in Begg's funnel plot, we do not have enough information to reject our null hypothesis about publication bias using Egger's test ($P = .67$).

Discussion

This meta-analysis of observational studies showed a significant association between vitamin D deficiency and risk of pre-eclampsia. Subgroup analysis indicated that the association between vitamin D deficiency and risk of pre-eclampsia was significant when the deficiency was defined as serum 25(OH)D concentrations of <50 nmol/L (20 ng/mL); however, when the deficiency was considered as <38 nmol/L (15.2 ng/mL), the association was not significant. The inverse association between vitamin D status and risk of pre-eclampsia was significant for both "cohort and nested case-control" and "cross-sectional and case-control" studies. However, when the analysis was done

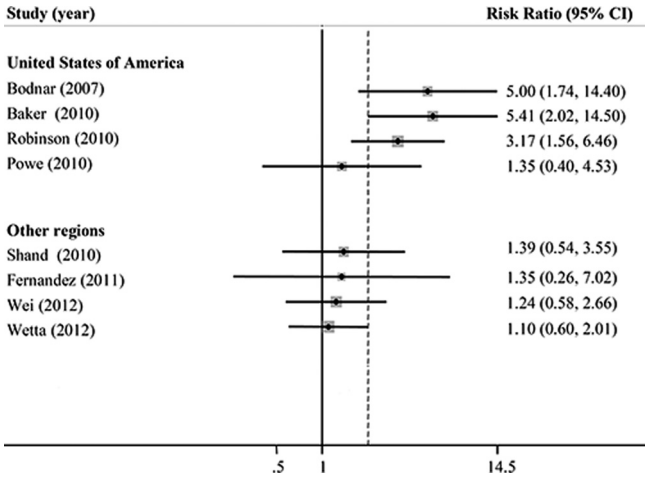

Figure 6. Forest plot of observational studies that assessed the association between serum vitamin D levels and pre-eclampsia separately by study location.

separately by study location, we found a significant association only for studies from the United States. To our knowledge, this is the first meta-analysis of observational studies that examined the association between vitamin D status and risk of pre-eclampsia.

Pooling information from all observational studies can provide more precise and powerful findings than those obtained from individual studies. Most cross-sectional studies showed a significant association between lower concentrations of $1,25(OH)_2D$ and pre-eclampsia at the third trimester of gestation (36–38). One cross-sectional study that had assessed maternal 25(OH)D levels at the first trimester (at 11–14 week gestation) reached no significant association with pre-eclampsia (36). Almost all cross-sectional studies had assessed serum vitamin D levels only 1 time after the diagnosis of pre-eclampsia. Very few studies had examined vitamin D status several times and before clinical diagnosis of pre-eclampsia. The inherent limitations of cross-sectional studies must be taken into account. Findings from these studies cannot prove casual relationships. The majority of case-control and nested case-control studies have shown lower levels of serum vitamin D levels in pre-eclamptic women than in healthy pregnant women (31, 32, 39–41, 43). However, 2 nested case-control studies (29, 33) assessing vitamin D status either at the first or second trimester of pregnancy reported no association between serum total or free 25(OH)D levels and risk of pre-eclampsia. The different findings from traditional or nested case-control studies might be explained by the quantification of vitamin D status in different times of pregnancy. Furthermore, some studies had considered 25(OH)D levels as a surrogate marker for vitamin D status, whereas others had used $1,25(OH)_2D$. Earlier studies have shown similar but independent biological effects for 25(OH)D and $1,25(OH)_2D$ (48). In this meta-analysis, we had 2 cohort studies with inconsistent findings. Shand et al (30) failed to detect any association between serum vitamin D levels and risk of pre-eclampsia, whereas Wei et al (34) reached a significant association between vitamin D deficiency (<50 nmol/L or <20 ng/mL) at 24–26 week gestation and increased risk of pre-eclampsia. We were unable to obtain the raw dataset from all 8 studies to do the separate analysis for both vitamin D cutoff points [<50 nmol/L (20 ng/mL) or <38 nmol/L (15.2 ng/mL)]. A suggestion for future studies is to report the estimates of association for both cutoff points of serum vitamin D levels.

Among the included papers, 10 studies had been performed in the United States (31–33, 35, 36, 38, 39–41, 43), and only 1 of these studies (33) had not reached the association between maternal serum 25(OH)D levels and pre-eclampsia. Of the 5 studies (29, 30, 34, 37, 42) that had not been performed in the United States, 3 reports (29, 30, 42) had failed to find a significant association. It is likely that different latitude, different skin color, and different covariates can explain the different results between the United States and other countries. In addition, different populations are likely to have a different amount of conversion of 25(OH)D to a biologically active form of vitamin D [$1,25(OH)_2D$].

Although the mechanisms through which low serum vitamin D levels can affect the risk of pre-eclampsia are still unclear, it is biologically plausible. Vitamin D has been shown as a potent endocrine suppressor of renin biosynthesis to regulate the renin-angiotensin system (49, 50). The renin-angiotensin system is a regulatory cascade that plays a critical role in the regulation of blood pressure and electrolyte and plasma volume homeostasis (50). Therefore, normal serum vitamin D levels help prevent hypertension through suppression of the renin-angiotensin system. In addition to the effect of vitamin D on the renin-angiotensin system, vitamin D can influence blood pressure through the suppression of vascular smooth muscle cell proliferation. It can also ameliorate insulin resistance, improve endothelial cell-dependent vasodilatation, and inhibit anticoagulant activity (51). Vitamin D may modulate macrophage activity and cytokine production. Pre-eclampsia is hypothesized to be a 2-stage disorder (52). At its first stage, placental perfusion is reduced, often secondary to abnormal implantation. The poorly perfused placenta is proposed to produce materials that, in an appropriate maternal environment, initiate the ensuing multisystem sequelae (second stage). These pathophysiological changes are proposed to be secondary to abnormal endothelial function, which is a component of a generalized increase in the inflammatory activation (34). The active form of vitamin D, $1,25(OH)_2D$, has been shown to regulate the transcription and function of genes associated with placental invasion, normal implantation, and angiogenesis (53). Therefore, insufficient serum vitamin D levels can impair normal functioning of these processes.

Several points need to be considered when interpreting our findings. One might believe that meta-analyses of this kind were developed for randomized clinical trials, and may not be entirely appropriate for use in observational nonrandomized studies. It might also be mentioned that no amount of statistics can compensate for a poor design and reduced precision in observational studies. Although a meta-analysis of randomized trials is more likely to provide unbiased information than that of observational studies, it must be noted that despite the inherent limitations, a meta-analysis of observational studies can provide much more valuable, summarized, and reliable information than a single study. This is particularly relevant for

observational studies on pregnant women, for whom conducting randomized trials is not so easy. It must be kept in mind that some studies included in this meta-analysis had small sample sizes. Furthermore, some studies had evaluated serum vitamin D levels just once, whereas single measurement of serum vitamin D levels might not reflect whole body vitamin D status perfectly. In addition, some studies had considered 25(OH)D levels and others considered $1,25(OH)_2D$ concentrations as a biomarker. Moreover, some studies included in this meta-analysis had not been specifically designed to assess the association between maternal vitamin D status and pre-eclampsia, and this relationship had been reported as an accessory finding. As with all meta-analyses, the publication bias needs to be discussed. Although we do not have enough information to reject our null hypothesis about publication bias due to the limited number of studies that our findings were based on, further investigations are required. Other limitations of observational studies, including selection bias and attrition bias, must be considered.

In conclusion, this meta-analysis of observational studies indicated that low (<50 nmol/L, or 20 ng/mL) serum concentration of 25(OH)D was associated with increased risk of pre-eclampsia. The inverse association between vitamin D status and pre-eclampsia was significant for both "cohort or nested case-control" and "cross-sectional or case-control" study designs. We failed to find a significant association in non-U.S. nations.

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References

1. **Sibai B, Dekker G, Kupferminc M.** Pre-eclampsia. *Lancet*. 2005; 365:785–799.
2. **Guo HX, Wang CH, Li ZQ, et al.** The application of serum cystatin C in estimating the renal function in women with preeclampsia. *Reprod Sci*. 2012;19:712–717.
3. **Sibai BM.** Magnesium sulfate prophylaxis in preeclampsia: lessons learned from recent trials. *Am J Obstet Gynecol*. 2004;190:1520–1526.
4. **Benedetti TJ, Cotton DB, Read JC, Miller FC.** Hemodynamic observations in severe pre-eclampsia with a flow-directed pulmonary artery catheter. *Am J Obstet Gynecol*. 1980;136:465–470.
5. **Drislane FW, Wang AM.** Multifocal cerebral hemorrhage in eclampsia and severe pre-eclampsia. *J Neurol*. 1997;244:194–198.
6. **Morriss MC, Twickler DM, Hatab MR, Clarke GD, Peshock RM, Cunningham FG.** Cerebral blood flow and cranial magnetic resonance imaging in eclampsia and severe preeclampsia. *Obstet Gynecol*. 1997;89:561–568.
7. **Smith GC, Pell JP, Walsh D.** Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. *Lancet*. 2001;357:2002–2006.
8. **Irgens HU, Reisaeter L, Irgens LM, Lie RT.** Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ*. 2001;323:1213–1217.
9. **Hoirisch-Clapauch S, Benchimol-Barbosa PR.** Markers of insulin resistance and sedentary lifestyle are predictors of preeclampsia in women with adverse obstetric results. *Braz J Med Biol Res*. 2011; 44:1285–1290.
10. **Lopez-Carbajal MJ, Manriquez-Moreno ME, Galvez-Camargo D, Ramirez-Jimenez E.** Risk factors associated to preclampsia [in Spanish]. *Rev Med Inst Mex Seguro Soc*. 2012;50:471–476.
11. **Liu NQ, Kaplan AT, Lagishetty V, et al.** Vitamin D and the regulation of placental inflammation. *J Immunol*. 2011;186:5968–5974.
12. **Grant WB.** Adequate vitamin D during pregnancy reduces the risk of premature birth by reducing placental colonization by bacterial vaginosis species. *MBio*. 2011;2:e00022–11.
13. **Diaz L, Noyola-Martinez N, Barrera D, et al.** Calcitriol inhibits TNF-α-induced inflammatory cytokines in human trophoblasts. *J Reprod Immunol*. 2009;81:17–24.
14. **Holick MF.** Vitamin D deficiency. *N Engl J Med*. 2007;357:266–281.
15. **Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier MC.** Vitamin D and inflammation. *Joint Bone Spine*. 2010; 77:552–557.
16. **Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR.** 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension*. 2011;57:63–69.
17. **Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM.** High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. *J Nutr*. 2007;137:447–452.
18. **Huotari A, Herzig KH.** Vitamin D and living in northern latitudes—an endemic risk area for vitamin D deficiency. *Int J Circumpolar Health*. 2008;67:164–178.
19. **Tsiaras WG, Weinstock MA.** Factors influencing vitamin D status. *Acta Derm Venereol*. 2011;91:115–124.
20. **Rostand SG.** Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension*. 1997;30:150–156.
21. **Turner M, Barre PE, Benjamin A, Goltzman D, Gascon-Barre M.** Does the maternal kidney contribute to the increased circulating 1,25-dihydroxyvitamin concentrations during pregnancy? *Miner Electrolyte Metab*. 1988;14:246–252.
22. **Gray TK, Lester GE, Lorenc RS.** Evidence for extra-renal 1 α-hydroxylation of 25-hydroxyvitamin D3 in pregnancy. *Science*. 1979; 204:1311–1313.
23. **Lindheimer MD, Fisher KA, Spargo BH, Katz AI.** Hypertension in pregnancy: a biopsy study with long-term follow-up. *Contrib Nephrol*. 1981;25:71–77.

24. **Finberg L.** Vitamin D deficiency and rickets. *J Pediatr Endocrinol Metab*. 2006;19:203.
25. **Kogawa M, Findlay DM, Anderson PH, et al.** Osteoclastic metabolism of 25(OH)-vitamin D3: a potential mechanism for optimization of bone resorption. *Endocrinology*. 2010;151:4613–4625.
26. **Bodnar LM, Catov JM, Zmuda JM, et al.** Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. *J Nutr*. 2010;140:999–1006.
27. **Dror DK, Allen LH.** Vitamin D inadequacy in pregnancy: biology, outcomes, and interventions. *Nutr Rev*. 2010;68:465–477.
28. **Lapillonne A.** Vitamin D deficiency during pregnancy may impair maternal and fetal outcomes. *Med Hypotheses*. 2010;74:71–75.
29. **Wetta L, Biggio J, Cliver S, Abramovici A, Barnes S, Tita A.** Midtrimester vitamin D status is not associated with preeclampsia prior to 37 weeks. *Am J Obstet Gynecol*. 2012;206:346–347.
30. **Shand AW, Nassar N, Von Dadelszen P, Innis SM, Green TJ.** Maternal vitamin D status in pregnancy and adverse pregnancy outcomes in a group at high risk for pre-eclampsia. *BJOG*. 2010;117:1593–1598.
31. **Baker AM, Haeri S, Camargo CA, Espinola JA, Stuebe AM.** A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia. *J Clin Endocrinol Metab*. 2010;95:5105–5109.
32. **Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM.** Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab*. 2007;92:3517–3522.
33. **Powe CE, Seely EW, Rana S, et al.** First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. *Hypertension*. 2010;56:758–763.
34. **Wei S, Audibert F, Hidiroglou N, et al.** Longitudinal vitamin D status in pregnancy and the risk of pre-eclampsia. *BJOG* 2012;119:832–839.
35. **Woodham P, Brittain J, Baker A, Haeri S, Camargo C, Stuebe A.** Maternal 25(OH)D levels and sFLT-1/PlGF ratio improves predictability of severe preeclampsia in early pregnancy. *Am J Obstet Gynecol*. 2011;204:S22.
36. **August P, Marcaccio B, Gertner JM, Druzin ML, Resnick LM, Laragh JH.** Abnormal 1,25-dihydroxyvitamin D metabolism in preeclampsia. *Am J Obstet Gynecol*. 1992;166:1295–1299.
37. **Frolich A, Rudnicki M, Storm T, Rasmussen N, Hegedus L.** Impaired 1,25-dihydroxyvitamin D production in pregnancy-induced hypertension. *Eur J Obstet Gynecol Reprod Biol*. 1992;47:25–29.
38. **Halhali A, Bourges H, Carrillo A, Garabedian M.** Lower circulating insulin-like growth factor I and 1,25-dihydroxyvitamin D levels in preeclampsia. *Rev Invest Clin*. 1995;47:259–266.
39. **Halhali A, Diaz L, Avila E, Ariza AC, Garabedian M, Larrea F.** Decreased fractional urinary calcium excretion and serum 1,25-dihydroxyvitamin D and IGF-I levels in preeclampsia. *J Steroid Biochem Mol Biol*. 2007;103:803–806.
40. **Halhali A, Tovar AR, Torres N, Bourges H, Garabedian M, Larrea F.** Preeclampsia is associated with low circulating levels of insulin-like growth factor I and 1,25-dihydroxyvitamin D in maternal and umbilical cord compartments. *J Clin Endocrinol Metab*. 2000;85:1828–1833.
41. **Halhali A, Villa AR, Madrazo E, et al.** Longitudinal changes in maternal serum 1,25-dihydroxyvitamin D and insulin like growth factor I levels in pregnant women who developed preeclampsia: comparison with normotensive pregnant women. *J Steroid Biochem Mol Biol*. 2004;89–90:553–556.
42. **Fernandez-Alonso AM, Dionis-Sanchez EC, Chedraui P, Gonzalez-Salmeron MD, Perez-Lopez FR; Spanish Vitamin D and Women's Health Research Group.** First-trimester maternal serum 25-hydroxyvitamin D-3 status and pregnancy outcome. *Int J Gynaecol Obstet*. 2012;116:6–9.
43. **Robinson CJ, Alanis MC, Wagner CL, Hollis BW, Johnson DD.** Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. *Am J Obstet Gynecol*. 2010;203:366.e1–e6.
44. **Yu CK, Ertl R, Skyfta E, Akolekar R, Nicolaides KH.** Maternal serum vitamin D levels at 11–13 weeks of gestation in preeclampsia. *J Hum Hypertens*. 2013;27:115–118.
45. **Kasawara KT, do Nascimento SL, Costa ML, Surita FG, e Silva JL.** Exercise and physical activity in the prevention of pre-eclampsia: systematic review. *Acta Obstet Gynecol Scand*. 2012;91:1147–1157.
46. **Patrelli TS, Dall'asta A, Gizzo S, et al.** Calcium supplementation and prevention of preeclampsia: a meta-analysis. *J Matern Fetal Neonatal Med*. 2012;25:2570–2574.
47. **Ma R, Gu Y, Zhao S, Sun J, Groome LJ, Wang Y.** Expressions of vitamin D metabolic components VDBP, CYP2R1, CYP27B1, CYP24A1, and VDR in placentas from normal and preeclamptic pregnancies. *Am J Physiol Endocrinol Metab*. 2012;303:928–935.
48. **Zella LA, Shevde NK, Hollis BW, Cooke NE, Pike JW.** Vitamin D-binding protein influences total circulating levels of 1,25-dihydroxyvitamin D3 but does not directly modulate the bioactive levels of the hormone in vivo. *Endocrinology*. 2008;149:3656–3656.
49. **Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP.** 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest*. 2002;110:229–238.
50. **Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J.** Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem Mol Biol*. 2004;89–90:387–392.
51. **Cardus A, Parisi E, Gallego C, Aldea M, Fernandez E, Valdivielso JM.** 1,25-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. *Kidney Int*. 2006;69:1377–1384.
52. **Roberts JM, Hubel CA.** The two stage model of preeclampsia: variations on the theme. *Placenta*. 2009;30(suppl A):S32–S37.
53. **Evans KN, Bulmer JN, Kilby MD, Hewison M.** Vitamin D and placental-decidual function. *J Soc Gynecol Investig*. 2004;11:263–271.